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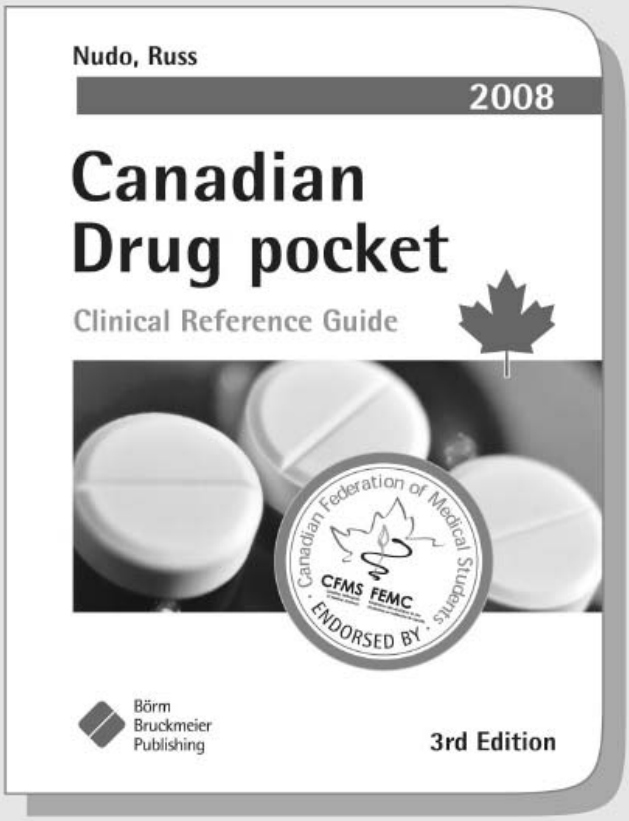

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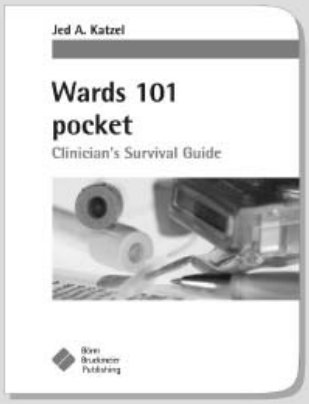

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EDITORIAL

From the Editor's Desk:
Frontiers in medicine: the McGill Journal of Medicine celebrates ten years, and insights into interventional medicine

This issue of the McGill Journal of Medicine marks something of an anniversary for the Journal, and before commenting on the articles in this issue, we thought this a fitting time for reflection on the state of the MJM. Though we chart our recent history through a period of beginning, ending, and beginning and ending again, we have embarked with conviction on the course which was once set for the Journal. The involvement of students from around the world during the past ten years – we estimate the participation of over five hundred students in the MJM since its re-establishment in the 1990s – has made this international effort possible. The MJM provides exposure for young aspiring clinicians and scientists to medical science – something that we feel is insufficiently invited by many medical school curricula. The experience is valuable to both our staff of over 120 current editors from around the world, and to those reading and submitting articles to the Journal. Additionally, though, we believe that the Journal empowers people in traditionally under-represented areas. We are proud to feature, for example, in this issue an article from students in North Carolina, U.S., on dentition in Costa Rica. Garcia and Guzzo provide excellent insight into the strengths and weaknesses of the oral hygiene programs in selected communities throughout Costa Rica, as influenced by allocation of dentists and the construction on the Inter-American Highway. Their work represents proficient medical research, with important implications for public health in developing countries. The staff at the MJM hope that we will open a dialogue between young people interested in bettering health in countries that have traditionally not had the representation in medical science and public health literature that North America, Europe, and parts of Asia have enjoyed. The Journal in this respect is a forum for young, ambitious people to meet, to brainstorm medical challenges by discoursing and sharing insights, and to solve problems throughout the world.

Impetus for this perspective is exemplified by a recent research project of one of the Editors-in-Chief of this issue, who had the opportunity to work with a group studying leishmaniasis in Peru. Leishmaniasis is a parasitic infection that manifests as either cutaneous, muco-cutaneous (which can develop as a sequelae to the cutaneous form), or visceral disease. The parasite is transmitted by the sandfly, which feeds on humans or dogs (the reservoir for the disease), in a manner reminiscent to malaria. The disease is common among people living in endemic regions of Peru, Brazil, Bolivia, and much of Africa and the Middle East. Presently, medical treatment consists of a 20-day course of pentavalent antimoy injections, which is not always successful, and often results in deforming scars (particularly when the disease is contracted on the face, where more farmers work without protection). Non-medical treatment, employed by the local people, consists of drinking a tintiucre referred to locally as "sangre del arbol" – a tree extract – and additionally by applying battery acid to the superficial lesion.

Our group has been working on developing a topical approach to disease management. We are testing a topical cream, imiquimod, which has been approved by the American F.D.A. for use in treating HPV cutaneous warts. Imiquimod is a toll-like receptor (TLR) agonist, activating fixed tissue macrophages which engulfed the parasite in the tissue as part of the innate host defense process. Once activated in this site-specific manner, the macrophages fuse the phagosome (the intracellular compartment containing the parasite) with lysosomes (the macrophage's acidic compartments that digest debris and foreign particles). We hope that these studies will become part of a cadre of evidence from this lab that supports a shift away from the archaic antimony treatment to a more topical, and less offending treatment of this infection. The premise of the work is, simply put, empowering people in the countries with need. The problem originated in Peru, but the tools used to address it (used, furthermore, by the team of local doctors helping in the study), comes from our end. We hope to recapitulate collaborations like these among young people in expanding the McGill Journal of Medicine.

In addition to the Costa Rica study herein, we are pleased to feature a Focus on "interventional medicine." The future of medical science rests heavily on the decisions of the rising generation. As such, we have expanded the Focus section in recent years, inviting reviews from experts in developing fields of medicine, and hopefully inviting discussion among readers about these new topics. In this issue, we feature four expert reviews on interventional medicine, which uses minimally invasive procedures to effect medical outcomes. These articles are eloquently written, and are an excellent amalgam of insights into the diverse applications of interventional medicine. Additionally, we have also chosen an article from a group at the University of Manitoba that studied "door-to-balloon" times at the authors' hospital in Winnipeg. Primary

percutaneous coronary intervention is preferable for patients in whom the first medical contact (the "door-to-balloon" interval) has occurred within 90 minutes. Additionally, it is preferred to conventional fibrinolysis in certain patients even if the interval between the first medical contact and the procedure exceeds 90 minutes (namely, patients with fibrinolysis contraindications, patients over 75, and patients in cardiogenic shock). As such, establishing the efficiency of putting patients' vessels in the hands of an intervntionalist become of the utmost importance. Only with analysis like the Manatoba study herein will efficiency be improved and lives saved.

Finally, this issue of the MJM also features a special Abstract Section wherein abstracts presented at the 2nd Annual Conference of the Canadian Society for Life Science Research (CSLSR) are published. The CSLSR is a non-profit organization created by life science students that is dedicated to bringing together young Canadian researchers at all university levels to share their knowledge, research, and discoveries. This year's CSLSR conference was held at McGill University and brought together students from across the country. The MJM is a proud supporter of this wonderful student initiative. The CSLSR and MJM will continue to support young researchers in their endeavors. It is our hope that students in life science from Canada and across the world will continue to involve themselves in such student forums in order to further their knowledge and encourage each other's work in research.

We hope you enjoy these abstracts as well as the other articles herein. We invite you to consider becoming a part of the MJM team: as a contributor, editor, or reader. Our door is always open.

PRL, WM, PW

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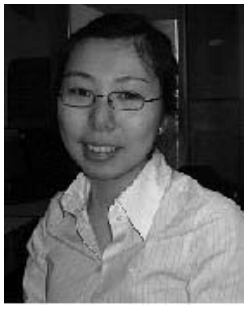

Patrick Lawler, M.D.C.M. (2009) and **Wenya Miao,** M.D.C.M. (2009) are the eleventh Editors-in-Chief of the MJM. Patrick completed his B.Sc.at Washington and Lee University. His prior research interests began in cancer biology and angiogenesis, but have shifted to atherosclerosis and ischemic heart disease. He has spent time researching the latter with groups in Boston, Stockholm, and Montreal. Wenya's research interests are in neurology, specifically in the field of neuron regeneration.

Patrick Williams (M.D.C.M., PhD. 2012) is the Executive Senior Editor of the MJM. He received a B.Sc. (Hons) in Biological Sciences from the University of California, Irvine. He is currently an MD/PhD at McGill, conducting research in cancer immunotherapy.

LETTERS TO THE MJM

Dear *MJM*,

It was a delightful experience to read the print copy of the McGill Journal of Medicine. Our previous experience of your journal was through your website. We congratulate the student team on producing an extremely professional journal. The paper and the print quality are comparable to the best journals in the world. The 9.2 issue carried a good collection of articles from various areas of medical science. We especially liked the Commentary article 'Who do I serve?'. The feature reviews in the special forum on Reflections by Neuroscientists were interesting.

Being medical students and clinical pharmacologists from a developing country, we were especially interested in the article by Junaid Subhan on ‘’TRIPS agreement and public health. In Nepal, the domestic pharmaceutical industry is rapidly developing and some manufacturers have obtained the WHO Good Manufacturing Practice (GMP) certification. However, domestic manufacturers meet only about 50% of the country's requirements and the rest is met by imports from India, Bangladesh and China. India became TRIPS compliant in 2005 and Nepal yet has few years to do so.

The author has given a very lucid elucidation of WTO and TRIPS. A major drug manufacturer in South Asia and the world is India. As Indian medicines are imported into Nepal, India also influences the Nepalese market. Before 2005, India did not allow molecules to be patented and only allowed process patents. Molecules introduced into the Indian market by one company could be manufactured by another company using a different process without infringing on the patent. This kept drug prices low and medicines were affordable to the vast majority. A downside, however, was that innovator companies were reluctant to introduce new molecules into the Indian market.

Post 2005, new molecules are introduced faster into the Indian market and many Indian multinationals are investing heavily on research and development of new molecules. Compulsory licensing, as suggested by the author, may help in making essential medicines available at a low cost in the developing world. Parallel importing is also another good strategy to ensure access. India, China and Brazil are the major generic manufacturers that have provided cheap antiretrovirals and anti-TB drugs to other developing and even to developed countries.

We basically agree with the author that the term 'essential medicines' should be defined within TRIPS. The change in the definition of essential medicines may be a good strategy. However, as rightly said by the author, companies may concentrate more on drugs which are strongly protected by patents and on which they will get a greater return on investment. This has already happened and tropical diseases and diseases of the developing world were neglected. Recently a number of initiatives like Medicines for Malaria Venture (MMV), the Drugs for Neglected Disease Institutive (DNDi) and the Global Alliance for TB Drug Development (based on public-private partnerships) have been started to encourage research and development of medicines for diseases of developing countries. Looking at the history of product patents in India, a long product patent may not by itself be an incentive for an innovator company to concentrate on developing a product. This had happened with molecules like ciprofloxacin and roxithromycin. Indian companies had also created copies of Sildenafil citrate.

Restricting process patents only to essential medicines may be a good idea in principle. Again however, the Indian example tells us that the innovator company may enjoy protection only for a very short period of time. We personally think it is much more difficult to develop and test a new molecule while is is much easier to manufacture an already introduced molecule using a different technique. Placing restrictions on the second developer may be a good idea in principle. However, the practical details will require a lot of work.

We congratulate the author on a well-written article and for exploring a possible means of balancing the interests of the innovator companies and the public health needs of developing countries. This is an area of debate. Medical students and doctors, we believe, should have at least a broad idea about TRIPS, patent protection, and its likely effects on the cost and availability of medicines.

Sincerely,

Bishnu R Giri, eighth semester medical student
Manipal College of Medical Sciences, Nepal

P.Ravi Shankar, MD, Department of Pharmacology
Manipal College of Medical Sciences, Nepal

Bishnu Rath Giri is an eighth semester student at Manipal College of Medical Sciences, Nepal, on a Nepalese government scholarship. He is interested in issues of community health and the measures to alleviate the health status especially of the rural areas.

P. Ravi Shankar is Assistant Professor of Pharmacology at the Manipal College of Medical Sciences, Pokhara, Nepal. His research interests are communication skills teaching and learning, teaching about rational use of medicines and pharmacoepidemiology. He has published a number of articles in national and international journals.

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ORIGINAL ARTICLE

Reperfusion times for ST elevation myocardial infarction: a prospective audit

Kendeep S Kaila, Kapil M Bhagirath*, Malek Kass, Lorraine Avery, Lillian Hall, Alex H Chochinov, James W Tam

ABSTRACT: ***Background*: New published guidelines recommend treatment of ST elevation myocardial infarction (STEMI) within 30 minutes of first medical contact to thrombolysis and 90 minutes to primary percutaneous coronary intervention (PCI).** ***Objectives*: To determine how a tertiary care center compares to these new guidelines and to evaluate the success of measures directed to shorten delays.** ***Methods*: This was a prospectively designed audit loop using retrospective chart review. Specific time intervals were evaluated: 1) T2 (ER presentation to diagnostic EKG; 2) T ER (ER presentation to reperfusion); and 3) T AHA (first medical contact to reperfusion). Results of the initial 12-month data were conveyed to Emergency Room staff and a dedicated EKG machine was placed in the ER for the subsequent 12 months, and the results were then re-analyzed.** ***Results*: In 2003-4, 58 patients with STEMI were identified, with 41 (70.7%) receiving reperfusion. Of those receiving thrombolysis, median T AHA was 54 [37-72] minutes, with 12.0% <30 minutes, while those receiving PCI, median T AHA was 58 [43-78] minutes, with 25.0% <90 minutes. In 2004-5, 52 patients had STEMI, with 40 (76.9%) receiving reperfusion. The percentage of patients meeting the guidelines was 14.3% for the thrombolysis group and 11.1% for the PCI group. Introduction of a dedicated EKG machine led to a strong trend towards improvement in median T2 (22 vs 10 minutes; P=0.07), but other treatment times remained unchanged.** ***Conclusions*: Treatment times are longer than recommended guidelines. More comprehensive strategies and improved coordination of medical services are required to shorten pre-contact and post-contact response times.**

KEYWORDS: ST elevation myocardial infarction, reperfusion times, ACC/AHA guidelines

INTRODUCTION

Acute myocardial infarction (AMI) is a leading cause of morbidity and mortality in Canada. The Canadian Cardiovascular Outcomes Research Team (CCORT) showed that there were 139,523 new AMI cases in Canada between 1997/98 and 1999/2000, with an in-hospital mortality rate of 12.3% (1). In the treatment of acute ST elevation myocardial infarctions (STEMI), both thrombolysis and percutaneous coronary intervention (PCI) are proven reperfusion strategies to decrease mortality (2-8). The initial landmark thrombolysis trials demonstrating a mortality benefit were completed in the late 1980's and early 1990's (2-4). A meta-analysis of these trials published in 1994 demonstrated an absolute mortality benefit of 3% at 5 weeks in those patients treated within 6 hours of symptom onset (5). In a further advancement, the primary PCI trials demonstrated improved outcomes when compared to thrombolysis (6, 7). For example, in

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the DANAMI-2 trial, the primary end-point of mortality, re-infarction, and stroke at 30 days was seen in 8% of patients receiving PCI and 13.7% of patients receiving thrombolysis (7).

Nonetheless, the optimal treatment modality has continued to be an ongoing and contentious issue in cardiovascular medicine (9-10). A recent review of 23 clinical trials by Keeley et al. suggests that primary PCI is more effective than thrombolysis in reducing death and non-fatal reinfarction (8). An essential caveat to this is that the benefit achieved by timely reperfusion by thrombolysis may be just as effective as PCI if there are imminent delays foreseen in receiving PCI. Hence, more important than the choice of initial reperfusion strategy is the concept of receiving treatment in a timely fashion, thus producing a similar degree of myocardial salvage (11).

The 2004 American College of Cardiology/American Heart Association (ACC/AHA) guidelines recommended that thrombolysis or PCI be administered within 30 or 90 minutes of first medical contact, respectively (12). These new guidelines were also reviewed and adapted for Canada by the Canadian Working Group (CWG) (13). It should be noted that these modifications were made from previous ACC/AHA guidelines established in 1999, which required thrombolysis or PCI to be administered within 30 or 90 minutes of arrival to hospital (14). With the emphasis on timely treatment of STEMI, the objective of our present study was to investigate how a tertiary care center in Winnipeg compared to these new guidelines over a 12 month period, looking specifically at the various components of time delay in patients receiving either thrombolysis or primary PCI. In this prospectively designed closed-loop audit, we subsequently made an attempt to rectify in-hospital time delays by introducing a dedicated EKG machine to the Emergency Room (ER) and by providing feedback on the previous year's data to the ER staff. We then re-evaluated treatment times in the subsequent 12 months.

METHODS

Study Design and Patient Population

This was a retrospective chart audit designed prospectively to improve in-hospital time delays for timely reperfusion. The audit initiative was put forth by the Section of Cardiology within the Departments of Medicine and Cardiac Sciences and the Department of Emergency Medicine. The protocol was consistent with standards developed for audit-based research in the Winnipeg Regional Health Authority (WRHA) and the University of Manitoba. Coded 'myocardial infarction' charts were analyzed at St. Boniface General Hospital initially between September 1st, 2003 and August 31st, 2004. Then, on September 1, 2004, a dedicated EKG machine was introduced to the ER, and the next year's charts were subsequently analyzed (September 1, 2004 and August 31, 2005). Furthermore, information from the initial year's audit was conveyed to the ER staff. Inclusion criteria included patients of at least 18 years of age presenting with STEMI, as per the definition consistent with ACC/AHA guidelines. Patients included in the study were either direct walk-ins to the ER or presented via EMS. Patients with NSTEMI, transferred MI, and in-hospital or peri-operative MI were excluded. There was no established mechanism developed in deciding whether patients received PCI or thrombolysis. In general, patients received PCI if there were clear contraindications to thrombolysis, a pre-shock/shock state was present, or if the cardiac catheterization suite was conveniently available (ie: selective daytime PCI).

Data Collection

The data was collected by one of three authors (KB, MK, KK). A fourth investigator (JWT) was available in difficult cases, whereby decisions were made by consensus. Data was recorded on standardized forms designed by our investigative group, containing information pertaining to patient demographics, clinical presentation, and specific time intervals. Figure 1 below illustrates the specific time intervals.

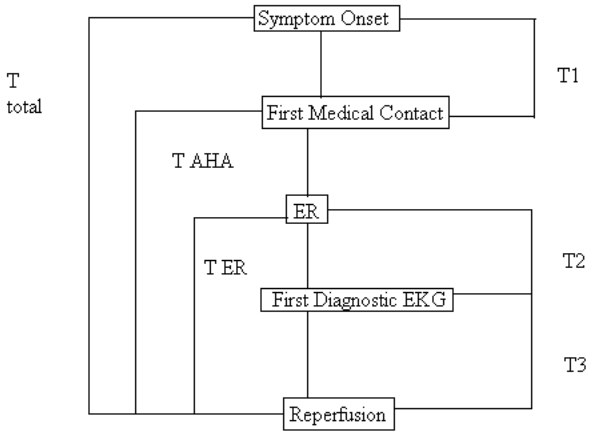

Figure 1: Flow diagram illustrating various components of the treatment times analyzed in this study.

The patient-dependent time interval T1 represents the elapsed time from symptom onset to first medical contact, which could be in the form of direct presentation to the ER or via EMS activation. Other important time intervals include: 1) T2 (time from ER presentation to first diagnostic EKG), 2) T3 (time from diagnostic EKG to reperfusion), 3) T ER (time from ER presentation to reperfusion, or 'door-to- needle/balloon' time), and 4) T AHA (time from first medical contact to

reperfusion, as defined by the ACC/AHA guidelines). Note that in cases with direct presentation to the ER by walk-in, T AHA and T ER were identical.

Other important definitions used in this study were: 1) RRS (received reperfusion strategy), 2) NRS (no reperfusion strategy), 3) RRS-L (received reperfusion strategy - thrombolytics), and 4) RRS-P (received reperfusion strategy - PCI).

Data Management and Analysis

The collected data was subsequently entered into a database system (MS Excel, USA). Statistical analysis was completed with web-based software (15-16). The c2 analysis was used to compare categorical data, while the Wilcoxon test was used for the comparison of non-parametric, continuous dataset. A p-value of <0.05 was deemed to be statistically significant. Statistics were used to compare the following time intervals between 2003-4 and 2004-5: 1) T2, 2) T3, 3) T ER, and 4) T AHA. Following the aforementioned change in the subsequent year, we anticipated direct improvements in T2 and T3, with corresponding improvements in T ER and T AHA.

Table 1: Demographic and clinical characteristics

	Total STEMI 2003-2004	Total STEMI 2004-2005
N	58	52
Age (Mean+/- SD)	64.5 +/- 14.0	63.6 +/- 15.0
Gender (%Female)	32.8	34.6
Mode (%)		
EMS	39.7	52.9
ER	60.3	47.1
Chest Pain (%)	90.0	96.2
Location (%)		
Anterior	41.4	38.5
Killip Score (%)		
1	75.9	74.5
CRF (%)	14.0	3.8
Previous MI (%)	27.6	8.0
Previous Angina (%)	37.9	28.0
Previous CABG (%)	6.9	8.0
Previous PCTA (%)	3.4	14.0
Treated DM2 (%)	15.5	6.0
Previous CHF (%)	5.2	6.0
Peak CK (median)	1173	960
Peak Trop (median)	4.02	2.76

RESULTS

Preliminary results were presented elsewhere (17). In 2003-4, a total of 58 patients diagnosed as having STEMI were identified, and 41 (70.7%) patients received reperfusion therapy. Of the patients who received reperfusion, 25 (61.0%) patients received thrombolysis, while 16 (39.0%) patients received primary PCI. In 2004-5, 52 patients presented with STEMI, with 40 (76.9%) patients having received reperfusion therapy. Of these, 21 (52.5%) patients and 19 (47.5%) patients received thrombolysis and primary PCI, respectively. Overall, the unadjusted, in-hospital mortality rate for the entire group was 17% in 2003-4 and 12% in 2004-5 (p = NS). Other significant demographic and clinical characteristics between the two groups are outlined in Table 1.

The specific time intervals for 2003-4 and 2004-5 are shown in Tables 2 and 3, respectively, with data recorded as the median time in minutes, including the 25th and 75th percentile.

In the initial year (2003-4), the time from ER to the first diagnostic EKG (T2) was 22 (13-33) minutes. For patients who received reperfusion, the treatment time (T3) was 20 (10-27) minutes in the thrombolysis group and 90 (73-214) minutes in the primary PCI group. In 2004-5, there was overall a non-significant, but positive trend toward improvement in T2, with 10 (7-39) minutes elapsing between ER presentation and the first diagnostic EKG (p=0.07). In comparison, T3 for thrombolysis (19 (11-34) minutes, P=0.27) and primary PCI (92 (71-118) minutes, P=0.39) was essentially unchanged.

Table 2: Specific time intervals for 2003-2004 (n=58)

	Total STEMI	RRS STEMI	NRS STEMI	RRS-L	RRS-P
T1 (Symptom onset - First medical contact)	106 (67-191)	91 (56-166)	156 (106-201)	112 (62-180)	85 (48-128)
T2 (ER - Diagnostic EKG)	22 (13-33)	17 (12-28)	29 (22-53)	17 (13-25)	18 (12-28)
T3 (Diagnostic EKG - Reperfusion)		42 (14-80)		20 (10-27)	90 (73-214)
T ER (ER - Reperfusion)		72 (31-119)		34 (21-67)	117 (91-246)
T AHA (First medical contact - Reperfusion)		81 (49-124)		54 (37-72)	124 (92-255)
T Total (Symptom onset - Reperfusion)		166 (123-330)		148 (110-313)	195 (144-483)

*All times stated as median (25th-75th percentile), in minutes
RRS - Received Reperfusion Strategy
NRS - No reperfusion strategy
RRS-L - Received Reperfusion Strategy - Lytics
RRS-P - Received Reperfusion Strategy - Primary PCI

Table 3: Specific time intervals for 2004-2005 (n=52)

	Total STEMI	RRS STEMI	NRS STEMI	RRS-L	RRS-P
T1 (Symptom onset - First medical contact)	96 (66-147)	101 (70-155)	96 (30-100)	94 (60-150)	108 (73-215)
T2 (ER - Diagnostic EKG)	10 (7-39)	10 (6-27)	21 (8-66)	10 (4-20)	10 (6-34)
T3 (Diagnostic EKG - Reperfusion)		42 (18-92)		19 (11-34)	92 (71-118)
T ER (ER - Reperfusion)		68 (25-123)		28 (18-59)	104 (91-137)
T AHA (First medical contact - Reperfusion)		91 (55-142)		58 (43-78)	139 (101-162)
T Total (Symptom onset - Reperfusion)		194 (160-269)		187 (120-260)	204 (176-272)

*All times stated as median (25th-75th percentile), in minutes
RRS - Received Reperfusion Strategy
NRS - No reperfusion strategy
RRS-L - Received Reperfusion Strategy - Lytics
RRS-P - Received Reperfusion Strategy - Primary PCI

As outlined in Tables 4 and 5, the percentage of patients meeting the previous standard vs. the newer ACC/AHA guidelines between the 2 years was compared for thrombolysis and primary PCI. In general, fewer proportion of patients met the more stringent guidelines. There was no statistically significant difference in the proportion of patients receiving treatment in the recommended time window between 2003-4 and 2004-5.

Table 4: Percentage of patients receiving thrombolysis within the previously recommended guidelines and the newer ACC/AHA guidelines between 2003-2004 and 2004-2005.

		Patients (%)	P-value
T ER < 30 min (Door-to-needle time)	2003-2004	40.0	
	2004-2005	52.4	
			NS
T AHA < 30 min (First medical contact to needle time)	2003-2004	12.0	
	2004-2005	14.3	
			NS

Table 5: Percentage of patients receiving thrombolysis within the previously recommended guidelines and the newer ACC/AHA guidelines between 2003-2004 and 2004-2005.

		Patients (%)	P-value
T ER < 30 min (Door-to-needle time)	2003-2004	25.0	
	2004-2005	22.2	
			NS
T AHA < 30 min (First medical contact to needle time)	2003-2004	25.0	
	2004-2005	11.1	
			NS

DISCUSSION AND CONCLUSIONS

The purpose of this prospectively designed closed-loop audit was to determine how one tertiary care center in Winnipeg compared to the new ACC/AHA prescribed treatment times for STEMI. This study is the first published audit of Canadian data specifically in comparison with the ACC/AHA guideline definition. The percentage of patients being treated in the optimal time period was unacceptably low in both years of the study, with even fewer patients meeting the more stringent guidelines.

Treatment times for hospital arrival to reperfusion (T ER) were comparable to previously published results (18-20). For example, in the FASTRAK II database, which is an ongoing, prospective registry of acute myocardial infarctions in Canada, the median time from hospital arrival to fibrinolytic treatment in 11,574 patients from 1998 to 2000 was 43 minutes, and only 27.4% of patients were treated within 30 minutes (18). Furthermore, in the AMI Quebec Study, where 1189 patients during the year 2003 were evaluated, the median door-to-needle time was 32 (20-49) minutes, with 48.8% of patients achieving the target of less than 30 minutes (19). The door-to-balloon time was 109 (79-150) minutes, and 35.5% of patients met the required goal of less than 90 minutes (19). In contrast, the more recent Calgary STEMI QIHI group showed an impressive median door-to-balloon time of 62 minutes, with 79% of patients meeting the prescribed time (21).

Following the implementation of a dedicated EKG machine in the ER and feedback of the previous year's results to the ER staff, it was expected that certain components of time delay would be shortened, leading to a concomitant improvement in reperfusion time in the subsequent year. Despite these changes, there was only a marginal improvement in the time to first diagnostic EKG, but this did not translate into meaningful reduction in time to reperfusion therapy.

The demonstration of the treatment times in this study indicates that systematic factors contributing to ongoing delay remain unidentified and unresolved. Improving patient-related factors to encourage earlier recognition of symptoms, and thus an overall reduction in the total ischemic time is essential, but interestingly, the REACT

Study Group in the United States was unable to demonstrate that community intervention had a significant impact (22). Attempts to ameliorate in-hospital delays such as the time to first EKG is the next logical time component to improve; however, we were unable to demonstrate this in our investigation. Furthermore, it would be provocative to look at the differences in reperfusion times in patients presenting via EMS or as a walk-in to the ER. We chose not to look at these differences in our study due to the small sample size, but certainly this would be important in a larger cohort of patients. Our institution is now in the process of attempting to coordinate EMS and ER services, as in many cases, the diagnosis of STEMI is known prior to ER presentation, but an ER EKG, for example, is invariably repeated (Personal communication; R.Grierson, WFPS/EMS Medical Director). Factors leading to delays in treatment after obtaining the EKG are uncertain, and we are looking further into local factors in an ongoing, prospective fashion. Finally, there is no local experience with pre-hospital thrombolysis, but published data in this area is increasingly provocative (23-26). Specifically, a meta-analysis comparing pre-hospital thrombolysis against in-hospital thrombolysis published in 2000 demonstrated a one hour reduction in time to thrombolysis and a 17% decrease in mortality (26). Furthermore, the pre-hospital diagnosis and transfer pathway for PCI developed in Calgary is another promising approach to finding a solution to this problem (21).

Limitations

There were some notable limitations in this study. Firstly, the sample size was small and included only 24 months of data. However, this was a homogenous sample representing patients from one tertiary care center within an urban region with five other acute care hospitals. Secondly, we were unable to provide a month-by-month evaluation of the hospital's performance; instead, we looked at the data annually. This is an important limitation, although it was imperative that we maintain the study in a true audit format, and by doing so, we were able to minimize the Hawthorne effect. The Hawthorne effect is a phenomenon whereby people may artificially change their behavior during a research study. If we had provided the ER physicians with monthly data as opposed to annual data, the Hawthorne effect would have likely been magnified, thus affecting our results. Thirdly, we looked at the time of administration of initial reperfusion strategy rather than the adequacy of reperfusion, defined as an open artery by EKG normalization and grade TIMI III flow. Finally, there is no local experience with pre-hospital thrombolysis and no defined established mechanism for the systematic use of primary PCI.

Conclusion

Treatment times are longer than the newly recommended ACC/AHA guidelines, with a minority of patients being treated in the optimal time period at our tertiary care center. More comprehensive strategies and improved coordination of medical services are required to shorten pre-contact and post-contact response times.

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ORIGINAL ARTICLE

Dientes ChiquiTICOS: an analysis of juvenile dentition and dental health in Costa Rican indigenous communities

Alfredo García*1, Christina Guzzo2

ABSTRACT: This study surveyed the dental health of three Costa Rican indigenous populations and two rural, non-indigenous communities. Sixty-six individuals, both children and adults, were interviewed regarding dental hygiene practices and the dentition of eighty-eight children from the ages of two to thirteen was examined. The indigenous populations, on average, showed a more important number of anterior dental pathologies as compared to a non-indigenous group (42% vs 20%). Collectively, both access to and utilization of dental healthcare were worse within the indigenous communities; however, there was still great variation amongst all five sites.

KEYWORDS: dentition, caries, dental health, hygiene, Costa Rica, idigenous.

INTRODUCTION

Costa Rica, with a total land area of 51,100 square kilometers, is a country slightly smaller than the state of West Virginia and has a total population of 4.02 million (1). The indigenous population, residing on twenty-two indigenous reserves, comprises only 1.7% of this population. There are currently six indigenous tribes in Costa Rica: Boruca, Bribri, Cabécar, Guaymi, Maleku, and Térraba. Three of these groups are discussed in this report: the Borucas, the Malekus, and the Bribris (Figure 1).

The Boruca population consists of approximately 2,000 individuals and is located in the province of Puntarenas in the southeastern section of Costa Rica. This study contains data collected at the community of Boruca, which is the largest of the three reserves. Due to construction of the Interamerican Highway, which now runs though the town of Rey Curré, transportation to and from the community has become much easier. Consequently, access to medical services has improved.

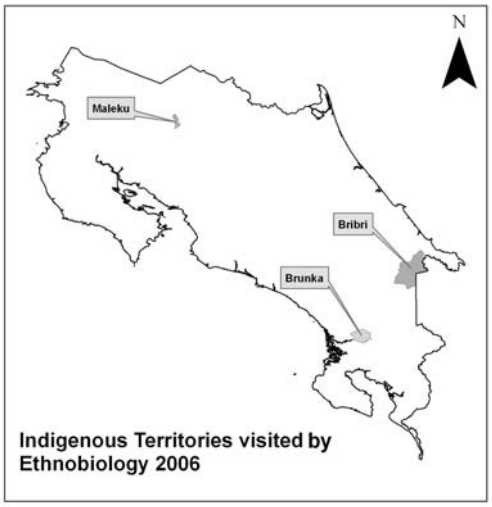

Figure 1. Location of indigenous territories within Costa Rica

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The Maleku are a group of roughly 500 individuals found in the province of Alajuela in Northern Costa Rica. The community at La Fortuna, which is the southernmost group of Malekus, is divided into the three different villages; Palenque El Sol, Palenque Tonjibe, and Palenque Margarita (2). This study contains data collected at the villages of Palenque Tonjibe and Palenque Margarita.

The Bribri of Talamanca is the largest of all Costa Rican indigenous reservations, spanning a territory of 43,690 hectares (3). For this study, two communities within this Bribri reserve were visited: Kachabri and Shiroles. Preservation of the Bribri culture can be seen in the maintenance of their language and in the continued use of *awapa* (shamans) for healing purposes. The Bribri health services have been characterized by state medical authorities as deficient in many aspects. The government has recognized the lack of attention, absence of house visits, difficulties regarding accessibility, and nutrition programs which are not meeting expectations (3).

In addition to the three indigenous reserves, two non-indigenous communities were visited to serve as comparison groups: La Gamba and Cahuita. La Gamba is a sustainable farming community located in the province of Puntarenas. It was established in 1978 as a *bananal* (banana plantation) and has since become an example of organic and subsistence farming practices. Cahuita is an Afro-Caribbean community located in the province of Limón along the east coast of Costa Rica. With the lure of Cahuita National Park and the region's deep historical roots, this community has become a well-known tourist destination. These sites were chosen because of the two different, yet common, Costa Rican communities that they represent. La Gamba gives information on the rural population devoted to cultivating land (known as the *campesino* culture). Cahuita gives insight into areas that are dominated by tourist influence and activity.

Collectively, due to their location in remote and inhospitable rural areas, the indigenous communities often lack access to health care, schools, electricity, and potable water. Socialized healthcare has reached the indigenous communities of Costa Rica only within the past decade. In 1999, the government completed distribution of identification cards to all indigenous people through the National Indigenous Commission (4). Consequently, all indigenous people were given the benefits of citizenship including *seguro social* (socialized health insurance). While this helped to facilitate access to public medical facilities for all citizens, the question remains as to how this has affected indigenous communities, in particular, their utilization of dental healthcare.

Some work has been done on the dental health of the Costa Rican indigenous populations, but nothing as extensive as this report. Statistical figures do exist illustrating the number of dental visits (5); however no real field work has been conducted by health specialists concerning these indigenous communities. Duke undergraduate Shaina Wahl of the 2005 Organization for Tropical Studies Field Ethnobiology program examined the dentition of the Boruca and Maleku communities, but difficulties in her investigation necessitated further research. Her sample size spanned from individuals 3 to 20 years of age, a range that incorrectly included both deciduous and permanent dentition, with her method of analysis consisting of simply counting the number of caries lesions present in the mouth. However, when evaluating the number of cavities present, it is essential to distinguish between deciduous (primary) and succedaneous (permanent) dentition; the two are considerably different anatomical entities (6). Some of these differences between the two types of dentition, such as enamel thickness, contribute to the appearance of more dental pathologies in deciduous dentition than in permanent dentition. Wahl's (7) discussion also failed to explore the underlying principles behind the answers she received and the severity of dental pathologies encountered.

In contrast, this study aims to fully and adequately represent the oral health of three indigenous communities by employing a series of more systematic approaches. A complete dental inventory was conducted using an age range that encompassed all the years of primary dentition up until the age in which the full permanent dental arcade develops. The severities of pathologies, not just the quantity of pathologies, were also analyzed. This study also includes the results of extensive interviews with children and adults on the dental hygiene of both indigenous and non-indigenous communities. Factors which influence indigenous children's adherence to self-care standards were appraised through a survey of access to and utilization of healthcare, pervasiveness of health education, attitudes towards self-care standards, and identifiable cultural factors. The results of this study can serve to educate indigenous groups on how to improve dental hygiene, utilization of healthcare, and ultimately the overall health status of their children.

MATERIALS AND METHODS

Research was conducted at the indigenous reserves of the Boruca, Maleku, and Bribri, the Afro-Caribbean community of Cahuita, and the *campesino* community of La Gamba. Community consent was obtained by the Organization for Tropical Studies (OTS) prior to arriving at these locations and verbal informed consent

was sought and obtained from each consultant prior to questioning. In the case of children, consent was received from their guardian before both dental examinations and general questioning. Consultants were chosen at random. A day was spent at each of the locations in which the researchers walked around the community and met with children in homes or schoolhouses (Figure 2). Each interview was conducted with both authors present.

Figure 2. Dental hygiene interview

Notes were made while looking into the open mouths of children and dental atrophies were recorded on standardized worksheets with as much detail as possible. The quality of dental health of each child was quantified using a system devised by the authors. One gradation point was given for each of the following dental atrophies: (1) pain and/or bleeding; (2) open mesial interstitial caries on central incisors (given only one gradation point for both incisors); (3) other anterior caries (each given a separate gradation point); (4) medium to very large untreated caries; (5) untreated caries on the permanent dentition; and (6) considerable plaque formation (given only one gradation point for overall appearance). Filled or treated cavities were not given a gradation point since these pathologies had been successfully addressed by a dentist.

Thorough semi-structured interviews were carried out with fourteen children at each community, seven of each gender. The guardian of each child was also interviewed. In addition, interviews were conducted with directors of the secondary schools, healthcare workers, elders, and parents of children too young to be interviewed directly (7). Both closed and open-ended questions were used for gathering dental data, using a list of thirty-five questions as a guide. Responses were recorded using notebooks and microcassette recorders, if given permission from the consultant (Figure 3). General observations were also recorded in hand notebooks, and digital cameras were utilized to aid with visual observations. Upon completion of each consultation, either a collection of toothbrushes, toothpastes, and dental educational materials or cookies were given to all children who contributed to our dataset. Adults were compensated for their time with dental health pamphlets or small gifts of food.

Figure 3. Dental examination

RESULTS

A total of 49 boys and 39 girls were examined (Figure 4) with the majority of participants being between the ages of 7 and 12. The breakdown of each community is given in Table 1. The average number of serious dental pathologies ranged from 1.6 to about 4 per mouth: 1.66 in La Gamba, 1.82 in Boruca, 3.85 in Maleku, and 3.14 in Bribri (Figure 5). Due to a lack of sample size, Cahuita was omitted from the dental examination

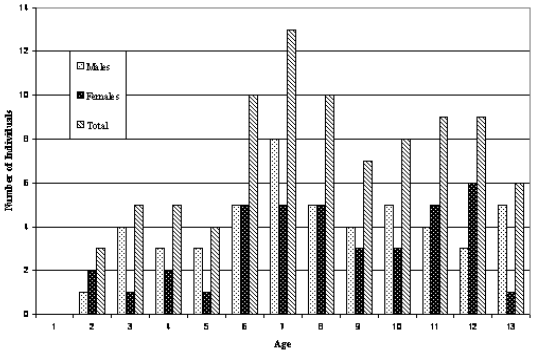

Figure 4. Distribution of sample size

Table 1. Community distributions

Community	Av. Dental Path.	Total #	Male/Female
La Gamba	1.66	15	9/6
Boruca	1.82	17	7/10
Maleku	3.85	28	15/13
Bribri	3.14	28	18/10

charts; dental hygiene information from interviews,

however, was still collected from Cahuita.

Pathologies of the anterior teeth (comprised of the incisors and canines) were noted in 34 out of a total of 88 individuals (38%). The percentage increases to 42% (31 out of 73 individuals) when data from La Gamba is

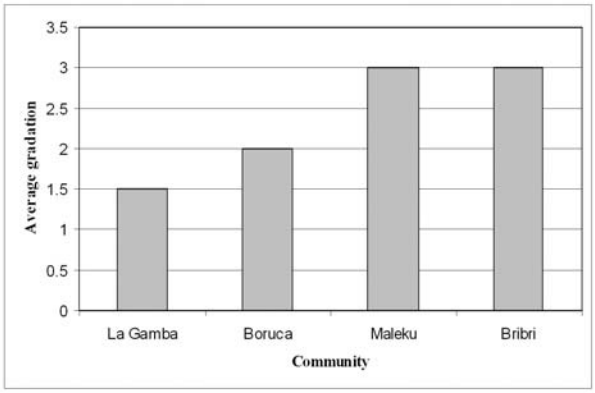

Figure 5. Average number of serious dental pathologies

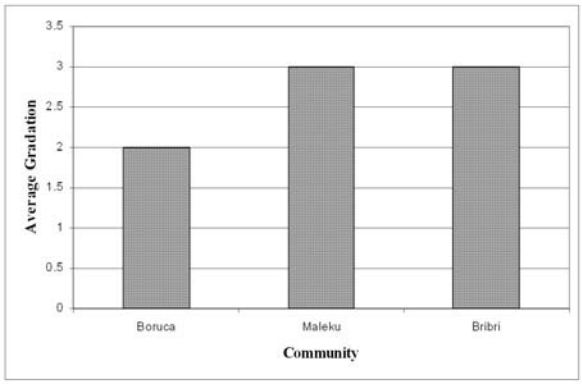

Figure 6. Average number of serious dental pathologies - indigenous

excluded, leaving only data obtained from indigenous communities (Figure 6).

Although distinguishable characteristics were observed in each of the five communities, there were also many similarities. Nearly all of the children reported brushing their teeth at least two times per day (76%). Altogether, 29 of 66 children interviewed (44%) reported brushing their teeth three times per day. Not all individuals cooperated with all the questions asked of them, therefore only 66 answers were available for the results.

Both adults and children use only a toothbrush and toothpaste to clean their teeth. Colgate is the toothpaste of choice throughout all five communities. Almost all teeth develop cavities and are pulled by the dentist before given the opportunity to fall out on their own. Only three adults reported knowledge of natural remedies for oral conditions. However, these are no longer utilized and none of the children were aware of such treatments. All parents reported brushing their children's teeth from birth through infancy and taking responsibility for teaching them how to brush their own teeth when capable. Most children also receive some form of dental health education at school. The diets among all of the consultants were very similar, consisting mainly of rice, beans, fruits, vegetables, and meat. The most common foods that children associated with being harmful to their teeth were candy, cookies, and chewing gum. The pervasiveness of these saccharines in the diet varied among the communities but overall consumption has increased in recent years.

DISCUSSION

Consultations with individuals residing in Cahuita and La Gamba were used as comparison groups to assist our evaluation of the dental healthcare situation in indigenous communities. Collectively, adherence to oral self-care standards was higher among consultants in Cahuita and La Gamba than in the three indigenous communities. This was indicated by the lower number of average dental pathologies (1.6 in La Gamba vs 1.8, 3.8, and 3.1). The better oral hygiene practices in Cahuita could be attributed to many factors, such as better school systems, higher income, easily accessible transportation, and the influence of tourists. The construction of the Interamerican Highway near the Boruca community has affected Borucan health in a positive light with the Borucas having considerably less average dental pathologies than the other indigenous communities. The qualitative data from dental examinations further substantiate this large inequity existing between the dental health of indigenous and non-indigenous children of Costa Rica.

The most significant finding was the frequency of anterior pathologies observed (Figures 7-8). Caries lesions are typically found in the posterior dentition where the deep occlusal crenulations (tooth chewing surface morphology) and difficult-to-brush areas are conducive to the formation of cavities. It is uncommon to find caries lesions in the anterior dentition where the tooth morphology is not conducive to residual food and sugar buildups. However, we observed anterior caries in 38% of the total children studied, and more strikingly, in 42% of the children in indigenous communities. This becomes even more interesting when one compares this figure to the 44% of children (all indigenous) who stated that they brushed their teeth 3 times per day.

Yet anterior pathologies are very preventable with simple brushing. They should only arise when food particles are allowed to remain in the interstitial areas for considerable amounts of time. This trend could have arisen from a number of possibilities. The children may

not be effectively brushing their teeth, or may not be brushing their teeth altogether. Since the numbers given in this document were self-reported, there is the considerable chance that the children over exaggerated their consistency in tooth brushing. Likewise, the amount of saccharines in the diet may directly affect this trend as well. There was a prevalence of eating sticky candies or of chewing sugar cane in all the communities visited, all of which directly contributes to considerable tooth damage without brushing.

Figure 7. Boruca child, age 4, displaying a case of anterior pathology

Figure 8. Bribri child, age 7, demonstrating anterior pathology

At the same time, the severity of other pathologies detected is of considerable importance. Many of the cavities had extended considerably deep within the tooth crown and were completely untreated. In some cases, the cavity succeeded in devouring the tooth crown entirely to the gum line. These results are noteworthy considering the majority of pathologies observed are preventable with simple dental care and attention. With early identification, caries can be treated quickly and easily, thus preventing the catastrophic damage we observed.

Through our comparison of indigenous and non-indigenous communities, it is clear that the governmental resources regarding dental health are not sufficiently reaching the indigenous people. The fact that these communities are isolated significantly impacts their access to dental care. Difficult terrain separates the indigenous communities from urban areas, where better services are often available. And although services are provided within or in close proximity to each of the indigenous reserves, these services are lacking in many areas. First, the clinics are understaffed and there is often only one public dentist to permanently serve an entire indigenous group. Second, the visiting dentists only come to the community sporadically and are not able to visit a significant proportion of the population in the duration of their stay. The lack of adequate dentists is mainly due to unwillingness of non-indigenous dentists to permanently reside in the indigenous communities. Since there are no governmental incentives for a dentist to work near these communities, many choose not to. Additionally, very few, if any, indigenous people attend medical or dental school and therefore cannot bring their expertise back to the community.

Consultants also attributed the cause of poor dental hygiene to a lack of information, education, resources, and help from the government. It should be noted, however, that the extension of governmental services to indigenous communities has only occurred within the past decade. Therefore, it is quite possible that these services provided are still being fine-tuned. The best solution would be for the government to pinpoint existing weaknesses in dental hygiene and develop a straightforward regimen for all citizens of Costa Rica to follow that would combat these issues. Consistent efforts should also be made to enforce this regimen. Members of the indigenous communities reported that the government currently provides minimal preventative education, and that this instruction is not professional and not delivered with authority. Furthermore, dental education has only been introduced to the indigenous communities within the last few decades, and many parents remain unaware of the importance of maintaining healthy oral hygiene. As a result, children also fail to recognize the consequences. Many consultants mentioned that education would significantly improve dental health. Colgate has sponsored a global dental awareness program entitled *Sonrisas Brillantes, Futuros Brillantes* for the past 15 years. Pamphlets for parents and worksheets for children depict the proper dental care techniques and practices. However, we were the first to introduce these educational materials to the indigenous families.

Overall, the results obtained through this study demonstrate the effects that all these variables have had on these communities. Although dental health education is considerably less in the indigenous populations, much of this can be attributed to the inadequate access to healthcare itself. As the results from Boruca

demonstrate, there could be a direct correlation with lower average dental pathologies and the Interamerican Highway. The disregard for dental health in the indigenous communities can also be attributed to the general lack of interest by governmental sources. Although the government provides a dentist for each community, the service is often unsatisfactory and insufficient.

Future studies should look at the dental health of adults to see whether there is a correlation across generations. To improve accuracy, a larger sample size should be used at each community and more detailed observations should be made. It would also be of benefit to visit and interview the dentists treating the members of each community. Interviews with residents of La Gamba concerning oral health practices and dental examinations with children of Cahuita would contribute to a more thorough study. Future studies should also look at the impact of the distribution of toothbrushes on the dental health of children. The dental health of these communities should be monitored for detection of any trends. Producers of dental hygiene products should be encouraged to launch *pro bono* campaigns in indigenous communities.

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ORIGINAL ARTICLE

Comparing knowledge of diabetes mellitus among rural and urban diabetics

Ahmad Ayaz Sabri*, Muhammad Ahad Qayyum, Naif Usman Saigol, Khurram Zafar, Fawad Aslam

ABSTRACT: A questionnaire-based cross-sectional study was carried out to assess the awareness of diabetes mellitus among rural and urban diabetics. After analyzing the awareness level of both populations, the urban diabetics were found to be more educated about diabetes. A 25-question survey was used to judge the awareness level of diabetes mellitus. A total of 240 diabetics were surveyed, 120 each from rural and urban areas. The mean awareness among the rural population was 13 (SD± 2) correct answers out of a possible 25. Similarly, in the case of the urban diabetics the mean awareness was 18 (SD± 2) correct answers. The survey was conducted on randomly chosen diabetics belonging to Lahore and Faisalabad, (urban areas), as well as Habibabad, Haveli Koranga and Baba Kanwal (rural areas). The results emphasize the interrelation between demography and awareness of diabetes mellitus. The rural diabetics are far less knowledgeable about diabetes mellitus, its management and its complications. Thus, there is an urgent need to improve the awareness level of diabetes mellitus in rural areas. Doing so will give rise to a healthier workforce and a lessened economic burden on Pakistan.

KEYWORDS: diabetes mellitus, awareness, rural, urban.

INTRODUCTION

Diabetes definition - diabetes is a disease which is caused by the inadequate production of insulin by the body or by the body not being able to properly use the insulin that is produced thereby resulting in hyperglycaemia (high blood glucose levels) (1).

Almost 10 % of the adult population of Pakistan suffers from diabetes mellitus (1). Diabetes mellitus plays an instrumental role in causing diseases like hypertension, cardiovascular diseases, diseases of skin appendages and gangrene (2). Other serious complications include retinopathy, neuropathy, nephropathy, and lower-extremity amputations (1, 2).

Although diabetes mellitus is an incurable disease, it can be managed very well. Training in self-management is integral to the treatment of diabetes. Proper management requires patients to be aware of the nature of the disease, its risk factors, its treatment and its complications (3, 4).

Diabetes mellitus along with its complications contribute a significant amount of burden on the society. Lack of awareness has resulted in an increased number of diabetics over the years. This has resulted in a less efficient workforce and a huge economic burden on Pakistan (5). This study was conducted to assess the level of awareness among the diabetics of rural and urban populations of Pakistan. The aim of this study was to find out the areas which need more attention in terms of resources and planning.

METHODS

This cross-sectional survey was conducted in July 2004 on randomly chosen diabetic patients of both rural and urban areas. A total of 120 diabetic patients were surveyed from urban areas. The urban areas included Lahore and Faisalabad. Similarly, 120 diabetic patients from rural areas were surveyed as well. The rural areas

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included Habibabad, Haveli Koranga and Baba Kanwal (all of which lie in Punjab province).

All the subjects answered a voluntary, confidential and self administered questionnaire. It contained true/false type of questions which were aimed to assess the awareness of diabetes mellitus in relation to its definition, types, causes, symptoms, control, management, treatment, complications and its relation to other diseases. A few examples of the questions asked are as follows: 1) To the best of your knowledge is diabetes a communicable disease True / False? 2) To the best of your knowledge does diabetes have anything to do with insulin True / False? 3) To the best of your knowledge does obesity have any relation to a person developing diabetes True / False? Apart from such questions the questionnaire also gathered data about sex, level of education and socio-economic status.

All the surveys were administered in the presence of at least one of the authors. Urban diabetic patients were surveyed in hospitals, schools, universities and their residences whereas rural diabetic patients were surveyed largely at village meetings (*chopaals*).

RESULTS

The total number of rural diabetic patients surveyed was 120. The majority of the population answered 6-10 questions correctly out of a total of 25 questions asked. Similarly, 120 urban diabetic patients were asked the same 25 questions. In this case, the majority of diabetic patients gave 16-20 answers correctly as shown in Figure 1. This contrast is the main feature of this study.

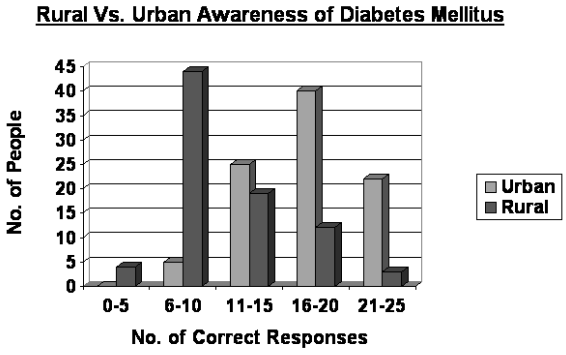

Figure 1. The distribution of the participants giving the number of correct responses.
Vertical axis: Number of diabetics
Horizontal axis: Number of correct answers
Total number of questions: 25

The mean awareness among the rural population was 13 (SD± 2) correct answers out of a possible 25. In the case of the urban diabetic patients, the mean awareness was 18 (SD± 2) correct answers.

Out of the 120 urban diabetic patients surveyed, 71 were males and 49 were female. In comparison, there were 88 males and 32 femals among the rural diabetics surveyed.

The data was also collected and tabulated (Table 1) based on the level of education of each participant. It was determined that that in the rural cohort, almost half of the participants had never gone to school at all; comparatively, a quarter of the urban participants had no formal education. The remaining breakup can be viewed in Table 1.

Table 1

	No Education	Primary (5^{th} Grade)	Middle (8^{th} Grade)	Matric (10^{th} Grade)	Inter (12^{th} Grade)	Graduate
Urban	30	15	17	23	11	24
Rural	53	20	19	11	1	16

The table shows the break up of participants from both urban and rural population in relation to their educational level.

DISCUSSION

In the present study, we have sought to determine the awareness level of both urban and rural diabetic patients about the disease. The strategy of this study was to prepare a questionnaire that would test the basic knowledge of diabetes mellitus, its definition, types, causes, symptoms, complications, management, treatment and relation to other diseases. This survey was administered to both rural and urban diabetic patients. Our study shows that the urban diabetic patients are much more educated about diabetes when compared with the rural diabetic patients.

Upon reviewing the answers to certain questions, a few more findings were discovered. One was preference to use '*desi ghee*' (a condiment high in cholesterol and saturated fatty acids) instead of cooking oil among both the rural and urban diabetic patients. Another one was the preference to consume meat instead of legumes and vegetables. One other finding which was prevalent among rural diabetic patients was their preference to consult *hakeems* (Muslim physicians) over doctors when it came to diabetes.

One factor which was observed to play a role in improving awareness was the level of education attained. A well-educated person had a much higher awareness level than a person who had received no education whatsoever. As a result, we want future

studies to look into this factor, and assess to what extent this factor plays a role.

Female diabetic patients were found to be far less aware of diabetes mellitus when compared with the males. One reason for this finding could be the low female literacy rate in Pakistan (6). Again, it emphasizes that future studies should be carried out to determine the link between education and diabetes awareness.

The link between awareness and socio-economic status is another factor that should be studied more deeply. It was observed during our study that diabetics belonging to poor socioeconomic status were less aware than those belonging to the higher class. While conducting this research, the authors have inculcated ideas on how to improve awareness about diabetes mellitus. In the short run, the authors believe that increasing the level of awareness among village elders in India and Pakistan would be a very potent strategy.

The organization in rural areas is such that all people are required to meet on a weekly or monthly basis at meetings known as *chopaals*, where the village elders dispense their experiences. In making the elders more aware, the trickling effect may make the general rural population more aware about diabetes mellitus.

Another strategy is to make the *hakeems* in the rural population more informed about the population's level of awareness, and help guide them to increase the awareness of their patients. The common rural citizen has a lot of fears concerning doctors and allopathic medication. As a result, increasing the level of awareness of the *hakeems* could to some extent help the general population as well. However, in the long run improving education standards and socioeconomic status is no doubt the cornerstone in enhancing diabetes mellitus awareness.

In conclusion, our study has provided direct evidence that urban diabetic patients are more aware than rural diabetic patients about diabetes mellitus. We have provided some discussion with regards to other factors that determine the awareness level of diabetes mellitus.

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CASE REPORT

Case report of an intra-abdominal desmoid tumour presenting with bowel perforation

Mushtaq Shah*, Bushra Azam

ABSTRACT: Desmoid tumours are benign tumours originating from the musculo-aponeurotic structures of the body. They are mainly composed of collagen. These tumours commonly occur in post-partum women in whom they originate from the rectus abominus and in old surgical incisions. Here we present a case report of a young gentleman who presented with an acute surgical abdomen and subsequently underwent a laparotomy and was found to have an inflammatory mass. Histological analysis of this mass revealed mesenteric fibromatosis (desmoid tumour). I wish to present this interesting case because the patient was neither female nor someone who had previously undergone any surgery. It is rare for mesenteric fibromatosis to present with intestinal perforation and only one case been reported in the literature thus far (1). An important learning point from this case is that rare pathology can manifest itself with common signs and symptoms.

CASE

History & examination

A 33 year old healthy male of Afro-Caribbean origin presented to the emergency department with acute generalised abdominal pain of three hours duration. He had not noticed any recent change in bowel habit, bleeding per rectum or any systemic symptoms. His past medical history was unremarkable. It was of note that there was no family history of inflammatory bowel disease or gastrointestinal malignancy. He was a non-smoker and did not consume alcohol. On examination he had a heart rate of 110 beats per minute, temperature of 37.2^{O}C, stable blood pressure but he was cold and clammy. Examination of his abdomen revealed a peritonitic abdomen that was not distended. No mass was palpable and there was no organomegaly. Bowel sounds were absent and rectal exam revealed an empty rectum and no palpable masses.

Investigations

An erect chest x-ray revealed a pneumoperitoneum. Blood tests revealed a neutrophilia of 25,000 but urea and electrolytes were within normal range.

Treatment

He was resuscitated with intravenous fluids and an emergency laparotomy was performed. During the laparotomy, a 0.5 cm perforation was noted in the ileum 25 cm proximal to the ileo-caecal valve on the mesenteric border. An inflammatory mass measuring 5 x 3 cm was noted directly adjacent to the perforation in the mesentry. 30 cm of small bowel was resected with a right hemicolectomy, hence removing the mass and the perforation. A side-to-side anastomosis was done.

Post-operative course

The patient had a swinging pyrexia for a period of one week. No obvious cause of sepsis was identified and it was thought that he may have a subphrenic collection. Therefore a computed tomography (CT) scan was arranged. However, he had a severe reaction to the intravenous contrast medium which resulted in hypotension. Fortunately, the patient made a full recovery. The scan confirmed the suspicion of a subphrenic abscess. The patient underwent a second laparotomy to drain the subphrenic abscess and no masses were seen during this procedure. Histological

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examination of the resected specimen from the first laporotomy revealed mesenteric fibromatosis (desmoid tumour) incompletely excised. The resected right hemi-colon did not show evidence of dysplasia, malignancy or polyposis. The estrogen receptor status was not reported. Due to continuing abdominal discomfort on the ward after the second laparotomy, an ultrasound scan was arranged that revealed the following:

> A rather ill-defined, fairly ovoid, inhomogeneously-hypoechoic mass lesion is identified in the lower abdomen (infra- umbilical) para-sagittal plane. It measures about 5 x 3 x 4.5 cm in maximum dimensions surrounded by excess peritoneal fat and some bowel loops representing a residual of a previously resected abdominal desmoid.

The pain eventually resolved and after making a good recovery, he was commenced on oral tamoxifen, 20 mg once a day. Although estrogen receptor status was not reported, the patient was commenced on this therapy as it was assumed that most desmoid tumours have estrogen receptors. He was subsequently referred to a specialist in a distant tertiary unit for further evaluation of the residual tumour and for the prospect of further elective surgery.

DISCUSSION

Desmoid tumours (fibromatosis) are benign fibrous neoplasms originating from the musculo-aponeurotic structures throughout the body. These tumours are locally aggressive. Their treatment is rather difficult as they have a strong tendency to recur. Desmoid tumours can arise from any skeletal muscle but commonly affect the rectus abdominus in post-partum females and in old surgical scars of the abdomen. Our patient did not fit into either category. Desmoids can present in two forms, peripheral or intra-abdominal. Peripheral tumours are smooth, firm and mobile. They are adherent to the surrounding structures. The overlying skin is unaffected (2). Gardner's syndrome and familial adenomatous polyposis coli (FAP) should be suspected in patients with such soft tissue growths (3).

An intra-abdominal desmoid, also known as mesenteric fibromatosis, is the most common solid primary neoplasm of the mesentry. However, it is the least common subtype of fibromatosis. Approximately 80% of intra-abdominal desmoids involve small bowel mesentry, as was the case with our patient. Involvement of the transverse mesocolon, retroperitoneum, omentum and the ligamentum teres have also been reported (1, 4). Intra-abdominal desmoids are usually asymptomatic until their growth and infiltration causes compression of the viscera. This can lead to intestinal obstruction, ischemic bowel secondary to vascular compression and hydronephrosis due to ureteric compression. Bowel perforation is extremely rare and my research has yielded one previous case report (1). Other rare manifestations reported in the literature include deep vein thrombosis, pyrexia of unknown origin, gastrointestinal bleeding and intra-abdominal abscess formation (5, 6). The subphrenic abscess that our patient developed is very likely to have been a complication of the initial laparotomy. Histologically, these tumours are composed of collagen that surrounds spindle cells which are poorly circumscribed (Figure 1-2). The cytoplasm is pale with regular nuclei, with neither mitoses nor giant cells. Macrophages, giant cells and lymphocytes are present in the periphery (7).

Exact etiology is uncertain but hormonal factors are implicated as there are estrogen eceptors present in some desmoid tumours. They appear in young women during and after pregnancy (2). They begin to regress during menopause and with tamoxifen therapy and even oral contraceptives. The adenomatous polyposis coli (APC) gene located on chromosome 5 is responsible for familial adenomatous polypsois coli (FAP). Biallelic mutations of the APC gene induces desmoid tumour formation, hence the association between these two disorders (8). In our patient, there was no polyposis in the resected right hemi-colon. Trauma has also been suggested as another causative factor but there was no such history in our patient (1).

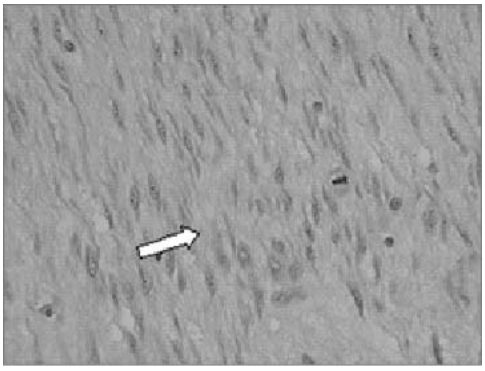

Figure 1. Spindle shaped cells (arrow) (1).

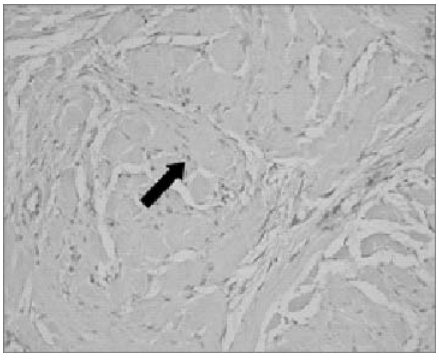

Figure 2. Collagen deposition (arrow) (1).

Both CT and magnetic resonance imaging (MRI) are useful in diagnosing and monitoring recurrence. They allow accurate staging and also enable one to delineate accurate anatomy prior to embarking upon major resection. MRI has the advantage of defining the extent of involvement and monitoring post operative recurrence, which can be as high as 70% (6). These patients should also undergo colonoscopy and examination of the eye to exclude Gardner's syndrome. Colonoscopy may reveal multiple colonic polyps and fundoscopy may demonstrate multiple pigmented lesions affecting the fundus of the eye (2, 3).

Complete surgical excision may be the only effective way of providing a cure but complete excision is often impossible and therefore adjuvant treatments have been employed with various degrees of success (1, 2). Other treatment modalities include radiotherapy, if there is recurrence or as primary treatment to avoid radical surgery (9). Anti-estrogens (e.g. tamoxifen), prostaglandin inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs) have also been used. Cytotoxic chemotherapy may be useful in those with disease recurrence or where surgery is contraindicated (10).

CONCLUSION

Desmoid tumours originate from the musculo-aponeurotic structures of the body and can be peripheral or intra-abdominal. Patients should be screened for Gardner's Syndrome and familial adenomatous polyposis coli since these can often present with dermatoid, epidermoid or other benign tumours. Emergency presentations are not common, but are usually secondary to mass effect on the viscera. CT and MRI are the imaging modalities of choice, particularly if surgery is contemplated and also in post-operative follow up to detect recurrence. Surgery is usually the mainstay of treatment but recurrence is common and adjuvant therapy is used. Some tumours possess estrogen receptors, hence they regress in response to anti-estrogens, such as tamoxifen. Post-operative monitoring is essential as recurrence can be as high as 70%.

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CASE REPORT

"Last supper with mushroom soup": a case report of amatoxin poisoning

R.N. Das*, S Parajuli, J Jayakumar

INTRODUCTION

Unlike Europeans' pastime of mushroom hunting or lactovegetarian's craze for "organic " food (1), in Himalayan developing nations like Nepal, either poor man's delicacy or hunger, often drives people living in hills to forage for wild mushrooms. In Nepal, unnumbered and medically unpublished small epidemics of mushroom poisoning take a heavy death toll annually. The present case depicts one such incident of accidental death of four youths of a 5-membered family, owing to toxic toadstools.

CASE REPORT

A 55-year-old woman with the two daughters (23 and 18-year-old) collected jungle mushrooms and had its soup for dinner along with one 20-year-old son and a 9 year-old grand-daughter on Tuesday evening at about 8:00 pm. The clinical presentations analyzed retrospectively from the mother and neighbors were as follows: on Wednesday, early morning onwards, the two daughters presented with burning in the throat, stomachache, and vomiting and diarrhea innumerable times. The grand-daughter and son also started complaining of stomach upset at about 10:00 am. The mother began experiencing similar nausea, vomiting, abdominal cramps and bloody diarrhea at noon. On Wednesday afternoon, the village physician diagnosed them cholera and treated them with saline at home. On Thursday morning, the daughters developed jaundice and renal shut down. They died at night within 72 hours post-ingestion, owing to excessive bleeding from venepuncture site, nose, mouth, vagina and rectum that lead to circulatory failure. On Friday morning, neighbors brought the two deceased daughters and the other three family members, who were in a shocked and comatose state, to Manipal teaching hospital. On examination, all of them had a feeble rapid pulse; unrecordable pressure, cyanosis, jaundice, labored breathing, motor weakness, delirium and confusion (Figure 1). Their serial hemogarm, coagulation profile, liver and renal function tests have been shown (Table 1). The grand-daughter and son died of fulminant hepatic and renal failure on the 2nd and 5th day of hospitalization respectively, despite all conventional treatment with assisted ventilation and dialysis. The post-mortem report revealed massive centrilobular hepatocellular necrosis and proximal convoluted renal tubular fatty degeneration with obstruction owing to haemoglobinuria. The mother recovered, within 4 weeks time, with high dose penicillin, dopamine, dobutamine, blood transfusion, balanced electrolyte infusion, dextrose, silymarin and vitamin K. The principal author and the mother in follow-up, together with help of a toxicologist, identified the poisonous Amanita phalloides when recollected from the same

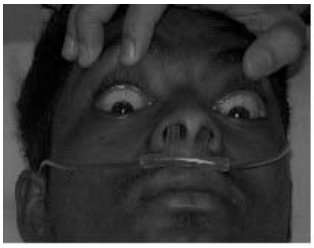

Figure 1. Comatose and deep icterus.

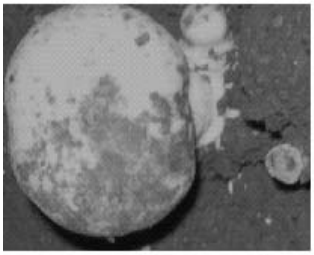

Figure 2. Wild mushroom.

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Table 1: Analysis of laboratory results in all patients

S.N	Age (1) /Sex	Blood sugar (2)		Urea/creatinine (2)		Tb/Db (2)		ASL.ALT (3)		PT (6)		Na^+/K^+ (4)		Hb (5)	
1st	10/F	34		36/2.5		4.4/2.9		7350/7600		120		130/5.9		10.8	
2nd	20/M	65	60	44/2.9	96/5.9	5.5/3.4	8.2/6.6	1110/800	3510/3750	67	180	128/6.2	122/6.8	12.2	11.8
3rd	55/F	70	96	57/1.7	14/0.7	2.5/1.7	2.0/1.1	7815/3600	490/1210	45	22	132/4.5	138/4.1	9.8	11.6
Day*		1	5	1	5	1	5	1	5	1	5	1	5	1	5

NB: *Day of hospital admission (1st & 5th day). Daily routine investigations were done. 1st patient died on 2nd day morning. So no tests were done afterwards. 2nd patient died on 5th day evening. (1) age in years, (2) urea,creatinine, blood sugar, total bilirubin (Tb) and direct bilirubin (Db) in mg%, (3) serum transaminases (AST/ALT) in IU/L, (4) electrolytes in mEq/L, (5) haemoglobin(Hb) in gm% and (6) prothrombin time (PT) in seconds.

spot and compared the resemblance from the picture book," Poisonous Plants - a colorful field guide" (Figure 2) (2).

DISCUSSION

Here we present the first ever reported case of amanita poisoning in mid-western region of Nepal in medical literature. There are many such unaccounted cases of fatal mushroom poisoning (mycetismus) which occur in Nepal annually, owing to misidentification of edible mushrooms especially during its fruiting stage in spring and fall. It is difficult, even for an expert mycologist, to visually differentiate the edible one from a toxic toadstool. Although over 5000 varieties of mushrooms are present, only a 100 are toxic (3). The poisonous mushrooms cannot be detoxified by cooking, freezing or by other means. The majority of toxic mushrooms cause early onset, self-limiting gastroenteritis due to unidentified gastrointestinal irritants while a few are fatal, resulting from late onset, amanitin or gyromitrin type protoplasmic toxins (4).

In Nepal, *A.muscaria* and *A. phalloides* are the two main species found. The former produces a toxic alkaloid, ibotenic acid, which has a rapid onset muscarinic effect (dizziness, muscular jeking ,staggering, confusion and coma) while the later produces a thermostable nitrogenous cyclic octapeptide, amanitine or amatoxin (alpha and beta) which selectively inhibits the nuclear RNA polymerase II, resulting in hepatic and renal cellular derangement. Besides producing thermolabile hemolytic glycoside, amanita hemolysin, another putative toxin, phalloidin, also causes hepatocellular damage. In amanita poisoning, the first phase starts with sudden severe gastrointestinal symptoms 8-10 hours post ingestion, followed by a temporary second phase of apparent remission of abdominal symptoms but overt features of hepatic and renal failure, as evidenced here in the postmortem report. In the final and third phase, death results from coagulopathy (epistaxis, hematuria, melena and hematemesis), encephalopathy (muscular twitching, excitement, delirium, coma, convulsion) and rarely cardiomyopathy (5).

The symptomatology varies with individual susceptibility, even within the same individual on different occasions, in addition to other factors i.e., amount, variety, age, geographical location and premorbid hepatic and renal conditions. The mother survived while all the others died, though they had soup at the same time. The plausible explanation is that the mother received the least share of the soup after serving the it to the rest. The two daughters and the grand - daughter had died earlier to the son, possibly owing to lesser capacity of hepatic enzymes in the female sex to metabolize amatoxin.

There was no facility to do high performance liquid chromatography (HPLC) or radioimmunoassay (RIA) for detection of amatoxin from plasma, feces, urine or vomitus. The progressive deterioration of hepatic, renal and coagulation function in the 1st and 2nd patients and improvement in the 3rd patient had been reflected in laboratory tests shown above.

The mother received benzyl penicillin 1800000 units per day, which was supposed to displace amatoxin from plasma protein binding site, increase renal excretion and inhibit hepatocyte penetration. Silymarin (MicroLab, Pharma, India) 420 mg a day, was given empirically in addition to conventional treatment. Silymarin, a potent antioxidant was known to prevent hepatocyte membrane lipid peroxidation, free radical damage and blockage of α-amatoxin uptake (6).

In Nepal, neither thioctic acid was available nor was liver transplant possible. Whereas, in Europe, early hospitalization, rapid diagnosis and aggressive management with charcoal hemoperfusion, thioctic acid, plasma exchange, extracorporeal liver assist device (ELAD) or orthotopic liver transplantation have shown to reduce mortality to 10% whereas 60-hour

delay increases it to 50-90% (7).

The mortality is higher in children (>50%) as compared to adults (20-30%). The fatality depends on amount and particular species consumed. If unattended, death ensues in 6-8 days in adults and as early as 4-6 days in children (8).

Every physician should consider amanita toxicity in the differential diagnosis of acute gastroenteritis and renal failure, especially in high prevalent regions. The essential steps of management would be early hospitalization, charcoal gastric lavage conventional fluid, dextrose, penicillin and silymarin therapy with hepatorenal support. Aiming at primary prevention, the government should hasten to establish regional toxicology centres which would impart public education and identify toxic toadstool, in order to reduce the mortality.

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CASE REPORT

Barrett's esophagitis related bronchoesophageal fistula - the diagnostic value of persistent air leak in the ventilated setting

O Halaweh, SZ Usmani*, R Abouasaleh

ABSTRACT: A case of acquired tracheoesophageal fistula (TEF) is presented in a 44-year-old female who presented with acute respiratory failure due to bilateral aspiration pneumonia. The patient had persistent air leak while on mechanical ventilation and underwent bronchoscopy which revealed the above etiology. Histopathology showed Barrett's esophagitis. The patient underwent primary closure followed by a short course of proton pump inhibitors. There are only two prior reported cases of acquired TEF associated with Barrett's esophagitis. This condition should be taken under consideration when investigating of an explained persistent air leak in a mechanically ventilated patient.

INTRODUCTION

Tracheoesophageal fistula (TEF) is a congenital or acquired life threatening complication of a communication between the esophagus and the tracheobronchial tree or the other mediastinal structures, due to pathological conditions affecting these structures. The most common etiology of nonmalignant acquired TEF is a cuff-related tracheal injury in an intubated patient. Esophageal cancer is the most common malignant etiology (1, 2). Patients with TEF on mechanical ventilation tend to have increased secretions, and are at increased risk of aspiration pneumonia from gastric contents that reflux through the TEF into the tracheobronchial tree. Barrett's esophagus refers to the replacement of normal squamous epithelium in the lower esophagus by columnar epithelia caused by chronic exposure to gastric acidic secretions. It is associated with increased risk for adenocarcinoma of the esophagus. This report describes an unusual case of Barrett's esophagus presenting with ulcerating TEF which was detected while investigating a persistent air leak on a mechanical ventilator.

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CASE REPORT

A 44-year-old female was admitted to the hospital with a sudden onset of respiratory distress. She had a three year history of gastro-esophageal reflux disease (GERD) but no history of smoking or alcohol use. The patient was intubated and mechanically ventilated. On physical examination, she had bilateral crepitations and rhonchi on chest auscultation. Her complete blood count and comprehensive chemistry profile were within normal limits. Chest radiograph demonstrated bilateral aspiration pneumonia. During ventilation, she was noted to have a constant leak of 150cc of tidal volume with each respiration. Suspecting a cuff leak, the treating team decided to replace the endotracheal tube. However, there was no improvement in the consistent loss of tidal volume.

Emergency bronchoscopy revealed a 1cm left-sided TE fistula near the carina. Esophagogastro-duodenoscopy (EGD) confirmed the diagnosis at 30cm from the incisor teeth (Figure 1). Biopsies and brushings were obtained to exclude

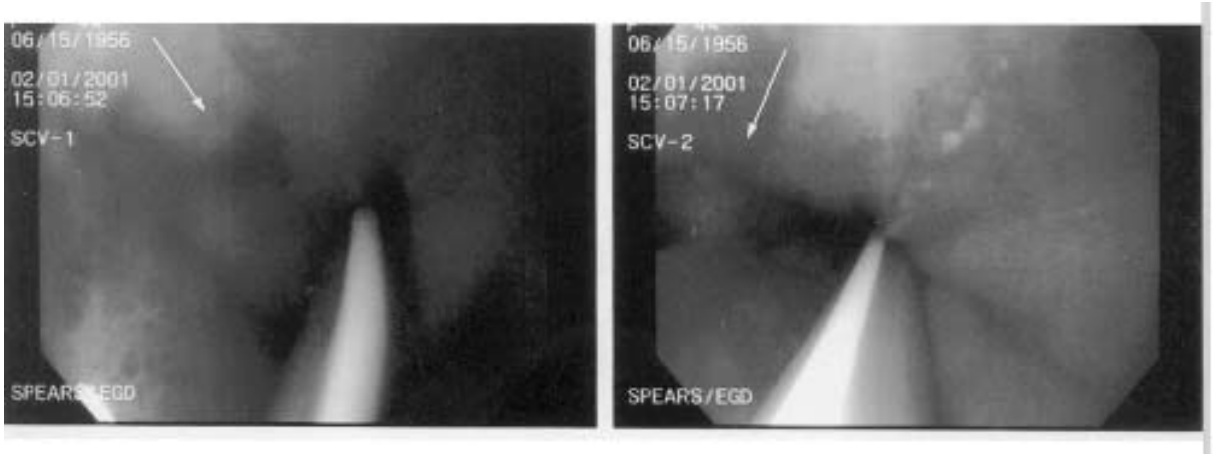

Figure 1. Tracheoesophageal fistula (TEF) at the level of the distal esophagus, as seen on esophagogastroscopy. White arrow points to the site of the TEF.

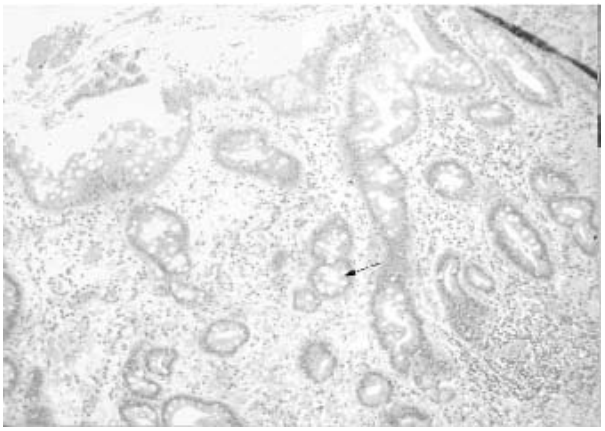

Figure 2. Medium power view of the non-dysplastic epithelial metaplasia in squamous epithelium of the esophagus (Hematoxylin & eosin stain).

malignancy. Histopathology was consistent with Barrett's mucosa with ulceration and active inflammation (Figure 2). No dysplastic or neoplastic changes were present. Computed tomography was obtained to investigate for an associated mass, but showed only a left sided tracheoesophageal fistula with no other mediastinal or abdominal abnormality. Primary surgical repair was undertaken with longitudinal suturing of the individual bronchial and esophageal components of the fistula. A small post-operative leak was identified on the barium esophagogram (Figure 3). It was successfully treated with conservative management consisting of percutaneous endoscopic jejunostomy (PEJ) tube feeding and a proton pump inhibitor therapy for 2 weeks. The patient improved and was extubated on post-surgical day 5 and discharge home in a stable condition after 2 weeks.

DISCUSSION AND CONCLUSION

Barrett's esophagus is an intestinal metaplastic response of the esophageal squamous mucosa to chronic gastroesophageal reflux (3). It can present as tongue-like extensions from the gastroesophageal junction or scattered islands of columnar epithelium in the early stages. Circumferential involvement of the esophagus is seen in advanced cases. Ulceration is identified in 10% of Barrett's esophagus. Deep wide-mouthed Barrett's ulcer can penetrate or perforate adjacent mediastinal organs. An air leak of more than 100 cc per tidal volume in mechanically ventilated patients is very unusual. Such a leak can be caused by inappropriate cuff inflation, misplacement of the endotracheal tube, pneumothorax, bronchopleural fistula, chest tube air leak and fistulas in-between the airways and the mediastinal structures. In this case, the etiology was found to be TEF. It is likely that the erosive nature of Barrett's esophagus and the proximity of the esophagus and tracheobronchial tree anatomically that led to the TEF. Proton pump inhibitors were used to decrease the gastric acid secretion to aid in epithelial healing. There are only two prior reported cases of acquired TEF associated with perforated Barrett's esophagitis (4, 5). TEFs are usually diagnosed by bronchoscopy or barium swallow studies. Our experience suggests that the detection of persistent and unexplained air leak in a mechanically ventilated patient should lead the clinician to consider TEF as a causative condition. Conditions that predispose patients to TEF should be taken into account when considering this etiology. Recognizing the presence of acquired TEF early on prevents the development of complications such as aspiration pneumonia, which could prove to be life-threatening especially in our current population of inpatients with multiple comorbidities.

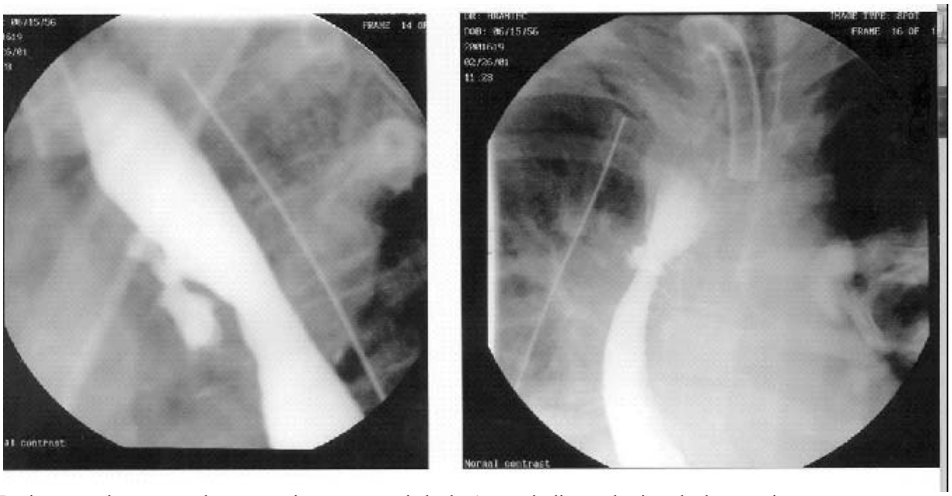

Figure 3: Barium esophagogram demonstrating post-repair leak. Arrow indicates barium leakage point.

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REVIEW ARTICLE

β-Adrenergic receptor signaling in cardiac function and heart failure

Aasakiran Madamanchi*

INTRODUCTION

Congestive heart failure (CHF) is a major public health problem. Nearly 5 million Americans are afflicted with CHF and about 550,000 new cases are diagnosed each year (1). CHF is a pathophysiological condition in which the heart is unable to pump enough blood to meet the metabolic needs of the body. It is the endpoint for several cardiovascular diseases including coronary artery disease, myocardial infarction, hypertension, cardiomyopathy, congenital heart defects, myocarditis, and valvular heart disease resulting from rheumatic fever or endocarditis. Regardless of the etiology, compensatory and adaptive changes occur in the heart to preserve cardiac output. Some of these changes are mediated by enhanced sympathetic nervous system activity (2). Increased catecholamine outflow from this system induces sustained elevated activation of the β-adrenergic receptors (β-ARs), which results in abnormalities in the β-AR signaling system that may ultimately lead to the pathogenesis of CHF (3).

In this review, I will discuss (i) β-AR subtypes in the heart; (ii) the functional role of β-AR signaling in CHF; and (iii) the recent studies in genetically engineered mice to elucidate the functional effects and therapeutic potential of critical genes in the cardiac β-AR signal transduction pathways.

β-ARs IN THE HEART

The β-ARs belong to the superfamily of membrane proteins known as G-protein-coupled receptors (GPCRs) (4). GPCRs are characterized by a conserved core structure with extracellular amino terminus, intracellular carboxyl terminus and seven transmembrane α-helices, which are connected by three extracellular and three intracellular loops. They transduce extracellular signals from endogenous hormones and neurotransmitters, ambient physical and chemical stimuli, as well as exogenous therapeutic agents. GPCRs are involved in regulation of a vast array of physiological processes including sensory perception, cell growth, metabolism and hormonal homeostasis.

The transmembrane signaling by GPCRs is initiated by the binding of ligands such as hormones or neurotransmitters (Figure 1). Ligand binding induces a conformational change in GPCRs that causes coupling with heterotrimeric G-proteins (5). G-proteins consists of α, β, and γ subunits and GPCR coupling leads to the exchange of G-protein-bound GDP for GTP and the dissociation of the G-protein into active Gα and Gβ subunits to mediate downstream signaling. Based on their amino acid sequences and function, Gα subunits are grouped into four subfamilies - $G\alpha_s$, $G\alpha_i$, $G\alpha_q$ and $G\alpha_{12}$ (6). Subunits of the diverse G-proteins differentiate the cellular signal by modulating the activity of various effector molecules such as adenylyl cyclase (AC) or phospholipase C-β. These effector molecules regulate the concentrations of second messengers in the cell, activating a number of different downstream signaling molecules.

There are four subtypes of β-ARs-β_1-AR, β_2-AR, β_3-AR and the β_4-AR (6). The β_1-AR is found primarily in the heart and comprises 75-80% of the β-ARs found in the heart (Figure 2). The β_2-AR is expressed in the lungs, kidneys and blood vessels as well as the heart and comprises 20-25% of cardiac β-ARs. The β_3-AR is found primarily in the adipose tissue, and minimally in the heart. The β_4-AR is considered a low affinity state of β_1-AR, which awaits genetic and pharmacologic characterization. Epinephrine and norepinephrine serve as the primary agonists for all β-ARs. However, recent data have revealed significant differences in the signaling pathways and cellular responses of the β-AR subtypes (7).

When stimulated, cardiomyocyte β_1-AR primarily binds to the G stimulatory (Gs) protein. The Gα subunit of the Gs protein ($G\alpha_s$) activates AC, which generates the second messenger cyclic adenosine monophosphate

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(cAMP); heightened cAMP levels activate cAMP-dependent protein kinase A (PKA). Activated PKA then phosphorylates troponin I, the L-type Ca^{2+} channel and phospholamban (PLB), resulting in greater contractility (8).. In addition to Gs, the β_2-AR couples to pertussis toxin - sensitive G inhibitory (G_i) protein. G_i coupling releases the activated $G_i\alpha$ subunit, which inhibits AC activity, and the $G_i\beta\gamma$ subunit, which causes downstream activation of the mitogen-activated protein kinases (MAPK). $G_i\alpha$ coupling also activates the cytosolic effector molecule phospholipase A2 (cPLA2), which causes cAMP-independent enhancement of calcium signaling and cardiac contraction. Recent studies indicate that β_2-AR-cPLA2 pathway substitutes for a deficient β_1-AR-Gs-cAMP pathway; decreased β_1-AR-AC coupling promotes the β_2-AR-cPLA2 pathway, whereas efficient β_1-AR-AC coupling suppresses it by the activity of PKA (9). The effect of cardiac β_2-AR activation varies depending on the species, and the developmental or pathophysiological state of the heart (9). The ability of β-ARs to couple with different G-proteins allows for the receptors to activate multiple signaling pathways and initiate diverse cellular responses.

The β-AR signaling system, like all GPCR systems, has a number of post-agonist stimulation regulatory mechanisms that serve to prevent overstimulation (10). These processes, together known as receptor desensitization, act as a negative feedback to the β-AR signal, under conditions of extended agonist stimulation, by repressing the intensity of β-AR signaling and decreasing the total number of receptors in the cell. The first of these processes is the deactivation of trimeric G-proteins. Soon after the dissociation of the coupled G-protein into active Gγ and Gβγ subunits, conformational changes occur that cause re-formation of the inactive G-protein. The rapid termination of the G-protein signal maintains the dose-dependent nature of β-AR signal propagation and resets the protein for future signaling. Although apparently contradictory, β-AR desensitization (discussed later) much like chronic receptor stimulation, contributes to the pathogenesis of CHF (3).

Continuous catecholamine stimulation overwhelms the short-term active/inactive cycling of the G-protein signal and triggers further negative feedback regulation of GPCR activity. Activation-dependent regulation of receptors, also known as homologous desensitization, proceeds within minutes of agonist stimulation (11). Homologous desensitization begins when the Gβγ subunit of the activated G-protein binds with the active form of a G-protein-coupled receptor kinase (GRK). GRK2, also known as β-AR kinase 1 (βARK1), is translocated to the stimulated receptor after binding with the activated Gβγ subunit, where it phosphorylates the agonist-occupied β-AR (8). βARK1is the most prominent cardiac GRK; GRK3 and GRK5, which are also capable of phosphorylating β-ARs, are present in cardiac myocytes in minimal levels. Phosphorylation of the receptor occurs at the C-terminus of the receptor

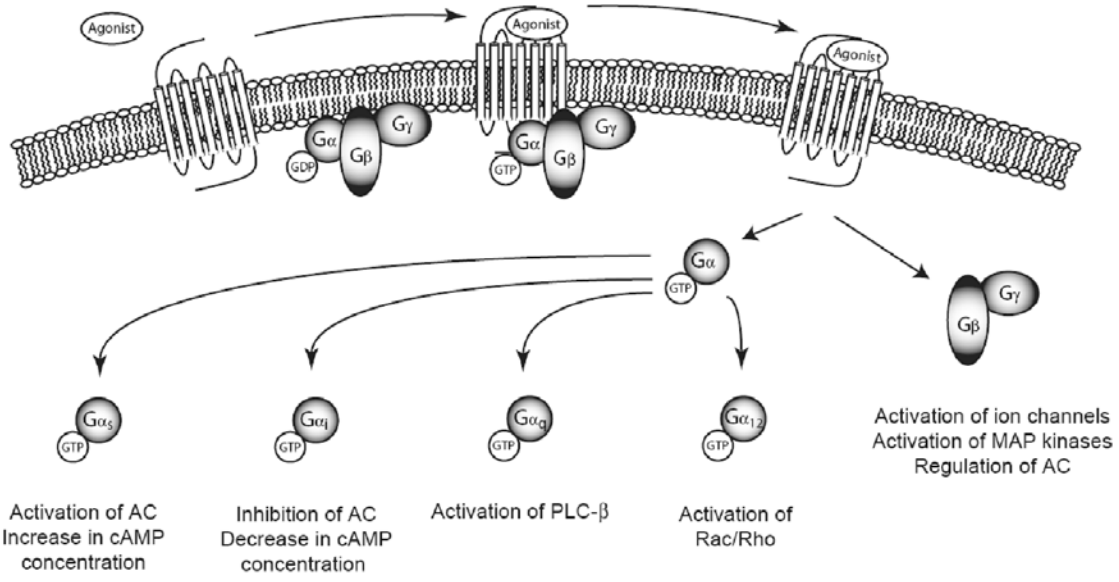

Figure 1. Classical GPCR signaling. Agonist binding to the receptor results in the coupling with G-proteins and exchange of G-protein-bound GDP for GTP. The activated G-protein dissociates into Ga and Gbg subunits, both of which independently affect cellular signaling through the activation or inhibition of effectors such as adenylyl cyclase (AC) or phospholipase C-b (PLC-b). Ga subunits are grouped into four subfamilies - Ga_s, Ga_i, Ga_q and Ga_{12} - based on their structure and function. The members of stimulatory Ga_s family couple to AC to cause an increase in intracellular cAMP levels, whereas members of Ga_i family inhibit AC and decrease cAMP levels. The members of Ga_q activate PLC-b, whereas members of Ga_{12} family activate Rac and Rho. Gβγ dimers activate large number of effectors including ion channels, mitogenactivated protein (MAP) kinases and activate or inhibit AC.

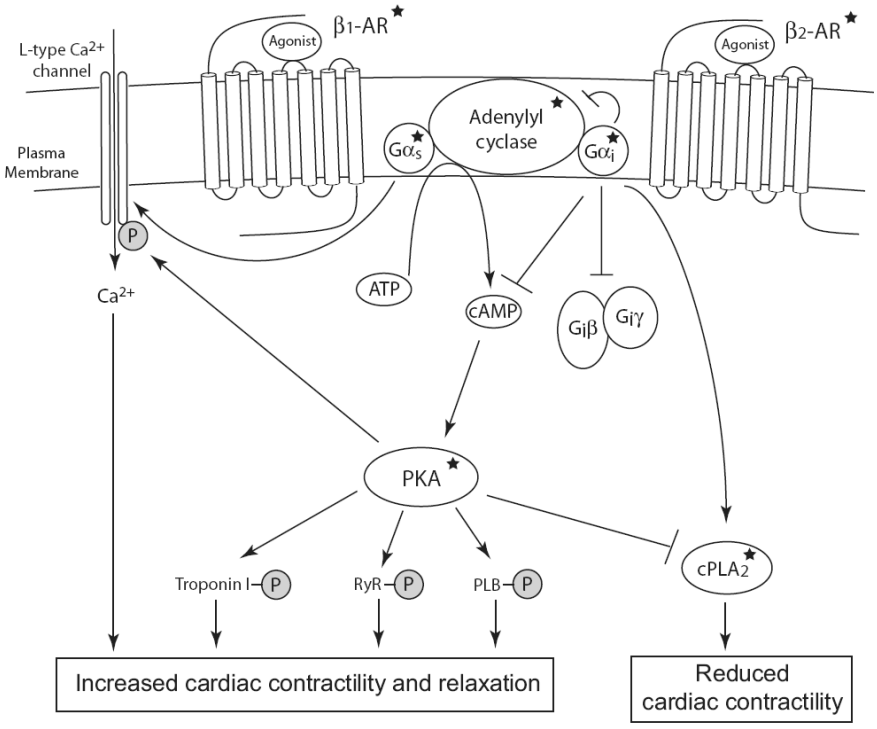

Figure 2. β-AR-mediated cardiomyocyte contractility. Agonist binding stimulates β_1-AR and results in coupling with and activation of heterotrimeric Gs, which dissociates into Ga_s and $G_i\beta\gamma$ subunits. The Ga_s activates both adenylyl cyclase (AC), which increases intracellular cAMP levels and L-type calcium channel, which allows Ca^{2+} to enter into cardiomyocytes. The cAMP activates PKA, which phosphorylates (P) several substrates including phospholamban (PLB), L-type Ca^{2+} channels, troponin I and the cardiac ryanodine receptor (RyR) resulting in increased cardiac contractility and relaxation. In addition to Gs, β_2-AR couples to pertussis toxin-sensitive G_i upon agonist binding. Activated-G_i releases Ga_i subunit, which inhibits AC and $G_i\beta\gamma$ and activates phospholipase A2 (cPLA2) leading to reduced cardiac contractility. The β1-AR-induced cAMP suppresses the β2-AR/cPLA2 pathway, via PKA. Asterisks denote activated proteins and ⊣ indicates inhibition.

protein and targets it for arrestin-induced functional uncoupling from the G-protein. An alternate internalization pathway involves phosphorylation of the receptor protein through the secondary action of a downstream signaling product of β-AR activation. This process, known as heterologous desensitization, is initiated when the second messenger-regulated kinase PKA phosphorylates the β-AR (12). As opposed to homologous desensitization, heterologous desensitization indiscriminately phosphorylates both stimulated and unstimulated receptors. In both cases, receptor phosphorylation allows for β-arrestin to bind and interfere with future association with G-proteins, functionally uncoupling the receptor (13). Binding to β-arrestin increases the receptor's affinity for adaptor protein (AP)-2 and clathrin (14). Internalization of receptors via clathrin-coated vesicles follows rapidly, within minutes of stimulation (15).

Following internalization, receptors are transported to endosomes. At the endosomes, the receptors can be recycled back to the plasma membrane where they return to a complete functioning state (14). In response to persistent receptor stimulation, the internalized receptor can instead be degraded in the liposome. This process, known as down-regulation, lowers the total number of receptors in the cell to relieve chronic overstimulation. Down-regulation is initiated within hours to days after consistent receptor internalization.

β-AR SIGNALING IN HEART FAILURE

As the major component of the interface between the sympathetic nervous system and the cardiovascular system, the β-AR signaling pathway emerges as a key actor during the progression of heart failure. Prominent irregularities in β-AR signaling changes include a reduction of the β_1-AR levels of up to 50% (interestingly β_2-AR levels remain constant), a sharp increase (up to 200%) of $G\alpha_I$ levels, and significantly elevated βARK1 activity (8). All of these adjustments reflect severely diminished β-AR signaling, likely the result of sustained, elevated catecholamine levels.

Cardiac hypertrophy often directly precedes the onset of CHF. Excessive stress activates a complex network of interacting pathways that initiate myocardial hypertrophy to preserve cardiac function. Conventional wisdom held that cardiac hypertrophy is an adaptive process that improves ventricular function in the face of a growing workload. However, as the cardiac efficiency of the hypertrophied heart further diminishes, the sympathetic nervous system is increasingly activated in an attempt to maintain cardiac output and systemic blood pressure (16). Furthermore, many studies indicate that sustained cardiac hypertrophy is closely associated with reduced contractility and an increased risk of heart

failure (17). Also, significant desensitization of β-ARs and other β-AR signaling irregularities are shared characteristics in the development of cardiac hypertrophy and the progression of heart failure. Transgenic mice that lack a hypertrophic response mechanism suffer less cardiac dysfunction than normal mice when facing long-term mechanical stress (18). In light of this evidence, a new paradigm has emerged in which it is argued that the signaling pathways stimulated during the hypertrophic process may actually play a greater role in the pathogenesis of heart failure than the original stress on the heart.

According to this model, heightened stimulation by the sympathetic nervous system elicits a hypertrophic response in the heart that is initially beneficial but becomes maladaptive when sustained. The hypertrophic response causes the activation of a number of signaling pathways, at a time when consistently elevated catecholamine levels are already overstimulating the β-AR signaling pathways. Sustained activation of the β-AR system combined with biochemical changes produced by hypertrophic processes strongly activate desensitization and down-regulation pathways, which ultimately lead to diminished β-AR function and loss of contractility.

LESSONS FROM TRANSGENIC MOUSE MODELS

Over the last decade and a half, cardiovascular research has been revolutionized through the use of genetically engineered mouse models. Mouse lines have been developed to model every aspect of cardiac pathology. Furthermore, the use of murine α-myosin heavy chain (αMyHC) gene promoter in ventricular myocytes has enabled cardiac-specific gene expression. The αMyHC promoter,which is inactive until around birth, avoids developmental complications arising from the specific transgene overexpression (15). The ability to genetically manipulate molecular components of the β-AR signaling system in murine heart failure models provides a powerful experimental system for uncovering potential roles of various elements of this system in the pathophysiology of cardiac failure.

β_1-AR

The β_1-AR has been studied in both gain and loss of expression studies. β1-AR knockout mice were largely embryonically lethal, while the surviving mice showed no response to catecholamine stimulation despite the presence of β_2-ARs (19). Cardiomyocyte-targeted overexpression of the β1-AR using the αMyHC promoter generated mice with 5 to 15-fold overexpression of β_1-ARs. The β_1-AR overexpressing mice developed dilated cardiomyopathy and heart failure at young age, which is similar to the pathology caused by chronic catecholamine overstimulation (20). This data reinforced the view that increased β-AR signaling, as happens in chronic receptor stimulation, is ultimately detrimental to cardiac function.

β_2-AR

Experiments using transgenic mouse models have made it clear that β_1-AR and β_2-AR are not identical signaling molecules. In contrast to β_1-AR knockouts, the β_2-AR knockout mice do not suffer from developmental defects and show no significant differences in cardiovascular phenotype when compared with wild-type mice (21). The β_2-AR knockout mice also differ from β_1-AR knockouts in that they show typical response to catecholamine stimulation. This indicates that the β1-AR is the signaling conduit for the catecholamine-induced changes in cardiac contractility.

Mice with cardiac-specific overexpression of β_2-ARs displayed enhanced basal contractility and cardiac function with minimal pathology (22). Adenoviral-mediated gene transfer of β_2-AR to myocytes from a rabbit heart failure model rescued β-AR signaling (23). Crossing transgenic mice overexpressing moderate amounts (30-fold above wild-type levels) of cardiac β_2-AR with transgenic mice overexpressing $G\alpha_q$ improved cardiac function and hypertrophy in the $G\alpha_q$ overexpression heart failure model (24). These results are encouraging for the prospective use of β_2-AR overexpression as a therapeutic approach. However, conflicting data clouded the issue as β_2-AR overexpression has failed to rescue other heart failure models (25) and has proven deleterious in the face of pressure overload (26). More information about appropriate levels of β_2-AR expression levels is needed to clarify the potential therapeutic value of β_2-AR gene transfer.

βARK1

The prevalence of βARK1 activity in the progression of maladaptive hypertrophy and cardiovascular disease has made it an object of extensive research. The role of βARK1was investigated by studying the contrast between hearts of transgenic mice overexpressing βARK1 and those expressing a peptide inhibitor of βARK (βARKct). βARKct, a peptide expressing the terminal 194 amino acids of βARK1, contains the Gβγ-binding domain and competes with endogenous βARK1 for Gβγ subunits necessary for βARK1 activity (27). Overexpression of βARK1 in transgenic mice showed a diminished β-AR response to catecholamine stimulation. This mirrors the augmented βARK1 activity observed in human heart failure. In stark contrast, the βARKct mice had enhanced cardiac function under normal conditions and an augmented

response to catecholamine stimulation (28). While homozygous βARK1$^{-/-}$ mice were embryonic lethal, heterozygous βARK1$^{+/-}$ mice had a cardiac phenotype of enhanced contractility similar to the βARKct transgenic animals (29, 30).

To determine the significance of increased βARK activity in the progression of heart failure, a number of studies were conducted involving the overexpression of βARKct in mice from various existing heart failure models. Remarkably, in each case, it was shown that cardiac overexpression of βARKct leads to prevention of progressive deterioration in cardiac function, prevention of hypertrophy, improved exercise tolerance, and correction of β-AR dysfunction (31). Furthermore, adenoviral delivery of βARKct to larger animal models of heart failure has shown therapeutic effects (32). Taken together, these studies indicate that inhibition of βARK1 activity can preserve β-AR signaling and lead to an improved response to catecholamine stimulation. This suggests that inhibition of βARK1 activity via introduction of βARKct or other small molecule inhibitors is a viable therapeutic approach for disease states characterized by increased βARK1 activity.

It is important to note that the effects of βARK1 inhibition may not be completely relegated to the β-AR system as βARK1 is involved in a number of non-β-AR signaling pathways (33). Furthermore, the demonstrated effectiveness of βARKct expression may not be entirely attributable to βARK1 inhibition. βARKct functions by binding to and interfering with activated Gβγ subunits, so it is very possible that inhibition of alternative Gβγ pathways contributes to part of the βARKct effect. In fact, recent evidence has shown that another Gβγ inhibitor, a N-terminally truncated phosducin, which acts independently of β_1-AR activity, replicates βARKct's protective effects (34).

PI3K

Recently, phosphoinositide 3-kinase (PI3K) has begun to garner interest because of its role at the intersection of the hypertrophic and β-AR signaling processes. PI3K, which belongs to a conserved family of lipid kinases that are involved in the regulation of a variety of cellular functions including cell growth, survival, signal transduction and apoptosis, is activated in cardiac myocytes during the hypertrophic response (35). PI3K activity is also closely associated with attenuation of β-AR function resulting in decreased contractility (36, 37). Recent studies demonstrated that βARK1 interacts with the phosphoinositide kinase (PIK domain) of PI3K to form a cytosolic complex and facilitates agonist-mediated PI3K translocation to the plasma membrane (38). PI3K, via its lipid kinase activity, catalyzes the production of D-3 phosphoinositides, which recruit adaptor proteins essential to receptor internalization. Overexpression of the PIK domain, which competitively displaces endogenous PI3K and prevents βARK-PI3K translocation to the membrane, significantly decreased agonist-stimulated β-AR internalization. Recently, we demonstrated that internalization of the receptor also requires the protein kinase activity of PI3K involving tropomyosin phosphorylation (39). Furthermore, overexpression of PI3Kγ, a catalytically inactive mutant, inhibited agonist-induced β-AR internalization and rescued β-AR function in calsequestrin overexpressing (CSQ) mice, a common heart failure model (40). Restoration of β-AR signaling via PI3Kγ overexpression resulted in significant improvement in cardiac function and survival, which indicates that inhibition of membrane-targeted PI3K represents a novel therapeutic approach to ameliorate cardiac dysfunction.

CONCLUSIONS AND NEW DIRECTIONS

In recent years, great progress has been made towards understanding the β-AR system and its role in heart disease. Increasingly, researchers are viewing the myocardial hypertrophic response and concurrent β-AR desensitization as maladaptive responses. However, this evolving notion is counterintuitive to the successful use of β-blockers which favor a reduction of β-AR stimulation to preserve cardiac function. Understanding the differences between β_1-AR and β_2-AR signaling may explain the apparent effectiveness of these β-antagonists in restoring β-AR levels and cardiac function in the failing heart. It is possible that β-blockers function by inhibiting sustained β_1-AR activity and the associated hypertrophic, pro-apoptotic, pro-necrotic effects (24). Another possibility is that β-blockers act to re-sensitize the β-AR system, reversing the abnormalities in β-AR signaling that result from prolonged catecholamine stimulation.

Studies using genetically altered mice have identified Gβγ and PI3K as particularly promising targets for therapeutic intervention. Further investigation of the secondary signaling pathways these molecules are involved in, is needed to fully understand how best to target these molecules for the treatment of CHF. Potential approaches include the.use of gene therapy (with βARKct or PI3Kγ) or the development of pharmaceutical inhibitors for heart-specific βARK1 or PI3K activity.

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REVIEW ARTICLE

'Miracle stents' - a future without restenosis

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ABSTRACT: Over the last three decades, percutaneous coronary intervention (PCI) technology has revolutionized the field of cardiology. PCI began in the form of balloon angioplasty, and was followed by coronary stenting. In-Stent restenosis is the main limitation of coronary stenting, and has been delayed to some extent by the development of drug eluting stents. Coronary angioplasty with stenting is currently the most popular non-medical treatment of coronary artery disease therefore solving the problem of in-stent restenosis could change the future role of other types of coronary intervention. This review examines the types of percutaneous coronary interventions, the mechanisms leading up to in-stent restenosis, and how previous and current treatments of in-stent restenosis influence the vascular response to injury.

BACKGROUND

For several decades, coronary artery bypass graft (CABG) and percutaneous transluminal coronary angioplasty (PTCA) have been the main procedures used to treat coronary artery disease. CABG is a surgical procedure whereby an obstruction in the coronary arteries caused by an atherosclerotic plaque can be treated using saphenous vein grafts or arterial grafts such as internal mammary arteries to bypass the obstruction (1).

PTCA was introduced over 20 years ago as an alternative coronary intervention to the more invasive CABG. The procedure involves making small incisions in the vessels of the groin or arm under local anaesthetic where a catheter can be fed through to the obstructed coronary vessel. A balloon attached to the end of the catheter is used to unblock the vessel and consequently restore the blood flow (1).

However, a number of studies in the early 1990s that compared the outcomes of CABG with PTCA revealed higher re-intervention rates with PTCA (2). During PTCA, the balloon is inflated at the site of atherosclerotic narrowing, dilating the vessel lumen, and causing compression of the plaque and stretching of the vessel wall. Thus, the result is fracturing and fissuring of the atheroma due to its inelastic components and an extension into the vessel wall, causing either superficial or deep arterial injury (Figure 1) (3).

The response of vascular tissue to the injury caused by balloon angioplasty accounted for its two major limitations, which were acute vessel closure and restenosis (renarrowing of the vessel). The incidence of acute vessel closure was 3-5%, and would occur within the first 24 hours of the procedure due to vessel dissection or acute thrombus formation (3).

Restenosis can be defined as a reduction in the luminal diameter of more than 50%. It had a high incidence rate of 25-50% in patients having undergone balloon angioplasty, with the vast majority of patients requiring revascularisation within 6 months (3). This occurred because of elastic recoil and intimal hyperplasia as a response to vascular injury.

Coronary artery stenting was then introduced with the hope that the limitations of balloon angioplasty could be overcome (4). This involved placing a stent, a metal

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meshwork device, into the vascular lumen to keep the vessel open and prevent acute vessel closure and restenosis (5). Coronary stenting was shown to significantly improve the outcome, including a 10% reduction in restenosis rates compared to angioplasty alone (6).

This paper reviews the definition, pathophysiology, and treatment of in-stent restenosis, as well as its implications in the development of new stent designs.

PROCEDURES FOR BALLOON ANGIOPLASTY AND STENTING

Under local anaesthetic, a guide catheter is introduced into the femoral artery in the groin or the brachial artery in the arm. The catheter reaches the aorta through the artery, and is lodged within the aorta at the origin of the coronary artery. To identify the areas of narrowing, a radio-opaque contrast is injected into the coronary artery and x-rays are used to produce a continuous image (3).

A guide wire that runs along the middle of the guiding catheter is passed down the coronary artery and through the obstruction. A balloon-tipped catheter is then passed over the guide wire placed at the stenosis ,and the balloon is inflated. The coronary artery lumen is widened, and the guide catheter, guide wire, and balloon are withdrawn (3).

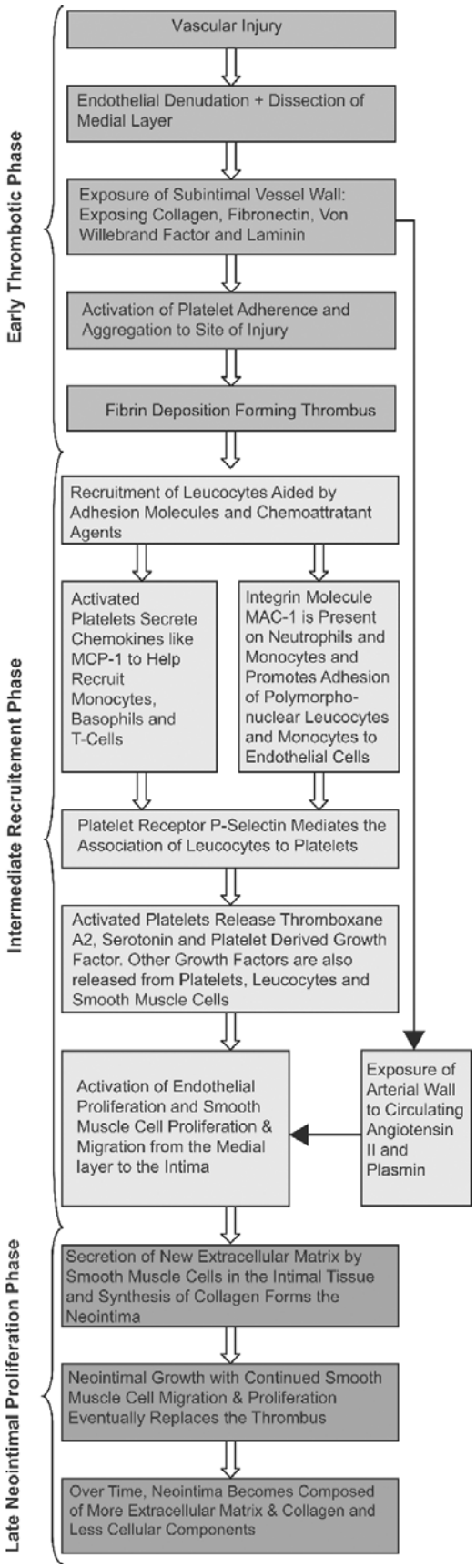
With coronary stenting, the stenosis may be stented directly or dilated with a balloon before stenting (3). With direct stenting, the stent (a tubular wire mesh) is stretched into a constrained state with a special delivery catheter and then gradually released at the site of the stenosis. The radial forces of the stent on the vascular wall lead to dilation of the vessel.

With a balloon-expandable stent, the balloon is inflated to expand the stent so that it holds the narrowed vessel open. Finally, the balloon is deflated and withdrawn (5).

DEFINITION OF IN-STENT RESTENOSIS

In-stent restenosis is defined as a decrease in the luminal diameter by greater than 50% in the stented area of the vessel, similar to post-angioplasty restenosis (PARS). This is known as angiographic restenosis. As with post-angioplasty restenosis, in-stent restenosis also occurs within 6 months of stenting (6).

Clinically, in-stent restenosis can present as recurrent angina or myocardial ischaemia although angiographic restenosis does not always lead to clinical symptoms such as angina. In-stent restenosis can also be defined by the relative length of the restenotic lesion: if the lesion measures less than 10 mm, it is known as focal, whereas if it measures more than 10 mm, it is known as diffuse (6).



PATHOPHYSIOLOGY OF RESTENOSIS

Restenosis occurs as a result of the response of the vascular tissue to the injury caused by coronary angioplasty (7). In the case of in-stent restenosis, it is the result of vascular injury that occurs after stenting. The injury caused by insertion of a stent is different from the injury caused by angioplasty alone. This results in differences in the pathophysiological mechanisms that lead to restenosis in response to injury.

The struts of the stent cause deep, focal injury to the vascular tissue whereas the balloon inflation is less controlled in stretching and fracturing the vessel wall. Also, in stenting, the extensive, early thrombus can act as a scaffold whereby cell proliferation and neointimal hyperplasia can occur. Additionally, inserting a stent causes permanent strain on the vessel wall as opposed to the transient strain applied by the balloon. Finally, with stenting, a foreign material remains in the vessel unlike the withdrawal of the balloon in coronary angioplasty (8).

The response to injury hypothesis used to describe the mechanisms that lead to atherosclerosis plays an important role in restenosis. In this hypothesis, repeated vascular injury causes endothelial denudation and subsequently results in platelet adhesion, thrombus formation and monocyte infiltration (7).

The leukocytes and platelets release cytokines, vasoactive agents, and growth factors, which promote an inflammatory response (9). Platelet-derived growth factor can lead to smooth muscle cell proliferation and migration into the intima to form the neointima (10).

Three mechanisms that lead to the development of restenosis are elastic recoil, neointimal proliferation, and negative remodelling (11). Inflammation plays an important role in linking the early vascular injury of deendothelialization and thrombus deposition, to the more chronic response of healing whereby cell proliferation, cell migration, and extracellular matrix synthesis leads to neointimal growth and a reduction in lumen size (10).

Elastic Recoil

The intimal, medial, and adventitial layers of the coronary arteries are separated by elastin fibres, namely the internal and external elastic lamina. Elastic recoil is a passive process that occurs in seconds to minutes as a result of the elastic laminae applying opposing force to the overstretch caused by balloon inflation. This reduces the lumen by up to 40% (12, 13).

In studies comparing balloon-injured and stented arteries, the rigid scaffolding of the stent prevented elastic recoil and subsequently stented arteries were found to have a much larger initial gain in lumen size (14, 15).

Inflammatory Changes

Inflammatory response within the vessel wall following PCI can be divided into three stages: early thrombotic phase (hours to days), intermediate recruitment phase (3-10 days), and the late neointimal proliferation phase (weeks to months) (11).

Early Thrombotic Phase

Vascular injury caused by angioplasty and stenting leads to endothelial denudation. Consequently, exposure of the subintima of the vessel wall activates platelet aggregation, which is followed by fibrin deposition and formation of a thrombus (11, 13). With stents, the injury is deeper and more focal, and thick platelet rich mural thrombi form on the stent struts (8).

Intermediate Recruitment Phase

In this phase, there is recruitment of leukocytes aided by adhesion molecules and chemoattractant agents (6). Firm adhesion and transplatelet migration of the leukocytes are aided by the integrin class of adhesion molecules (16). For example, integrin molecule MAC-1 (CD11b/CD18) promotes the adhesion of polymorphonuclear leukocytes and monocytes to the endothelial cells (10). MAC-1 expression is increased following stenting (17).

Chemokines are chemoattractant cytokines, which lead to further leukocyte recruitment and infiltration. The monocyte chemoattractant protein (MCP-1) is an example of a chemokine secreted by activated platelets (18). MCP-1 expression has been found to be elevated in endothelial cells and vascular smooth muscle cells following stenting (19) and levels of MCP-1 have been shown to be persistently elevated in patients with restenosis (20).

Activated platelets release factors that activate endothelial proliferation and smooth muscle migration from the media to the intima (11). There is also exposure of the arterial wall to circulating angiotensin II and plasmin (13). Medial smooth muscle cells undergo phenotypic modification from contractile to 'synthetic', which leads to their migration to the intima and subsequent proliferation there (6).

Late Neointimal Proliferation Phase

Formation of new extracellular matrix in the intimal tissue is coupled with synthesis of collagen to form the neointima (6).

Over time, the thrombus is replaced by the neointima and this increases up to 3 months after the procedure (11). However, over subsequent months the neointimal hyperplasia becomes composed of more extracellular matrix and less cellular components (6).

Negative Remodelling

Vascular remodelling consist of the compensatory changes that occur to the arterial size due to expansion of the media and external elastic membrane. "Positive remodelling" describes expansion in the external elastic membrane area, and "negative remodelling" describes shrinkage of the external elastic membrane at the lesion site (12).

Examination of restenosis after experimental angioplasty revealed that arterial remodelling in the form of chronic constriction correlated more with restenosis after angioplasty than neointimal-medial growth (21). Intravascular ultrasound studies show that negative remodelling accounts for 2/3 of lumen loss in restenosis following angioplasty (22).

Stenting, however, was found to cause greater neointimal growth but prevented arterial remodelling. Their overall benefit over angioplasty was attributed to the larger initial gain in lumen size (23).

Oxidative stress is increased post angioplasty and may also be involved in constrictive remodelling by enhancing the breakdown of nitric oxide, a vasodilator, thereby contributing to endothelial dysfunction (24).

TREATMENT OF IN-STENT RESTENOSIS

Prior to the relatively recent introduction of drug eluting stents, brachytherapy was the only treatment effective in significantly reducing in-stent restenosis. The mechanisms that govern these treatments are described below.

Brachytherapy

This non-pharmacological treatment delivers intra-coronary sources of ß or γ radiation thereby inhibiting cell proliferation in both media and adventitia, reducing both neointimal accumulation and adventitial myofibroblast accumulation.

Intracoronary brachytherapy also prevents constrictive remodelling thought to be a result of reduction in the healing process and can even induce positive remodelling resulting in luminal enlargement. In the SCRIPPS trial, catheter-based brachytherapy was shown to reduce restenosis rates and this was maintained up to three years (25).

However, there are serious limitations to this treatment. One of these is edge restenosis whereby stenosis occurs at the ends or outside the stents, which could be a proliferative effect of low radiation doses on damaged tissue. Late thrombosis can also occur resulting in higher rates of late myocardial infarction.

Other limitations include failure of healing of medial dissections, acceleration of neointimal thickening at some doses or after chronic therapy and delayed reendothelialization. This type of non-specific treatment is able to target multiple mechanisms of restenosis but the non-specificity also limits this treatment by inhibiting the healing process (13).

Drug Eluting Stents

The introduction of drug eluting stents has been seen as another breakthrough in percutaneous coronary intervention. This is because of the dramatic reduction in restenosis rates not achieved by any previous treatments. Clinical studies showed rates of 5% or lower (26).

Drug eluting stents achieve these rates by inhibiting multiple biological processes that lead to restenosis. The scaffolding of the stent prevents recoil while the drugs delivered work on the surrounding tissue to prevent neointimal hyperplasia. Also drug eluting stents can deliver higher concentrations of a drug locally without systemic effects (11).

Rapamycin (Sirolimus)

Rapamycin modulates immune function and acts on T-lymphocyte activation and cell proliferation. It enters cells easily because of its lipophilic property and then binds to an intracellular receptor called FKBP12. This complex then increases cellular cyclin dependent kinase inhibitor (CDKI) p27 and inhibits the action of retinoblastoma protein (pRb), which regulates vascular smooth muscle cell proliferation (11, 13).

Inhibition of vascular smooth muscle cell proliferation and migration occurs as a result of growth arrest between G1 and S phases of the cell cycle. Rapamycin also inhibits inflammation after injury by inhibiting T-lymphocyte proliferation and activation. The biological effects of rapamycin lead to overall inhibition of neointimal formation thereby reducing restenosis (11, 13).

Paclitaxel

Paclitaxel belongs to a group of drugs called taxanes which are antiproliferatives used in cancer treatment. It inhibits cell proliferation and migration by promoting polymerisation of tubulin dimers and subsequently stabilising microtubules into an assembled state (11).

Microtubule disassembly is required for transition between G2 and M phase of the cell cycle. Therefore cell proliferation (such as in smooth muscle cells) is inhibited by this drug. It is also lipophilic and therefore rapidly taken up by cells. However, by altering the cytoskeletal structure, Paclitaxel exerts long-lasting effects in vascular smooth muscle cells (13).

Clinical trials have shown the positive outcomes of using this drug, such as the ELUTES and ASPECT trials which revealed a dose-dependent reduction in percent diameter stenosis (11).

The multiple actions of rapamycin and paclitaxel suggest they are the ideal agents to inhibit multiple processes leading to in-stent restenosis. However, one of the main concerns about this non-specific feature of both brachytherapy and drug eluting stents is the reduction in the healing process, which leads to loss of reendothelialization and possible late thrombosis (13).

DES vs. BMS

The reduction in the need for new revascularisation procedures has been the main clinical benefit of drug eluting stents (DES) over bare metal stents (BMS), shown in a great number of randomized controlled trials since 2002 (27). This finding resulted in their widespread use with estimates of DES accounting for more than 90% of stents used in the USA and Switzerland (28).

Initial concerns over increased stent thrombosis with DES compared to BMS were followed by several reports evaluating the safety of the drug eluting stents. These reports showed no significant differences between DES and BMS in the incidence of stent thrombosis during the first year of follow-up (29).

However, there has been a growing concern about the long-term safety of DES. At the World Congress of Cardiology in September 2006, the results of two independent meta-analyses were presented, showing a higher incidence of death and MI in the first generation DES compared to BMS at the latest follow-up of four years and this is thought to reflect the incidence of stent thrombosis. This has led to a heated debate about the widespread use of DES (28).

A series of meta-analyses evaluated the risk of very late stent thrombosis (>12 months) with the use of DES. One of these comprised of 6675 patients from 14 clinical trials and found that the incidence of very late stent thrombosis (>1 year post procedure) was low but that there was an increased risk of late thrombosis with DES (30). In another meta-analysis comprising nine double-blind trials and assessing the safety of sirolimus and paclitaxel-eluting coronary stents, stent thrombosis was more common in both DES compared with BMS after 1 year. However, there was no significant difference in the cumulative rates of death or MI at four years.

Recently, several meta-analyses have been carried out and some of these have found there to be no significant differences between the use of BMS and DES in the rates of death, MI or stent thrombosis.

One recent meta-analysis of seven randomized trials comparing DES and BMS in acute myocardial infarction (AMI), involved a total of 2357 patients with a follow up of 8-12 months. The results showed the DES to reduce the need for revascularisation significantly in patients with AMI with no difference in the incidence of death or MI compared to BMS. Furthermore the DES were not found to increase the risk of stent thrombosis at 1-year follow-up. However, there was no data available on the higher risk group of patients and the follow-up was limited to 12 months (32).

Another recent meta-analysis of four randomized trials, comprising 1748 patients, compared the use of sirolimus-eluting stents (SES) with BMS, with follow-up of four years. Three of these trials included patients with higher risk and more complex lesions. The authors concluded there were no significant differences between the two stents in the rates of death, MI or stent thrombosis (27).

The evidence remains inconclusive about the risk of stent thrombosis in DES and an update of the current evidence regarding the safety of DES is due to be presented at the next World Congress of Cardiology in September 2007.

New Developments

Within the stent industry there has been ongoing research aimed at developing a type of stent that will overcome the limitations of both bare metal stents and first generation drug eluting stents. Thus the goal is to prevent in-stent restenosis while promoting healing and reendothelialization of the vessel to prevent late thrombosis.

The most promising of these new types of stents is the biodegradable stents. The idea behind these is that having widened the lumen of the vessel, the stent remains *in situ* only for the time needed to prevent elastic recoil and constrictive remodelling. Thereafter, the stent is slowly absorbed and metabolised by the vessel until it disappears allowing reendothelialization to take place (33).

Currently bioabsorbable magnesium stents are in clinical trials. In a recent multicentre study, results showed that the stents can achieve an immediate lumen enlargement similar to conventional metallic stents and can be safely degraded after four months. Ongoing research in this domain includes modifications to prolong the degradation period (34).

Furthermore, biodegradable stents can also be designed to deliver drugs and these biodegradable drug eluting stents would be able to prevent in-stent restenosis with the added advantage of preventing late stent thrombosis (35). The stents may also be used as a delivery vehicle for genes to exert a beneficial anti-proliferative effect on the arterial wall cells. Examples include genes for endothelial nitric oxide synthase and vascular endothelial growth factor. Gene transfer is now in phase I studies in humans (36).

Drug eluting stents are also undergoing development so that the next generation of DES overcomes the limitations of the first generation such as late thrombosis. Recently, one of these DES has been put forward to the Food and Drug Administration for market approval in the U.S. This is the Xience V everolimus-eluting stent, which has a thin metal platform and the new drug has been shown to reduce tissue proliferation in coronary vessels. Furthermore, results of the clinical trials (SPIRIT) demonstrated no stent thrombosis at 3 years (37).

Following on from this, a bioabsorbable everolimus-eluting stent has been developed and is also undergoing clinical trials (ABSORB). In a recent trial of 30 patients, at 30 days the device was successful in 93.5% of all cases. There was also 100% safety as no patients experienced a major adverse coronary event or stent thrombosis. At 180 days, 3.3% of patients had major adverse coronary events and no stent thrombosis. Unfortunately there was a restenosis rate of 11.5% and this was due to shrinkage of the stent. Currently the stent is undergoing further development to resolve this issue (38).

The other type of DES that is being developed is the EPC-capturing stent where the stent is coated with an antibody (CD34+) that attracts endothelial progenitor cells (EPCs). The goal of this DES is to promote endothelial growth so the artery can heal faster to prevent in-stent restenosis. In a recent study testing this new DES, results showed excellent procedural success with 3.7% target vessel revascularization at 6 months (38).

With the advent of newer and better developed DES, the problems of in-stent restenosis and late thrombosis could be a thing of the past with better long-term outcomes for patients treated with the next generation of DES.

CONCLUSION

Prior to drug eluting stents, brachytherapy was the only treatment that proved effective in reducing restenosis. However brachytherapy showed significant side effects and therefore the search was still on for a solution to the problem of in-stent restenosis.

This came with the introduction of drug eluting stents, which has revolutionized coronary stenting. They have been hailed as 'miracle' stents due to their almost zero in-stent restenosis rate. The safety concerns have been mainly directed at the first generation of DES. Therefore, current advancements in the field of DES could increase the efficacy and safety of future DES.

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REVIEW ARTICLE

Therapeutic cloning: promises and issues

Charlotte Kfoury*

ABSTRACT: Advances in biotechnology necessitate both an understanding of scientific principles and ethical implications to be clinically applicable in medicine. In this regard, therapeutic cloning offers significant potential in regenerative medicine by circumventing immunorejection, and in the cure of genetic disorders when used in conjunction with gene therapy. Therapeutic cloning in the context of cell replacement therapy holds a huge potential for *de novo* organogenesis and the permanent treatment of Parkinson's disease, Duchenne muscular dystrophy, and diabetes mellitus as shown by *in vivo* studies. Scientific roadblocks impeding advancement in therapeutic cloning are tumorigenicity, epigenetic reprogramming, mitochondrial heteroplasmy, interspecies pathogen transfer, low oocyte availability. Therapeutic cloning is also often tied to ethical considerations concerning the source, destruction and moral status of IVF embryos based on the argument of potential. Legislative and funding issues are also addressed. Future considerations would include a distinction between therapeutic and reproductive cloning in legislative formulations.

KEYWORDS: therapeutic cloning, SCNT, cell replacement therapy, gene therapy, mitochondrial heteroplasmy, oocyte availability, biomedical ethics

INTRODUCTION

The advancement in biotechnologies and stem cell research, although encountering many scientific difficulties, legal constraints and ethical roadblocks, offers a tremendous potential in regenerative medicine and in the treatment of genetic defects. Therapeutic cloning is the transfer of nuclear material isolated from a somatic cell into an enucleated oocyte in the goal of deriving embryonic cell lines with the same genome as the nuclear donor. Somatic cell nuclear transfer (SCNT) products have histological compatibility with the nuclear donor, which circumvents, in clinical applications, the use of immunosuppressive drugs with heavy side-effects. While the goal of reproductive cloning is the creation of a person, the purpose of therapeutic cloning is to generate and direct the differentiation of patient-specific cell lines isolated from an embryo not intended for transfer in utero. Therapeutic cloning, through the production of these autologous nuclear-transfer embryonic stem cells (ntESC), offers great promises for regenerative and reproductive medicine, and in gene therapy, as a vector for gene-delivery. This review focuses on the recent breakthroughs in research based on therapeutic cloning, their feasibility, and their potential applications in medicine. The second part of this review discusses current roadblocks of therapeutic cloning, both in science and biomedical ethics, as well as the main alternatives to therapeutic cloning.

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Procedure for SCNT and characteristics of the ntESC

The procedure for SCNT does not differ from that of reproductive cloning (1). The host oocyte is arrested at metaphase II (2), and immobilized through light suction exerted by a pipette tip. A glass needle is used to remove a small piece of the zona pellucida and is reinserted through this puncture to extract the polar body and the oocyte nuclei. The incorporation of the somatic nuclei

into the enucleated oocyte can be done through electrofusion, which is the application of an electric pulse to incorporate a mammalian cell into the oocyte (used to produce Dolly). Alternatively, a somatic nucleus can be injected in the perivitelline space, the fluid-filled region between the zona pellucida and the ooplasm, as was used for Cumulina, the first mouse cloned through SCNT. Mitosis occurs in vitro until the formation of the blastocyst, a fluid-filled hollow ball of cells (40-150 cells) to which is attached, from the inside, the embryoblast or inner cell mass from which ntESC are taken. Subsequent addition of cell-type specific markers and growth hormones promotes the differentiation of the ntESC into the desired cell-line to be implanted in vivo inside the nuclear donor for therapeutic purposes, in cell replacement therapy for instance. In vitro, the ESC can proliferate ad infinitum and are totipotent, capable of differentiating into any cell-type of the body, contrary to adult stem cells which are multipotent, namely committed to produce any type of cells pertaining to a particular lineage (3).

Current legal status of therapeutic cloning in relation to reproductive cloning

Laws regarding biomedicine are generally formulated in vague terms that do not distinguish reproductive from therapeutic cloning. The Convention on Human Rights and Biomedicine (Oviedo convention), formulated by the Council of Europe in 1997, is counterintuitive. Article 13 declares that "an intervention seeking to modify the human genome may be only undertaken for preventive, diagnostic or therapeutic purposes" and stipulates in Article 18 that "the creation of human embryos for research is prohibited (4)." The Protocol on Cloning, put forward in 1998 and signed by 19 European nations, bans reproductive cloning and was paradoxically signed by France and Germany, which both have permissive policies regarding the generation of human ntESC lines.

Committees are formed in different countries to debate and regulate cloning, such as the President's Council on Bioethics, created in the USA in 2002, which is a much less permissive group than the UK's Human Fertilization and Embryology Authority (HFEA). The legitimacy of the latter is being questioned by the Prolife movement under the pretext that they were not democratically elected (5). Canada's Assisted Human Reproduction Act, in vigor since 2004, allows stem cell research only on unimplanted embryos obtained from fertility clinics but forbids SCNT (6). Asia has the highest legal permissibility since the generation of human ntESC lines through SCNT is legal (25). Australia is currently reviewing its existing laws (7) to follow the Asian trend in Singapore, China and South Korea, and to legalize the generation of chimeras using human DNA.

Since both reproductive and therapeutic cloning require the in vitro generation of a human embryo, prohibiting reproductive cloning is likely to result in severely hindering medically important research based on therapeutic cloning. A worldwide ban on reproductive human cloning was proposed by France and Germany to the UN in 2001 and effective4 since September 2006 (4). A breakthrough in reproductive cloning was published a month earlier by Zavos and Illmensee, who injected a skin fibroblast nucleus from an infertile man into an oocyte provided by his wife. One out of three SCNT attempts was successful, and although the four-celled embryo failed to implant in utero, this is the "first evidence of the creation and transfer of a human cloned embryo for reproductive purposes (8)." One may infer, from the rigidity of the current legislature regarding therapeutic cloning and stem cell research, that legal constraints are motivated by the fear that scientific development will be faster than the legislative debate, which was almost the case with Zavros and Illmensee's breakthrough, and lead to the unregulated reproductive cloning of human beings.

Promises of therapeutic cloning

SCNT in the context of therapeutic cloning holds a huge potential for research and clinical applications including the use of SCNT product as a vector for gene delivery, the creation of animal models of human diseases, and cell replacement therapy in regenerative medicine. Furthermore, SCNT might, in the future, allow in vitro organogenesis and counteract senescence. The combination of therapeutic cloning and gene therapy offers a great potential for patient-specific rescue of a genetic mutation of the loss-of-function type, resulting in lowered or eliminated activity of a particular protein. Therapeutic cloning used in cell replacement therapy has the potential to create various types of tissues such as osteoblasts to counteract osteoporosis, and spinal cord regeneration following trauma, as shown by Deshpande et al, who transferred motor neurons derived from ESC to rats with a severed spinal cord (9). The resulting recovery of motility could lead to clinical applications for paralysis in humans through therapeutic cloning.

Applications in regenerative medicine: recent breakthroughs for diabetes and neurodegenerative diseases

Therapeutic cloning constitutes a promising tool in tissue engineering and might offer the possibility of synthesizing organs de novo, which would solve the problems of immune rejection and organ shortage for transplantation. The assembly of patient-specific cardiomyocytes, blood vessels and skin pieces fixed on

a scaffold (39) holds great hope in the treatment of infarctus, atherosclerosis and severe burns, respectively. Consequently, the feasibility of de novo organogenesis based on SCNT depends on the elucidation of the tissue-specific molecular pathways mediating differentiation as well as the improvement of current SCNT and tissue engineering methods in order to recreate in vitro the complex three-dimensional organization and different intercellular interactions in organogenesis.

D'Amour et al designed in 2006 a five-step protocol enabling human embryonic stem cells to differentiate into endocrine cells producing most pancreatic hormones (10), including glucagon and insulin, with implications for use in cell replacement therapy for the treatment of diabetes mellitus. The combination of growth factors and differentiation markers added at each stage of the method were designed to duplicate pancreatic organogenesis in vivo, a breakthrough which could concretize the hope of generating organs using therapeutic cloning. According to this protocol, patient-specific ntESC lines would be differentiated into successive cell-type intermediates representative of pancreatic organogenesis, from endoderm to the terminally differentiated ß-cell that would then be transplanted into the patient's pancreas to treat diabetes-related hyperglycemia.

The recent success of therapeutic cloning in a mouse model of Parkinson's disease foreshadows clinical applicability in humans to treat neurodegenerative diseases and conditions involving demyelination. Parkinson's disease is characterized by the deterioration of dopaminergic neurons resulting in constant tremor and muscular stiffness impairing motility. Barberi et al derived, by SCNT with somatic nuclei from mouse cumulus and tail-tip cells, two ntESC lines which were induced to differentiate into motor, GABAminergic, serotonergic and dopaminergic neurons (11) forming synapses and displaying normal electrophysiological properties in vitro. The dopaminergic neurons were directly injected into the cortical striatum of mice with Parkinson-like lesions induced by 6-hydroxydopamine. Long-term behavioral rescue was observed, and 80% of the ntESC derived neurons were alive 8 week post-transplantation, contrary to only 40% for stem cell-derived neurons. Hence, the therapeutic cloning approach was shown to be more permanent as a cell replacement therapy and could eventually be extended to the treatment of cortical atrophy resulting from stroke or Alzheimer's disease.

REJUVENATING POTENTIAL

It has been observed that the SCNT of the nucleus of a cell close to reaching senescence resets the lifespan of the cell, as seen by the ability of the resulting embryo to carry 31 rounds of division, compared to 33 for a wild-type embryo of the same developmental stage (39). The rejuvenating potential conferred by SCNT can be paradoxically thwarted by telomere shortening, which reflects both the biological age of the nuclear donor and the time during which the ntESC lines were grown (37), leading to premature aging also observed in cloned animals. However, the addition of a transgene containing the two coding regions needed for the production of telomerase could restore telomere lengths and thus increase the survival of the transplanted cells, which would increase the success rate of therapeutic cloning for regenerative medicine. This strategy needs more investigation to be feasible because, while telomere length would not trigger tumorigenesis (12), Stampfer et al showed that, knockout p16INK4a epithelial cells with no endogenous telomerase activity do not respond to the pro-apoptotic signals of the growth factor TGF-ß following the addition of high levels of hTERT, the telomerase catalytic subunit (13).

GENERATING ANIMAL MODELS ANIMAL MODELS OF HUMAN DISEASE

Animal models of human diseases can be designed through therapeutic cloning for research purposes. Although a viable nonhuman primate has not yet been produced by SCNT, the success of Mitalipov and Wolf in creating a monkey by embryonic cloning from the nucleus of an allogenic blastomere supported the possibility that, through gene targeting, genetic defects can be reproduced in a wild-type genome to express a loss of function (14). Hence, we are getting one step closer to patient-specific genetic engineering of animal models of human disease. With improved SCNT protocol, the nucleus of a patient's skin biopsy could be introduced into a primate or mouse enucleated oocyte so that the resulting clone expresses the condition in a patient-specific way. Hence, clinical testing would be done on the animal model to find an optimal treatment, such as the drug combination to treat epigenetically-triggered cancer, highly variable among instances.

CANCER DIAGNOSIS

SCNT has applications in cancer research to identify whether a particular type of cancer arises from a genetic or an epigenetic defect (15), such as the demethylation of a tumor suppressor gene. The epigenetic modifications of chromatin structure in cancerous cells involve altered histone methylation, phosphorylation and deacetylation, as well as DNA methylation, which are reversible unlike genetic mutations. Supportive evidence for oncogenesis resulting from epigenetic features includes studies where normal mice blastula were obtained through SCNT from a skin malignancy (16) and a medullar

tumor (17). These studies could lead to clinical applications for cancer diagnosis in humans since nuclear reprogramming signals from the host ooplasm variably reset the epigenetic profile of the nuclear donor DNA. The derivation through SCNT of a healthy patient-specific stem line would show that cancer onset was triggered by epigenetic alterations. Anti-methylation drug, such as 5-aza-2'-deoxycytidine inhibiting DNA methyltransferases (18) that inactivate apoptotic genes in cancerous cells and histone deactylase inhibitors against oncogene overexpression are currently under clinical trial (15) as a potential anti-cancer therapy.

However, epigenetic resetting (15) following SCNT is likely to disrupt normal phenotype of the embryo-derived cell lines and the adult clone, the latter displaying an abnormally low body weight and expression level of MUP encoding genes (Major Urinary Proteins) as shown by Reik et al in the mouse. The epigenetic pattern of imprinted genes that was established during gametogenesis is lost through SCNT (20) and the inactivation of early genes directing embryogenesis can explain low embryo viability and poor efficiency in the derivation of autologous ntESC lines. Blelloch et al found out, from studies on neurons, that stem cells used as the nuclear donor have a higher success rate (21) than fully differentiated cells in the derivation of autologous embryonic cells. The introduction of a genetic mutation to reduce the function of DNA methyltransferase-1, as investigated by the same team, improved the production of ntESC due to resulting "global hypomethylation" (22) of genomic nuclear DNA.

THERAPEUTIC CLONING IN THE CONTEXT OF GENE THERAPY: CURRENT HOPES AND DRAWBACKS

SCNT from genetically modified nuclei obtained from a patient's skin biopsy, for example, is an efficient strategy to restore normal expression of a missing factor or to facilitate in vivo survival of the graft generated. For instance, patient-specific cardiomyocytes produced through SCNT will not integrate into the scarred heart tissue resulting from myocardial infarction. A proposed strategy (39) would be the genomic integration of an exogenous gene, prior to transplantation, encoding an anti-scarring factor (22) such as TGF-ß (transforming growth factor-ß). In the case of haemophilia, characterized by a deficit in functional clotting factor IX and XIII (39), the addition of the genetically engineered missing DNA sequence, or the replacement of the dysfunctional gene through homologous recombination in a patient's biopsy prior to SCNT could produce patient-specific cell lines with a correction for the defect.

For instance, Duchenne Muscular Dystrophy (DMD) is an inheritable X-linked condition characterized by reduced intramuscular dystophin levels, causing cellular necrosis and weakening (23). Being a single-gene disorder, DMD can be treated by therapeutic cloning in combination with gene therapy to restore normal dystrophin production. In the case where ntESC are transplanted without prior differentiation in vitro, the insertion of a transgene encoding MyoD (35), a transcription factor responsible for commitment to the myogenic lineage, may promote muscle regeneration.

The combination of gene therapy and therapeutic cloning has exciting potential for the genetic rescue of missing alleles in heritable genetic disorders such as severe combined immunodeficiency (SCID), in which genetic mutations of specific genes such as RAG-1 and 2, essential for the DNA recombination allowing immunoglobulin and lymphocyte polymorphism, render the immune system completely inefficient. Hochedlinger et al took a somatic nucleus from the tail-tip of an SCID mouse-model, created through the double-knockout of the Rag-2 gene (recombination-activating gene 2), and rescued the genetic defect through the insertion of two copies of the Rag-2 gene by homologous recombination (24). SCNT was performed to clone viable Rag-2 (+/+) mice with a normal immune system, from which embryonic stem cells were differentiated in vitro into hematopoietic stem cells normally found in the bone marrow. Three weeks following transplantation into the Rag-2(-/-) knockout mouse model, partial rescue of immune function was observed, as well as the presence of lymphocyte precursors and functional antibodies. However, mature T lymphocytes were not observed, suspected to be due to selective differentiation of the transplanted stem cells into myeloid cells (bone marrow precursors) instead (25). Although more work needs to be done to elucidate the pathways leading to preferential differentiation in vivo, the combination of gene therapy for the rescue of a loss of function and therapeutic cloning to bypass graft rejection holds the potential to eventually cure other immune disorders. Oncogenic activation following transduction constitutes a major drawback to this approach. In 2002, the insertion of the transgene to treat X-linked SCID in the LMO2 oncogene caused the onset of leukemia in two out of seven patients recently treated (26). Repeated graft rejection, even when derived through SCNT, remains an unsolved problem in the case of autoimmune disorders such as pernicious anemia and multiple sclerosis.

ROADBLOCKS OF THERAPEUTIC CLONING

Legal and funding issues

Legislative constraints and the subsequent lack of funding constitute a major impediment to the

advancement of therapeutic cloning. For instance, although therapeutic cloning is not completely banned in the United States, federal funding is not permitted to be used in experiments involving the 20 cell lines in the NIH (National Institute of Health) registry (44) derived before August 9, 2001. Out of these cell lines approved by Bush, 12 died and the remaining is not useful for research purposes. Researchers have to therefore rely on the scarcity of private funding, although 4 American states, including California with a yearly investment of 295 millions, have a budget allowed specifically for stem cell research (44). Clinton expressed concern on chimera production through "experiments involving the mingling of human and non-human species" (58) but paradoxically all the cell lines in the NIH registry that got Bush's approval were grown on animal-feeder cell layers and therefore contain traces of animal contaminants.

Oocyte availability: regulations and ethical concerns

A major roadblock in the feasibility of human therapeutic cloning is the low availability of oocytes for research purposes. Currently, due to low SCNT efficiency, it is estimated that 280 human oocytes (35) would be needed in order to derive one observe patient-specific ntESC line. The Human Fertility and Embryo Authority, in England, allows women in fertility clinics to offer two oocytes for scientific research, provided that at least twelve oocytes are collected (25), although the extra oocytes taken are more likely to be donated for in vitro insemination. Oocyte donations are, by law, forbidden to be remunerated other than to reimburse the cost of the procedure (from 1000 to 2000$) for ethical reasons. Substantial financial gain would incite poorer women to surrender of part of a finite supply of gametes, in addition to the risks incurred through surgerical removal of the oocytes and hormonal treatments. Ovarian hyperstimulation syndrome (OHSS) results, in most cases, from the administration of drugs such as gonadotropin-releasing hormone agonists (27), to induce the simultaneous maturation of multiple follicles into oocytes. OHSS can lead to cardio-respiratory difficulties, renal problems and internal hemorrhage, due to the accumulation of fluid in the abdominal cavity caused by abnormal vascular permeability in the vicinity of the ovaries (28), and occurs in 2 to 5% of the patients (29).

Fertility clinics are the major source of human oocytes for research. The aged oocytes that did not fertilize during in vitro trials are not optimal for SCNT, as investigated by Hall et al, who observed overexpression of genes encoding for meiotic spindles proteins and a lower cleavage efficiency (30) of aged oocytes versus fresh ones. After two years of debate, Harvard is the only group currently allowed to use oocytes collected for the sole purpose of research, with informed consent. Eggan and Melton intend to generate human ntESC lines from patients with diabetes, sickle cell anemia and amyotrophic lateral sclerosis (ALS), a progressive neurodegenerative condition targeting motor neurons (31).

Possible solutions to the oocyte shortage for therapeutic cloning

Interestingly, SCNT could provide a solution to low human oocyte availability and a promising therapeutic approach to circumvent infertility. As reported by Nagy and Chang, artificial gametes (32) can be created by haploidization, through SCNT into an enucleated oocyte ready to undergo meiosis upon induction. Tesarik et al incorporated the nucleus of a human cumulus cell into an enucleated allogenic oocyte. They got a 50% success rate in haploidization, and two out of the six artificial oocytes were successfully fertilized in vitro and thawed for possible implantation later on (33). However, abnormal chromosomal segregation and mitotic spindle assembly, as observed in mice, need to be resolved before haploidization through SCNT can be safely wide-spread in fertility clinics.

Once the molecular pathways of oocyte maturation are resolved, immature follicles could be collected post-mortem (39) and induced to mature in vitro for research purpose. However, this option needs to be rigorously regulated, and is likely to stir an ethical controversy.

An alternative to low human oocyte availability would be to use an oocyte of a different species. Successful trials were done to generate blastocysts in vitro through the SCNT of skin fibroblast nucleus from different mammals (ungulates, rodents, pigs, monkey) (34) and human into an enucleated bovine oocyte. However, only 1% of the human-bovine hybrids developed beyond the 16-cell stage, allowing the derivation of a human ntESC line (39). The insertion of human nuclear genome into an animal oocyte, especially through electrofusion where both human and animal mitochondrial DNA coexist in the same ooplasm, raise objections among the detractors of therapeutic cloning, although the percentage of total residual animal DNA (nuclear and mitochondrial) is too low to consider the hybrid as a chimera.

Mitochondrial heteroplasmy

Immune rejection of the ntESC in cell replacement therapy is due to mitochondrial heteroplasmy as a consequence of SCNT since the nuclear donor and ooplasmic host cells are not autologous in most cases. Mitochondrial heteroplasmy is also a major cause of SCNT embryo inviability beyond the eight-cell stage because the mitochondrial-nucleus interactions necessary for the production of most mitochondrial

proteins are disrupted due to inter-species incompatibility (35). Also, antigens such as Mta are encoded by the mitochondrial genome and trigger an auto-immune response targeting the hybrid (36) after transplantation. Cyclosporine A, an immunosupressor drug already used in organ transplantation (37), and the addition of either hemoglobin or ß-mercaptoethanol might inhibit mitochondrial-induced apoptosis in the ntESC. Inter-species incompatibility can be circumvented to a certain degree if the donor and host species are closely related. For instance, embryonic cells derived from the injection of a human nucleus in a chimpanzee ooplasm were viable, contrarily to when SCNT was done with the ooplasm of a nonhuman primate such as the orang-utan (39).

The transfer of mitochondria isolated from patient-specific biopsies (39) might circumvent the immune rejection problem due to mitochondrial heteroplasmy, and a female patient could in theory donate both the somatic nucleus and oocyte necessary for cell replacement therapy in her own body. The latter option offers great promises since recent studies in bovine showed that autologous SCNT embryonic production was more efficient than when the nuclear donor and ooplasmic host are from allogenic origins, and epigenetic reprogramming occurs to a significantly less extend in autologous SCNT embryos (40).

Transfer of animal contaminants

The interspecies transmission of pathogens is a non-negligible issue when injecting a human nucleus into the oocyte of another species, such as bovine or pig (41). For instance, the porcine endogenous retrovirus (PERV), although inoffensive in pigs, disrupts the transcription initiation of genes in humans, as demonstrated in vitro by Moalic et al, by integrating within the CpG islands of promoters (42). Gene therapy approaches are being designed to reduce the infectivity of PERV due to the mannose-rich N-glycan integrated in the viral capsule (43). Thus, through the insertion of genes such as ManIb and ManII, encoding mannosidases involved in N-glycan catabolism, the infectivity of PERV was significantly reduced but not annihilated in human cells.

Animal serum and non-proliferative mouse fibroblast used as feeder-cell layer to direct the development of the human ntESC (44) is problematic since animal contaminants can be transferred to the ntESC which in turn might trigger an immune response post-transplantation, thereby revoking the goal of therapeutic cloning.

N-glycolylneuraminic acid (Neu5Gc) is a mammalian sialic acid (a sugar with acidic side-chains present in the membrane of all cells) not found in humans, although most of us have anti-Neu5Gc antibodies. When human ntESC are grown on animal feeder cells or in contact with animal-derived serum, the stem cells incorporate enough Neu5Gc to potentially elicit an immune response (45) in vivo, hence killing the transplanted cells. Although the murine leukemia virus is not pathogenic (44) when transmitted from mouse feeder cells to human ntESC, non-cellular matrices are being designed to counteract the problem of animal contaminants. Ludwig et al developed a combination of human growth factors (TGFß, LiCl, bFGF, GABA, pipecolic acid which enhances receptor sensitivity to GABA) on a basal lamina reconstituted in vitro with human extracellular matrix components (collagen IV, laminin, vitronectin and fibronectin) (46) as a substitute for an animal feeder-cell layer. Neu5Gc was not reported in the cell lines cultured on this human matrix. In sum, the issue of pathogenic transmission is in the process of being solved, bringing one step further the potential for clinical application of therapeutic cloning in cell replacement therapy.

Tumorgenesis and spontaneous differentiation

NtESC are subjected to the same tumorigenicity potential as wild-type stem cells. The formation of teratomas, after in vivo transplantation, is due to co-purification of pluripotent stem cells along with the wanted differentiated cells. Fujikawa et al noted tumor formation in the ß-pancreatic cells transplanted in diabetic mice (47). The teratomas resulted from a low concentration of 0.2% Oct4 and SSEA-1 positive cells, both markers of pluripotency (48) downregulated upon differentiation, indicating the presence of left-over undifferentiated stem cells. Although the hyperglycemia associated with type I diabetes was reversed, tumorgenesis occurred 20 days post-transplantation, rendering stem cells, whether wild-type or issued from therapeutic cloning, a non-viable option for clinical applications in this instance, unless better isolation methods for the exclusive purification of differentiated stem cells are designed.

ETHICAL CONSIDERATIONS

Destruction of IVF

Therapeutic cloning and stem cell research stir an ethical controversy due to the source of embryonic stem cells, taken from aborted fetuses, unutilized zygotes (49) and embryos morphologically incapable of in utero implantation, the latter representing 60% of all embryos (50) created through IVF. Some consider the use of discarded embryos diminutive since they imply "recycling" life products, as implied by Bush's statement that "there is no spare embryo (29)."

The apparition of the primitive streak directing polarized development confers to the two-week embryo a higher moral status (51) as a potential human

organism, compared to the earlier embryo at the stage of a randomly-organized group of cells. Consequently, laws prohibiting the culture of embryos for more than two-weeks, which marks the onset of gastrulation and the formation of the primitive streak, are in vigor in several countries such as the United States, based on a decision of the British Warnock Commission (29) in 1984.

The ethical debate on the moral impermissibility of deliberate destruction of an embryo can be circumvented by a new technique deviced by Chung et al. They successfully derived human ESC from a single cell without destroying the blastocyst in the process (52), using the same manipulations normally devoted to genetic screening in preimplantation embryos. This method seems to be promising for solving the ethical concern of killing a human embryo, rendering feasible the pre-natal generation of individual-specific cell lines for use in regenerative medicine later on in life. However, Chung's method does not modify the fate of the ex vivo embryo, since the latter has a slim chance of implantation.

Moral status of the IVF embryo and the argument of potential

The main ethical roadblock against therapeutic cloning is the destruction of the generated embryos in order to collect cells that would further be differentiated in vitro. Embryo destruction is viewed as morally objectionable by the Prolife partisans because they grant the early embryo potential for personhood following development to term. Knowing that only 1 to 2 % of cloned mice produce viable organisms (53), the probability of producing a viable cloned human embryo is even slimmer. However, the detractors of research using embryos would argue that the potential for personhood ought not to be granted on the basis of probability because a minority - one out of three (54) - of zygotes conceived by natural means might implant in utero and be carried to term. Hence, the argument from potential relies on possibility rather than probability and is based on the belief that morally significant human life beings at conception. According to Dawson and Singer, "since something is logically impossible only if its assertion involves a contradiction, it is not logically impossible for a human blastocyst in a laboratory to develop into a person (55)."

A counterargument put forth by the advocates of embryo research is that, left untouched, it is impossible that the in vitro embryo develops into the mature organism, and in utero implantation cannot occur if the transferred embryo reached the eight-celled stage and beyond (54). Nothing morally compelled one to generate a SCNT embryo that would have otherwise not existed, therefore we are not morally obligated to transfer the latter in utero. According to McMahan, "the idea that the potential to become a person confers a special moral status is plausible, if at all, only if the potential is identity-preserving (56). " He further argues that, contrary to the late-term embryo, the early embryo as an "insentient cluster of cells" has nonidentity potential since the latter is not identical in morphology to the mature organism, which possesses a neuronal network characteristic of higher mental life, is able to develop complex cognitive capacities including self-consciousness and sentience, of which the early-term embryo is deprived. The destruction of an embryo of lower moral significance in the context of justified research to improve the quality of life of existing people of higher moral status ought to be viewed as morally permissible. Furthermore, under the philosophical point of view, "cell replacement therapy does not involve the destruction of an embryo but only its transformation into an embryonic cell line (57)."

The engineering of mouse blastocysts lacking the Cdx2 gene , otherwise needed for the generation of the midgut endoderm and trophoblast differenciation, results in non-viable embryos that spontaneously stop dividing, providing a criticized alternative to the destruction of embryos by deliberate human action.

CONCLUSION

Although several scientific roadblocks remain unsolved, the medical benefits that could be gained from treatments based on therapeutic cloning outweigh the ethical dilemma and calls for further improvements to be clinically applicable. In sum, therapeutic cloning features great potential as a histocompatible method for cell replacement therapy to restore motility following paralysis, counteract senescence, and repair damages done by stroke, myocardial infarction, liver cirrhosis, severe burns and osteoporosis to name a few. Used as an alternative to viral vectors, patient-specific cell lines derived through SCNT can be used in conjunction with gene therapy to treat conditions caused by genetic defects among which diabetes, hemophilia, sickle cell anemia, SCID, neurodegenerative disorders such as Parkinson, DMD and many more. Transgene insertion could be used before in vivo transplantation of the ntESC in order to enhance graft survival, differentiation and integration. Other applications of therapeutic cloning include the diagnosis of epigenetically triggered cancer and the tailoring of a treatment using SCNT, the creation of animal models of human diseases, and could eventually lead to tissue engineering of organs de novo. Main scientific difficulties include tumorigenicity, in vitro spontaneous differentiation, interspecies transfer of pathogens, low oocyte availability, epigenetic reprogramming of the genome, mitochondrial heteroplasmy and the possibility of graft rejection.

Ethical controversy on the source and destruction of embryos as well as the contradictory legislations and scarcity of funding contribute to impede advancements in therapeutic cloning. Future considerations would be to unify federal and state laws, and establish a clear distinction between therapeutic and reproductive cloning in the redaction of laws pertaining to the SCNT generation of embryos. In this regard, a close understanding of the science and ethical issues pertaining to therapeutic cloning is necessary to ensure improvement in clinical applicability without falling into unregulated abuses.

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REVIEW ARTICLE

Asbestos: mining exposure, health effects and policy implications

Kristina Luus *

ABSTRACT: The purpose of this paper is to review research in the health effects and risks associated with exposure to asbestos and then to use this scientific evidence to analyze the implications of Canada's current policy on the use, manufacturing and export of asbestos. The review begins with a brief historical introduction to asbestos, and then moves on to look at the risks associated with asbestos exposure. Epidemiological and in vitro studies are then analyzed to determine the health risks of asbestos, with a specific focus on the different effects of serpentine and amphibole asbestos fibres. The paper then concludes with an analysis of Canadian policy in light of established scientific evidence and with a discussion of the possible implications of a gap between scientific knowledge and public policy.

INTRODUCTION

This critical examination of the historical and current health effects of exposure to asbestos will first focus on a historical introduction to asbestos, and then assess the risks of exposure, causes of health problems and resulting health effects of exposure to asbestos. Focus is placed on assessing occupational health risks associated with mining asbestos, with special emphasis on differentiating between the health risks of different types and lengths of asbestos fibres. The purpose of this focus is to assess the validity of claims made by policymakers and the Chrysotile Institute that the type of asbestos mined in Canada is significantly safer than other types of asbestos (1, 2). The review of scientific literature will then be used to inform a policy critique of Canada's asbestos policies.

Asbestos

Asbestos are fibrous, naturally occurring hydrated silicates that have long been mined and used for their fire-retardant and insulating properties as construction materials (3, 4). Asbestos can be found in amphibole and serpentine forms (5, 6). 95% of the asbestos mined globally is in a serpentine form of chrysotile type, with fibres that are long and curly (7). Amphibole forms of asbestos may be of amosite, crocidolite or anthophyllite types, and are shorter and straighter than serpentine varieties. According to the Stanton Hypothesis, amphibole fibres were originally believed to pose less risk (4, 6), but these fibres were then linked to increased rates of mesothelioma (8).

History of exposure

Dr. Montague Murray first recognized the negative health effects of asbestos in 1899 (9). However, dust control legislation for mines was not enacted in North America until 1971 (3). In the intermediate years, mining and use of asbestos increased dramatically by 120-fold, peaking upon the enaction of legislation in 1971, and decreasing exponentially until the present (Figure 1). The current decreases in the rate of mining are due to public health concerns and to the progressively more restrictive standards placed upon the level of asbestos dust allowed in mines, from 5 fibres/cm^3 in 1971 (3) to 1 fibres/cm^3 at present (10). Although the global levels of asbestos mined have decreased significantly, Canada continues to be one of the world's leading producers. 2.4 x 10^5 tonnes were mined in Canada in 2003 (11), which accounted for much of the world's production of asbestos (12).

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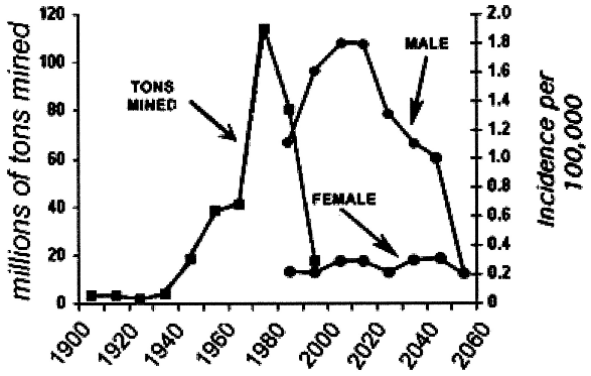

Figure 1. Correlation of Asbestosis and Asbestos Mined from 1900-2055 in the United States (Cudgell and Kamp, 2004)

Current risks

Adverse health effects from exposure to asbestos remain a serious concern to miners, mining communities and residents of buildings that contain asbestos. Miners and mining communities are at the greatest risk from asbestos related diseases, but are better prepared to limit their exposure to asbestos than homeowners who are unknowingly breathing in asbestos. There is a time lag of 15 to 40 years between exposure and asbestos-caused disease for both residents and miners, which often makes it difficult to relate historical exposure to current symptoms (Figure 2) (7, 3). Asbestos has far-reaching and long-lasting impacts for human health, both through occupational and environmental exposure.

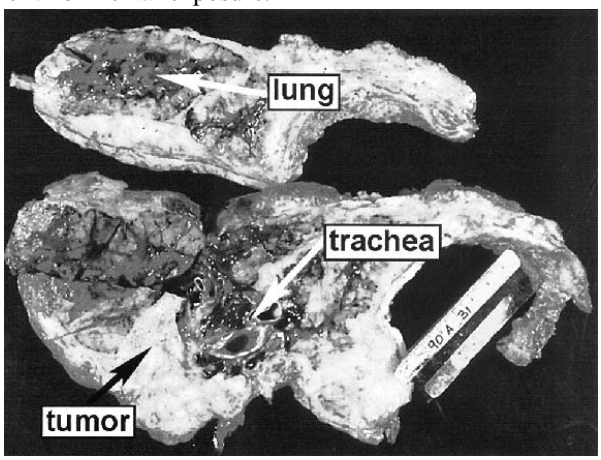

Figure 2. Malignant mesothelioma most frequently affects the lining of the lungs (3).

EXPOSURE

Pathways

Exposure to asbestos fibres occurs through ingestion, skin contact or inhalation (3, 9, 13). Inhalation of asbestos fibres is dangerous and results in asbestos-related diseases. Skin contact with raw asbestos fibres results in relatively harmless epidermal overgrowth. Ingestion of water from asbestos-contaminated pipes has not been found to increase the incidence of asbestos-related diseases (14). The remainder of this essay will therefore focus exclusively on inhalation of asbestos fibres.

Persistence

Asbestos occurs naturally underground in trace quantities. Amphiboles are naturally present in surface soils in specific regions of several countries including Finland, Greece and Afghanistan, and affect local residents (3). However, since asbestos is only dangerous when inhaled, subsurface deposits pose little risk. When these deposits are mined, airborne concentrations increase greatly In Libby, Montana, mining and processing of asbestos-contaminated talc and vermiculite increased airborne asbestos concentrations from <0.004 in 1847 to 0.022 fibres/cm^3 in 1995. These airborne concentrations become embedded in the tree bark, where the asbestos persists and can affect those who harvest the contaminated wood (15). Exposure to asbestos can occur when workers process products such as talc and vermiculite that are naturally contaminated by tremolite (3). Asbestos-contaminated vermiculite was mined in Libby, Montana for 70 years, leading to infection of both workers and the community since workers brought home materials from the mine and used them as clean fill in constructing driveways and gardens. Asbestos therefore persists in trace amounts in soil and in larger concentrations in buried waste sites (16).

Risk

The risks associated with asbestos are significant, and workers have historically been subjected to concentrations 10-100 times the Canadian legal limit of 1 fibres/cm^3 (3, 17, 13). These workers have suffered from a wide range of health effects. Asbestos has a significant risk even at lower concentrations. The families of workers have therefore had elevated rates of asbestos-related diseases from the asbestos inadvertently brought home on the clothing of miners. Radiographs of the spouses of workers exposed to asbestos indicated that 19% of them suffered from pleural changes, and that the only factor of significance in detailed questionnaires was the latent period since first exposure. The microscopic fibres of asbestos are therefore impossible to eliminate from the indoor air environment, and pose a significant risk for the workers, their families and those whose homes contain airborne asbestos.

CAUSES OF HEALTH PROBLEMS

Exposure to asbestos results in a variety of health problems caused by the autoimmune, genotoxic and irritative effects of asbestos. Within each of these categories, case studies and experiments will be referred to in order to illuminate differences between the mechanisms of different types of asbestos.

Autoimmunity

Autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus and multiple sclerosis have been found at increased rates in populations exposed to asbestos (13). An epidemiological study of the autoimmune effects of asbestos exposure was conducted on 7,307 residents of Libby, Montana who had undergone occupational and environmental exposure to amphibole asbestos from a local vermiculite mine (18, 13). Of these residents, 6.7% were diagnosed with auto-immune diseases, compared to a rate of 1% in an unexposed population in Missoula, MT. Furthermore, elevated levels of autoantibodies were detected in the exposed population at a rate 28.6% higher than the general Missoula population. However, despite the statistical significance of these findings, the causation between asbestos exposure and autoimmune diseases is still incompletely explored. Asbestos is believed to bring about systemic autoimmunity by suppressing the body's natural killer cells, and has been thought to increase the incidence of lung cancer by suppressing pulmonary parenchymal cells (19, 21). A study exposed mice to serpentine chrysolite asbestos and amphibole crocidolite asbestos to compare the effects of these two types of asbestos on autoimmunity.

The results from this experiment determined that crocidolite asbestos had a slightly more toxic impact than chrysotile on the immune function of pulmonary parenchymal cells (20). This finding confirms the more damaging health effects overall of amphibole asbestos in comparison to serpentine asbestos.

Genotoxicity

The genotoxic effects of asbestos have been found both in factory workers (17) and their wives (21). In these studies, asbestos has been found to damage DNA, gene transcription and protein expression (17, 21). The resulting genotoxic effects would lead to inflammation, cell death and errors in modulating cell proliferation (7). Although further research is still required to identify the mechanisms through which asbestos incites genotoxicity, multilocus deletions of DNA in hybrid-human cells have been induced through exposure to crocidolite (22). Research on in vivo rats has found that chrysotile promotes genotoxicity more rapidly than crocidolite (23). This difference may be due to fibre length, as indicated by an *in vivo* experiment by Keane et al which found that the magnitude of asbestos-related disease on Chinese hamster lungs grew with the length of the fibre (24). Genotoxicity is therefore related to length, which means that longer serpentine chrysotile fibres are more genotoxic than short amphibole asbestos fibres. This finding runs counter to reviews that have summarized the effects of amphibole asbestos as more damaging that the effects of serpentine asbestos (4, 6). It is possible that the genotoxic effects are either less important or take longer to manifest than the health effects of irritation and autoimmunity, and therefore have not yet appeared in research literature because earlier studies included all forms of asbestos together (25).

Irritation

Upon inhalation, asbestos causes significant irritation to the lungs and bronchioles. The resulting irritation causes the lungs to try to digest the asbestos. However, because of its chemical and physical stability, the asbestos cannot be digested and instead becomes encased in scar tissue. Growing masses of scar tissue result in benign fibrosis, effusions and plaques in the lung cavity (12). Since crysolite fibres are thin-walled sheets of silicates, they have a half-life of of 0.3-11 days, whereas the double-chained chemistry of amphibole fibres have a much longer half life that extends from 500 days to infinity (25). It is therefore clear that amphibole fibres have a much more irritative effect on the lungs than crysolite fibres and are therefore much more likely to lead to asbestosis, effusions and plaques through irritation.

RESULTING HEALTH EFFECTS

The diseases that result from the aforementioned causes will now be analyzed, starting with the benign effects of asbestosis, effusions and plaques, and then moving on to bronchogenic lung cancer and malignant mesothelioma. After describing the symptoms and causes of each diseases, the incidence of each disease will then be analyzed in terms of the type of asbestos that the population was exposed to, to determine the varying risks of various types of asbestos.

Asbestosis

Asbestosis is a disease characterized by bilateral interstitial pulmonary fibrosis due to the inhalation of asbestos fibres (26, 3). The fibrosis of the lungs causes shortness of breath and dry cough. In severe cases, patients have difficulty with oxygen diffusion, since the disease primarily affects the base of the lungs (26). Amphibole fibres have been strongly linked to asbestosis through a series of nine epidemiological

studies reviewed by Hessel et al (27). However, chrysotile fibres break apart so quickly that they result in no fibrosis at subchronic levels (3413 total fibres/cm^3) (25). Asbestosis is therefore contracted primarily through amphibole fibres.

Plaques

Pleural plaques occur when fibrosis is localized in a specific region of the lung. Plaques are a distinctive feature of asbestos exposure that cause functional impairment (28, 3). However, whereas Goldsmith found plaques to be benign (28), Sprince et al have linked the presence of plaques with the emergence of cancer and immune deficiency (5). It seems likely that disagreements over the prognosis of plaques in terms of their propensity towards malignancy may be better resolved with a more thorough look at the differences in the rates of malignancy caused by the two main forms of asbestos. With this in mind, we will now turn to examine the two carcinogenic diseases caused by asbestos.

Malignant mesothelioma

Malignant mesothelioma is a type of cancer caused by asbestos that occurs in the lining of the lungs or the abdomen (Figure 2) (3). In a mortality study of Prieska, a South African town that had been milled and mined extensively for asbestos, it was found that the risk of death by malignant mesothelioma was $277/10^6$ (29). The current incidence of malignant mesothelioma is projected to double in Europe over the next 20 years due to increased knowledge about this disease, which is often missed or misdiagnosed as pneumonia (30). Rates of malignant mesothelioma are much greater when populations have undergone exposure to amphibole fibres, because of the greater persistence of amphibole fibres, and their greater autoimmune effect (31). McDonald et al. (32) have shown through an epidemiological study of asbestos workers in Quebec that the diminished rates of malignant mesothelioma associated with serpentine asbestos were also due to the lower concentrations of fibrous tremolite, an asbestos mineral with significant pathology.

Bronchogenic lung cancer

Bronchogenic lung cancer is brought about through interstitial pulmonary fibrosis, which then becomes malignant when the body's autoimmune defenses break down (3). An epidemiological study of a town in Turkey with natural deposits of asbestiform minerals found elevated rates of bronchogenic lung cancer. Upon autopsy of a patient who died from lung cancer, many asbestos bodies were found. They were composed of tremolite fibres with few chrysolite fibres (33). This finding supports the evidence mentioned in the previous section that amphibole asbestos is more irritative and persistent in the lungs and has a stronger autoimmune effect. It also supports the findings that tremolite concentrations increase the incidence of cancer (32). The increased irritation causes the fibrosis that results in tumors. The breakdown of the autoimmunity in the lungs then results in bronchogenic lung cancer.

Effusions

Effusions occur when fluids accumulate in the lungs. These events can be acute with complete resolution, or can be chronic and result in significant accumulations of fluid with associated fever and pain (3). If severe, effusions can result in rounded ateclatasis, where the lung is left without air. Effusions occur frequently when mesothelioma sets in, but can also result from benign fibrosis, plaques and nonspecific fibrous thickening (34). Effusions are therefore symptoms of asbestos exposure that can result in death. Amphibole fibres have been associated with effusions more so than chrysolite fibres (34), likely because amphibole fibres are more irritative and long-lasting in the lungs (25).

CANADIAN POLICIES ON ASBESTOS

Despite the increased knowledge of the risks and consequences of exposure to asbestos, Canadians continue to be exposed to asbestos through unintended environmental exposure, and occupational exposure at levels of up to 1 fibre/cm^3. It is therefore vital that this analysis of the health effects of asbestos conclude with an analysis of current legislation for asbestos and its implications in light of scientific evidence, as well as a list of policy recommendations.

Current Legislation

The Canadian Government currently prohibits the spraying of mixtures of fibres that contain asbestos if the fibres are not fully encapsulated during spraying in Item 40 in Part 1 of Schedule 1 to the Hazardous Products Act (HPA) (35). Products containing crocidolite asbestos fibres are banned, but an exemption in 12(f) of the HPA states that these products are not banned if they are "packaged as a consumer product" (35). Finally, Item 37 in Part 1 of Schedule 1 of the HPA bans the advertising, sale and importing of all products that consist in their entirety of asbestos fibre. The background to this section of the HPA reads:

> the addition of this item to the Schedule does not affect the commerce of products that contain asbestos as an ingredient (irrespective of the concentration of asbestos) nor the sale/importation of pure asbestos to/by industrial users (35)

The ban is therefore so narrow as to have little effect on community-level exposure to asbestos.

Canada also continues to export chrysotile to the EU despite their ban under the justification that "Canada considers that the bans imposed by many EU Member States and the Commission cannot be justified by scientific risk assessments" (36). Meanwhile, a wealth of scientific evidence referred to previously in this review has demonstrated that evidence directly links chrysotile asbestos with health risks.

Workers who process chrysotile or have been exposed to asbestos for over 2,000 hours in their lifetime must undergo a mandatory chest x-ray (37). However, the implications of this regulation regarding compensation and health care are unclear when a problem is detected.

Implications

The laxness of the regulations concerning the sale, export and mining of asbestos has detrimental consequences for the health of Canadian communities. The Chrysotile Institute continues to receive federal subsidies to mine asbestos in Quebec, and sets safety regulations "in accordance with government" in Canada (1). The safety regulations developed were described in the previous section, and since these regulations protect corporate interests more than community health, there are serious implications for community health.

Since asbestos-related diseases appear many years after first exposure to asbestos (Figure 3), it is often difficult to establish a link between disease and exposure, especially since smoking and genetics are confounding factors in the incidence of asbestos-related diseases. Lawyers and corporations have therefore depended on scientists to prove or disprove this link. Miller (2006) found in his analysis of radiographic readings for asbestosis using International Labour Office classification that evidence has been misused by the media and attorneys to give undue compensation to victims. However, evidence has also been found that victims of asbestos exposure are often misdiagnosed, and fail to receive adequate protection (4). The lack of firm government guidelines for the use and mining of asbestos is therefore of great detriment to miners and their communities.

Recommendations

Since epidemiological analyses and animal testing have clearly shown that asbestos has health effects through occupational and environmental exposure, it is the responsibility of the government to protect the health of its citizens instead of yielding to economic corporate interests. The government of Canada must stop exporting asbestos to EU countries in which asbestos has been banned, instead of continuing to challenge the rights of these countries to refuse imports using WTO guidelines (36). Although chrysotile is less potent than other forms of asbestos, serious questions must be asked about whether the benefits of economic development in Quebec outweigh the long-term health consequences of exposure to asbestos. One potential way in which these costs and benefits can be better balanced is by forcing companies such as the Chrysotile Institute to assume responsibility for the compensation of people affected by asbestos.

Overall, better monitoring is required, both of the health of miners and their communities and of indoor air

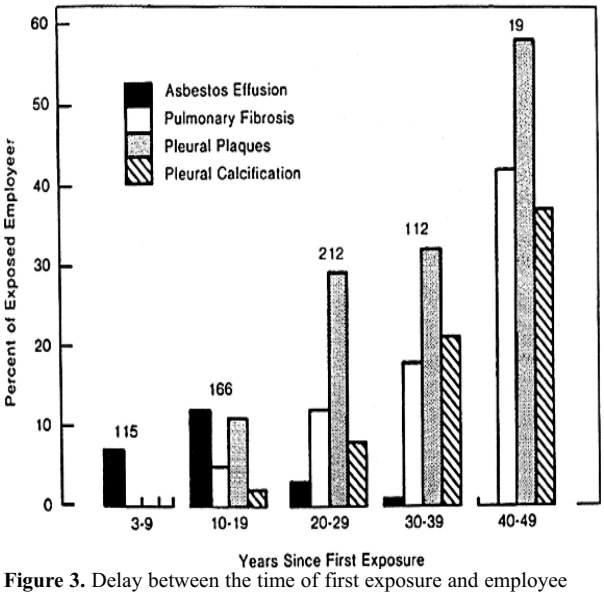

Figure 3. Delay between the time of first exposure and employee diagnosis with asbestos-related diseases (Kamp and Weitzman, 1999)

quality in buildings containing asbestos. Despite claims that indoor air quality testing of public buildings is unnecessary and unduly frightens the population, the overwhelming risks of exposure and subsequent health effects warrant testing these sites for indoor air quality. It is only through a thorough knowledge of the potential health effects of a specific site that informed policies can be created.

The purpose of this paper has been to review current scientific literature on the risks and health effects of exposure to different types of asbestos, and then use this scientific evidence to critically analyze current Canadian policies. The main problem in the existing policies is that they are not based on scientific evidence of the health effects of asbestos, and are designed not to mitigate these effects, but to create an appearance of maintaining public health standards while yielding to corporate interests. The continuing health consequences of asbestos are therefore a prime example of the way in which policymakers are choosing short-term economic benefits instead of minimizing scientifically established risks, to the great detriment of our communities.

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CROSSROADS

Anthropology speaks to medicine: the case HIV/AIDS in Africa

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INTRODUCTION

It is exceedingly rare for medical doctors and anthropologists to sit down and exchange ideas, even about an issue as important as the global HIV/AIDS epidemic. Does that mean that anthropologists have no knowledge of value to add to the epidemiological and biomedical understanding of the epidemic? This paper asserts that, despite the general neglect of anthropology by the biomedical and public health sciences, anthropology as a discipline has contributed valuable concrete knowledge that has enriched the epidemiological and biomedical understanding of the HIV/AIDS epidemic.

The paper argues that two schools of competing anthropological thought have contributed to this knowledge base. The first school is comprised of what will be called *traditional anthropologists*. These are classically trained anthropologists who see their role as adding socio-cultural depth to biomedical and epidemiological understandings of the HIV/AIDS epidemic. The second school stands for anthropological change. This group of *political economy (PE) anthropologists* argues that anthropology's 'special understanding' of society is not of primary relevance to understanding HIV/AIDS, as it is the political and economic structure in which individuals act that shapes their behaviour. This school proposes structural violence, the notion that societal structures such as racism, sexism and inequality cause direct and indirect harm to individuals, as the principal perspective for understanding HIV/AIDS[1].

The paper examines anthropology's contribution to our understanding of HIV/AIDS and sexuality, gender, risk groups, and behaviour change strategies. The paper argues that while the PE anthropologists provide an extremely valuable perspective, their approach does not capitalize on anthropology's comparative advantage (a rich understanding of the local cultural context) and therefore risks ignoring an important level of anthropological analysis - the local culture. Thus both types of anthropological knowledge have contributed to our understanding of HIV/AIDS, and without this knowledge, clinicians and public health practitioners would lack our current nuanced understanding of the epidemic.

The focus of the paper is on the HIV/AIDS epidemic in sub-Saharan Africa specifically as Africa is home to 64 percent of all people living with HIV (1). While UNAIDS asserts that "[t]here is no such thing as the 'African' epidemic'" because there is a tremendous diversity across the continent in patterns of HIV infection, there are nonetheless certain commonalities found across sub-Saharan Africa (2). First, both aggregate prevalence and incidence are the highest in the world, with profound human and socio-economic ramifications. Second, despite recent strides forward, treatment rates remain the lowest in the world, with an estimated treatment coverage of fifteen percent (1). Third, the epidemic occurs alongside a number of macro-level social shocks such as wars, macroeconomic crises, other infectious disease epidemics, and high levels of political instability (3, 4, 5, 6).

THEORETICAL AND HISTORICAL BACKGROUND

Manderson traces anthropology's interest in disease to the discipline's "professionalization as an applied science, the interest of other public health scholars in anthropological methods and theories, and the

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1 The author recognizes that there is no such person as a 'traditional anthropologist' and that PE anthropologists may equally use their traditional disciplinary tools. The classification is merely an attempt to present a stylized representation of two dissimilar *approaches* to anthropological understandings of HIV/AIDS.

involvement of anthropologists in international health programs of multilateral organizations and bilateral aid programs" (7). Building on anthropologists' earlier work with public health issues, the social science study of AIDS in Africa has required "the efforts of both anthropologists sensitive to public health, biomedical and non-Western healing issues, and anthropologists who seek to analyze the AIDS epidemic as they would any other phenomenon in the field" (8). Manderson evokes a common theme of both schools of anthropology by asserting that anthropological involvement has ensured that "some account is taken of local knowledge, cultural influence on the patterns of disease, and structural barriers to good health" (7).

We can identify four stylized phases of anthropological research into HIV/AIDS in Africa since the onset of the epidemic:

1) Anthropologists as *Handmaidens*: The Biomedical Paradigm;
2) Anthropologists as *Cultural Experts*: The Community Paradigm;
3) Anthropologists as *Political Economists*: The Structural Violence Paradigm;
4) The Future: An Anthropological Synthesis (9).

During the *Handmaiden* period, anthropologists supported biomedical research without challenging the traditional public health approach. This early paradigm was characterized by a heavily biomedical emphasis and a largely individualistic bias in understanding HIV/AIDS (8, 9).

In the *Cultural Expert* phase, there was a move away from individual-centric understandings of the epidemic. By the late 1980s it had become clear that a far more complex set of social, structural, and cultural factors mediate the structure of risk in every population group, and that the dynamics of individual psychology could not be expected to fully explain changes in sexual conduct without taking these broader issues into account (10). During the early 1990s, there was a growing focus on the interpretation of cultural meanings as central to a fuller understanding of both the sexual transmission of HIV/AIDS in different social settings and the potential to respond to HIV/AIDS through the design of more culturally appropriate prevention programs (11).

In the *Political Economist* phase, anthropological literature on HIV/AIDS began to increasingly focus on the linkages between local sociocultural processes that create risk of infection and global political economy (10, 11, 12, 13, 14). Farmer, a central figure in the structural violence school, is vituperatively critical of the earlier anthropological emphasis on cultural phenomena at the expense of political economy. He attributes these omissions of the structural and economic causes of HIV/AIDS transmission to "the ways in which anthropology 'makes it object'" (14). Farmer recounts that

> Animal sacrifice, zoophilia, ritualized homosexuality, scarification, and ritual beliefs all figure prominently in the early anthropology of AIDS. The only problem was that none of this had any onstrable relevance to HIV transmission or AIDS outcomes, and claims to the contrary were eventually revealed to be mistaken (14).

The HIV/AIDS epidemic, Farmer argues, requires broad biosocial approaches emphasizing structural forces such as racism, sexism and inequality, of which structural violence is the pre-eminent model (14).

Castro and Farmer propose *structural violence* as a conceptual framework for understanding the HIV/AIDS epidemic (12, 15). They argue that societies are shaped by large-scale social forces such as racism, sexism, political violence, poverty, and other social inequalities that are rooted in historical and economic processes (12). These forces, which together define structural violence, "sculpt the distribution and outcome of HIV/AIDS" (Ibid.). As an example, consider Schoepf's observation that one consequence of the economic crisis of the 1980s was a proliferation of multiple partner strategies, as poverty forced women to exchange sexual favors for financial support (4). With the onset of AIDS, "what once appeared to be a survival strategy has been transformed into a death strategy" (4) as "[m]acrolevel crisis generates conditions for microlevel dislocation" (16). We thus see the power of Farmer's observation that: "*fundamentally social forces and processes come to be embodied as biological events*" (14, 17).

Linking HIV/AIDS and structural forces, such as poverty, is critical to achieving effective prevention and treatment strategies. This is because the links between disease and poverty are profound though often ignored. In a major report on *AIDS as a Development Issue*, Collins and Rau argue that it is "commonplace for HIV/AIDS programme managers to acknowledge poverty as a causative factor, but to then say that 'poverty' is beyond the scope of their programmes" (18).

Thus by continually emphasizing poverty and its associated structure of inequality, PE anthropologists provide a very powerful policy proposal: poverty reduction should be our central goal (14, 19). The whole of anthropology, however, cannot focus on poverty reduction, as that would be poor use of anthropology's comparative advantage, which leads us to our next topic, sexuality.

SEXUALITY AND HIV/AIDS

Writing in 1932, Malinowski observed a 'surfeit of sex' in anthropology. "I alone," he confessed, "have to plead guilty to four books on the subject, two of which have the word sex on the title-page" (20). After

Malinowski, however, sexuality was given scant attention by social scientists until the AIDS epidemic provoked a renewed wave of research (21).

At the outset of the epidemic and even into the 1990s, the non-anthropological literature on HIV/AIDS contained sweeping statements about a special 'African sexuality,' based on traditional marriage patterns different from those of Europe and Asia (11). A common theme in early HIV/AIDS literature posited that the spread of the AIDS epidemic in sub-Saharan Africa was related to multi-partner sexual relations (22).

Anthropologists were employed to explain this hypothesis. Some early studies reported that sexuality outside of marriage is not disapproved of strongly in certain African societies (22, 24). Such research was not without its critics at the time, and ironically the notion that multiple sexual partners is more common in developing than developed countries was reversed by a 2006 epidemiological review in the *Lancet* which showed the opposite pattern (25).

A major theme of the critiques of early sexuality studies has been their emphasis on individual agency, the notion that individuals are able to make free and unconstrained decisions regarding their sexual behaviour. Since the 1990s, anthropological research has suggested that the range of factors influencing the construction of sexual realities is far more complex than previously perceived (16). With the rise of the structural violence paradigm, it has become more widely espoused that, as with all behaviour, not just cultural, but also structural, political, and economic factors shape sexual experience to a far greater extent than has previously been understood (26, 27). In particular, research has emphasized that political and economic factors have played a key role in determining the shape and spread of the epidemic and has further emphasized that these same factors have been responsible for many of the most complex barriers to effective AIDS prevention programs (10). This research has been important in changing our understanding of who is and is not at risk from HIV/AIDS, a debate addressed below.

CONCEPTUALIZATIONS OF RISK GROUPS

A major contribution of anthropological research to our understanding of HIV/AIDS came through the enhanced conceptualization of the much-abused term, 'risk groups.' *World Development Report 1993* expressed mainstream public health thinking by arguing that "*[h]igh-risk groups* may include sex workers, migrants, members of the military, truck drivers, and drug users who share needles" (28). This view was widely held in the early 1990s and prostitutes were the first and most prominent identified risk group in Africa. Ugandan President Museveni asserted in November 1990 that "the main route of AIDS is through prostitution" (29).

Because of frequent associations between identified 'risk groups' and blame, epidemiological research was criticized for creating "scapegoated 'risk groups'" (8, 11). Such discourse was criticised by anthropologists for:

> over-emphasizing symptoms, with depersonalized 'seropositives' which are seen to be typically 'prostitutes' or 'promiscuous people', members of so-called 'high risk groups,' or 'core transmitters,' or 'control populations,' all epidemiological equivalents, linked to 'reservoirs of infection'" (30).

In opposition to such essentialist understandings, Schoepf and others argue that "there are no empirically bounded 'risk groups'", it is instead the behaviour of unprotected sex, rather than a particular kind of relationship that puts people at risk (27, 31, 23). The categorization of empirically discrete risk groups was further undermined by anthropological research which emphasized the poor definitions of such groups, and the stigmatization which the earlier understanding of risk groups engendered. Moving away from the "trap of restricting our research to identified high-risk groups," anthropologists have been important in shifting the debate to the more useful concepts of 'vulnerable groups' and 'risk behaviours,' concepts which recognize that everyone is vulnerable to infection (32).

World Health Report 2004, which focused exclusively on HIV/AIDS, continued to employ the original understanding of risk groups, arguing that prevalence is higher among "people at higher levels of risk - sex workers, injecting drug users, men who have sex with men - and their sexual partners" (33). UNAIDS, by contrast, has taken up the discourse of vulnerable populations, which includes women and youth along with the original members of the high-risk groups. UNAIDS argues that "HIV/AIDS epidemics in many countries are concentrated in specific populations that are often marginalized and vulnerable to a broad range of health and psychosocial difficulties apart from, or in addition to, HIV/AIDS" (34). An important implication for prevention strategies is that as "AIDS thrives on exclusion ... including vulnerable people in all available responses is a way of increasing society's total resistance to the epidemic" (34). Building on the more recent understanding of vulnerable populations, we will now turn to the role of anthropology in understanding the gender dimension of the HIV/AIDS epidemic.

GENDER AND HIV/AIDS

Both traditional and PE anthropologists have made important contributions to our understanding of the gender dimension of the epidemic. As with all 'high risk groups,' women were implicitly blamed by the

traditional understanding of the epidemic for spreading the disease (29). This is a problem which has not gone away. O'Neil warns that "[e]thnographic and epidemiological research has the potential for blaming and further stigmatizing women, if the research focuses exclusively on female sex workers as 'vectors'" (35). This has been observed in the case of women in Costa Rica, where prostitutes are "portrayed as the vectors, rather than agents/subjects/victims of disease" (36). In Northern Tanzania, Dilger interviewed informants who expressed feeling that women, whether married or unmarried, are 'greedy' for money and, therefore, have fast-changing sexual relations thatcan result in disease transmission (37).

Both the 'promiscuity' and 'vulnerability' of female sex workers have been singled out. However, sex workers are not unique in their problems pertaining to sexual negotiations. Anthropological studies in Southern Africa indicate that women, in general, are relatively powerless in sexual negotiations with men (38). Risk situations are omnipresent for women (39). Akeroyd links the sexual abuse of women to "cultural assumptions about relations between men and women and the subordinate (personal and often legal) status of women (8). Paradoxically, risks for young women increased as AIDS consciousness spread and men began to seek very young partners whom they assumed to be free of infection (4).

Rather than seeing women as vectors, a structural violence perspective allowed us to further understand their deep vulnerability due to economic, social and physical factors. Over a decade ago, *World Development Report 1993* recognized that "[p]reventative efforts addressed to women, especially those of childbearing age, can protect both maternal and child health" (28). *World Health Report 2004* asserted that women are already facing severe hardships resulting from "inequality, discrimination and victimization, and HIV/AIDS often exacerbates the hardships" (33).

Women often lack the agency to escape their vulnerability, predominantly because they are poor. It is poor women who are most susceptible to HIV infections, for gender alone does not define risk (18). Higher levels of female poverty is thus another compounding risk factor which has been identified (40, 41). The World Bank has emphasized that it "is important not simply to provide information on condoms but also to ensure their availability and to empower members of the core group, especially female sex workers, to use them" (28). UNAIDS similarly argues that the root causes of female vulnerability - their legal, social and economic disadvantages - must be addressed (2). Ultimately, this type of empowerment will require a reduction of poverty, but in the interim, anthropologists have identified a number of important behaviour change strategies.

BEHAVIOUR CHANGES AND STRATEGIES

Diverse and 'factually incorrect' understandings of the HIV/AIDS epidemic has made prevention an often insurmountable challenge in Africa. As UNAIDS argues, educational programmes need to take account of traditional belief and value systems, as well as popular mythologies that circulate amongst the population (34). Anthropologists have helped ensure that education campaigns are, as far as possible, culturally appropriate. However, in the absence of widespread access to treatment, two major behaviour-change strategies which had little regard for local cultures have been employed: advocating abstinence/monogamy and promoting condom use (4, 5, 42). Anthropologists recognized that both strategies face huge practical difficulties.

The idea that knowledge of risk does not necessarily translate into behaviour change is "as much a truism in public health as is the awareness in anthropology that what people say is no clear guide to what they do" (15, 43). Indeed, as HIV/AIDS continues to spread rapidly in Africa, one of the most difficult issues is the apparent disparity between people's knowledge and awareness of HIV/AIDS and the extent to which they take measures to protect themselves (15, 10). The policy implication of this disparity is that education about risk of infection is not sufficient as cultural determinants of health behaviours serve as important barriers to health behaviour change (31).

In the realm of behaviour change, few changes have faced more socio-cultural, economic, political and religious barriers than condom use. UNAIDS declares that "[c]ondoms are key to preventing the spread of HIV/AIDS" (34). Smith argues that while one may simply ask whether people have access to condoms, a more sophisticated manner of asking this question requires attention to issues of how sexual relations and condom use are negotiated within contexts of poverty, age and gender inequality, and other configurations of power that influence people's priorities and constrain their choices (15). Lyons identifies attitudes towards condom use in Uganda as ranging from 'condoms are not African,' 'condoms will promote promiscuity and moral lassitude,' 'condoms are a ploy to control our population size,' 'condoms kill women,' 'condoms are evil' to 'condoms will hinder the reconstruction of Uganda'" (29).

The most prominent barriers to condom use cited by traditional anthropological research are grounded in cultural norms. Setel's observation is representative: for many men and women, "the very definition of sex was to ejaculate into a women or to receive a man's sperm; using a condom was said to be 'dirtying oneself'" (44).

Much anthropological research has observed that men in Africa frequently attach great importance to the notion of flesh-to-flesh sex, citing condoms for removing intimacy (37, 39). Smith recounts that "[m]any young people told me that suggesting condom use as protection from HIV/AIDS would be very difficult because it would imply either that one suspected one's partner was a carrier (or the kind of immoral person who could be a carrier) or that one's own sexual behaviour was sordid and risky" (15).

Invoking structural violence, Collins and Rau dismiss culture and argue that "[p]eople whose livelihood strategies expose them to a high risk of infection are, precisely because they are impoverished, less likely to take seriously...the threat of an infection that is fatal years from now" (p. 15). Others emphasize that risk-taking behaviour is not solely an individual matter: it is caused ultimately by social and economic factors, and "influencing the underlying causes of the epidemic will do much more to control the spread of HIV infection than the best education or counselling programmes" (8). Education is important, but heeding and being able to act on advice are complex matters often beyond the control of an individual (Ibid). A number of anthropologists have recognized that the ultimate barrier to condom use is poverty. This is the case not only because of the direct costs of condoms (34, 39), but because of the broader culture of education, risk-taking and self-preservation (15).

CONCLUSION

This paper has identified the major themes of knowledge anthropologists have contributed to our understanding of HIV/AIDS. From anthropological inquiry into sexuality in the African context, has come an awareness that individual choices and cultural norms encouraging 'promiscuity' cannot be exclusively blamed for spreading the epidemic. Our understanding of sexuality has also been deepened by embedding such behaviour in its political and economic context.

Regarding risk groups, anthropologists have been instrumental in shifting the discourse from empirically bounded 'risk groups' to more nuanced understandings of 'vulnerable groups' and 'risk behaviours.' We see in this sphere of knowledge the marriage between traditional anthropological analysis of behaviour with the PE anthropologists' emphasis of structures of risk and vulnerability. As with the risk group conceptualizations, the emphasis in the debate on HIV/AIDS and gender has shifted from women as 'vectors' to women as a vulnerable group.

Traditional anthropologists have ensured that behaviour change strategies accurately and sufficiently take into account the local culture. This has been especially important in terms of promoting behaviour change such as condom use, where cultural understandings such as the importance attached to flesh-to-flesh sex in certain African communities. The PE anthropologists have added, however that no amount of 'education' is enough, due to structural factors constraining and shaping people's behaviour. We can expect anthropologists to make important contributions to new debates surrounding male circumcision, which has been deemed efficacious at reducing HIV transmission, as well as microbicides, which may be shown to be efficacious in the near future (45).

In closing, it is not appropriate for the medical community to doubt the contributions of anthropology to the public health understanding of, and limited successes in the fight against, the HIV/AIDS epidemic. Setel cautions that "the formal health care sector can only add its voice to a social and cultural environment that already has its own very powerful epistemology of AIDS" (44). Thus broader social change grounded in anthropology is invaluable. Castro calls for anthropologists to act as 'advocates' for HIV patients and the poor generally (46). It is precisely through the synthesis of their traditional tools with a broader understanding of structural violence that anthropologists act, in conjunction with health care professionals, as advocates for HIV/AIDS patients.

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CROSSROADS

Elective advice

Shane O'Hanlon

INTRODUCTION

Going on a medical elective is an exciting opportunity. No matter where the destination, for most people it is an intensely rewarding experience, and the memories last a lifetime. However poor preparation can impinge on an otherwise productive elective, leading to lost opportunities. A little time spent considering your options will be time well spent.

THINGS TO CONSIDER

If this is your first time going on elective, you should talk to someone who already has some experience. Ask around your medical school or hospital. Ideally you should try to find someone who has already been to the hospital or project you are interested in.

Going in a group is easier than going alone. It can be an intense experience, and it is comforting to have someone you can relate to. It also helps when it comes to getting to know people when you arrive. Going it alone is the more challenging option, but can be a more interesting experience. Remember though that in remote areas in some countries it can be almost impossible to contact family and friends.

See if there are any local organizations which might provide sponsorship. Start your own volunteer group if you have the time. Choose a project which suits you, and do some research before you go. Many people decide to visit a developing country - it's not often that you have the chance to experience another culture while also providing benefits for the place you visit. Find travel information about the country, and get an idea of how the health system works there. Decide what skills you have to offer, and what time period you have free.

ORGANIZING THE TRIP

Make a shortlist of your preferred volunteer opportunities. Email or write the contact person, with any questions you have, and the dates you intend to go. Tell them how many are in your group, and what skills you can offer. If you are accepted in one placement, don't forget to let the others know you won't be coming. Once you have a confirmation, book your flights as soon as possible. If you are a student, try for student deals. For any obscure destinations, ask the contact person if they know which airlines fly there, or go to a specialized travel agent. As always the Internet is a good place to search for cheap airfares!

In the developing world, many hospitals are in financial difficulty, so any aid you can provide may literally be life-saving. Write to local businesses, medical organizations, or your local political representative for donations. Do not forget to hit up the other staff members in your local hospital/clinic who may have worked in the developing world in the past. Organize fund-raising events (coffee mornings, bag-packing, raffles, pub quizzes), and ask your local hospital if they have any equipment to spare. Bear in mind that some of the archaic stuff may not be suitable, while the stuff you think is useless may actually be a godsend... it is difficult to estimate this yourself but do make a serious effort to establish the level at which your hospital functions.

Pharmacies and GPs will also donate left-over or sample medications. You may receive some that are past sell-by date, but check with your contact person if these are useful. Unfortunately some hospitals charge patients even for donated drugs and this can be upsetting for volunteers who brought the drugs in good faith, not expecting them to be beyond their targeted patients' means... Again, check before you go.

Do not forget to see what the visa requirements are for your destination. Contact the embassy if you are unsure. Make copies of your passport, tickets, and other travel documents, and strongly consider travel insurance (if you're off to a remote destination check if you need extra cover in case you need the local flying

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doctors service, as this is not covered in many insurance policies).

Finally, try to plan some time at the end of your placement to relax, and see the local area.

WHAT TO PACK

Packing is an important consideration when planning your trip. Keeping your baggage weight to a minimum is vital, but you have to ensure to pack suitable clothes for the climate, and to bring work clothes too. Scrubs are essential - they are comfortable, cool in warm climates, and you can always leave them as a donation. Other essentials include: toiletries, basic medications, insect repellent, mosquito nets, washing powder, penknife, books...

Many volunteers bring useful medical equipment or medications, which are difficult to find locally. Unfortunately problems can be encountered at customs, so care should be taken. A letter from a head of your faculty, or other authority, stating that you are a volunteer with humanitarian supplies can help. Keep medications separate from other supplies, so that if they are confiscated you can keep the rest. An itemized list of everything you have is often required by the airlines. Also check if import tax must be paid on arrival - the embassy will advise you. Do not forget to ask for extra baggage allowance from the airline - they are usually very helpful, and this means more space for donations.

On the subject of books - a few compact ones are a great idea, especially if there is not much backup. They are also perfect as donations. Drug formularies are often requested, and other practical books like emergency manuals, or surgical atlases can be indispensable. Dental manuals and physiotherapy guides are also a possibility - check with your contact person to see what they need.

PROBLEMS

Once you get through customs, the next challenge is reaching your local destination. Transport is often far less comfortable and reliable than at home, but can be lots of fun! You may have to squeeze into packed minibuses with chickens on your lap, or negotiate at length with taxi-drivers for a reasonable fare. It can take hours to travel a few miles, so be patient.

Once you start work, it may not be what you expect. Remember you should try to fit in with the established schedules and practices, no matter how frustrating it might be! Always ask for help when in trouble, and if you have difficulties with staff members, talk it out. Stubbornness can be detrimental to patient care.

Living accommodations are usually very basic, and food may be a shock to the system. Remind yourself that you should try to adapt, and that it's not forever! A few home comforts, or high-energy food can help to ease the burden.

SAFETY

Always consider safety as your number one priority. While volunteers are usually welcomed everywhere, you may be targeted simply for being a foreigner. The worst time is usually on arrival, when you are getting orientated. Keep an eye on all your bags, and do not delay. You may receive many offers for help - politely refuse if you do not need it. For women, offers of marriage are common in some countries, but flashing a ring stops further solicitation! Take advice if you are a woman travelling alone.

Your safety while working is also important. If you are coming into contact with transmissible diseases, you should use preventive measures. Universal precautions are, as always, the rule. HIV prophylaxis packs are available, though costly. Note that policies regarding disposal of needles may not be the same as you are used to - be cautious and do not bring anything unwanted home with you. Your travel/health insurance may not cover you in this situation.

If you are going to a risky area, check the status before you go, and be aware of no-go areas. This information is usually available on government websites.

CONCLUSION

Going on an elective can be a once in a lifetime experience, so enjoy it! The vast majority of people come back relaxed, refreshed but also educated and fulfilled. If it works out, do not forget to pass on your tips!

Dr. Shane O'Hanlon (MB, BCh, BAO, BMedSci, MRCPI) is a specialist registrar in geriatric medicine at the Cork University Hospital in Ireland. As a student he went on electives to Africa, the Caribbean, France and Spain. This article gives some advice on how to go about organizing your elective, no matter what the destination.

CROSSROADS

An aide mémoire: working on acute medical assessment units

Naseem Naqvi*

Acute medicine is an emerging speciality that has now been approved by most postgraduate education and training boards as a distinct branch of medicine concerned with the immediate and early management of acutely ill patients. Acute medical assessment units are an interface between emergency medicine and in-patient care, and are the front door of hospitals. Working on these units is exciting as well as challenging. With my background of working on these units in National Health Service in the UK, readers, especially junior doctors, will find the article useful if they are intending to pursue a career in acute medicine.

Acute medical assessment units have rapid turn-over of patients; it is essential for staff and doctors working on these units to manage their time effectively, prioritise clinical needs, and learn to multitask to avoid redundancy and duplication of work. It is also vital to acknowledge and appreciate the contribution of your colleagues to boost up their morale and team spirit.

Information handling is an important area of acute care which is often mismanaged. Work loads and time constraints often prevent us from harmonizing in patient care. Firstly, it is essential to be fully aware of the trust protocols and local guidelines. It is always helpful to search for old case notes and to look up electronic patient case records if available. One should not hesitate to ring up the GP or nursing homes, as we may sometimes get valuable information which may not be readily available from patients, especially if they are demented, blind or deaf. Furthermore, when encountering a language barrier with a patient, it is worth considering the Patient Language Help Line (NHS Language line in UK).

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Since acute medicine has been introduced as a distinct discipline, there are concerns regarding the education, training, career pathways, and job plans of doctors who are drawn to this field, as well as the need for an academic base to ensure teaching and research of the highest quality. In most countries including the UK, curriculum will run from Foundation years to acquisition of CCST (certificate of completion of specialist training). For most junior doctors rotating through various medical specialities during their SHO/ Residency years, a work experience on acute admissions units is their only opportunity to learn and practice acute general medicine. Also, these are the best places to learn practical procedural skills: central venous catheter line placement, intercostal drain placement, lumbar punctures, temporary pacings etc. An expertise in these procedures is an essential skill in the armoury of a physician intending to pursue his career in acute medicine.

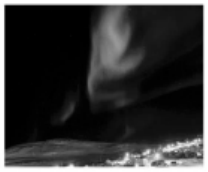
Acute health care is complex to manage; patients now seek assurance that doctors remain safe throughout their practicing lives and rising expectations are a mark of our success. The concept of safety-netting holds its vital importance when it comes to working on acute medical assessment units. It is imperative that one should know and realize his/her limitations and ask for a senior's help when one feels out of breadth. We must not hesitate to contact the consultant or Senior Registrar on call if any doubts regarding the management plan. Also, it is worth contacting specialist centres for advice if you work in a small district general hospital.

There has been much activity and growth in acute medicine as a speciality. For acute physicians, the challenge over the last decade has been to develop strategies to cope with the increasing demands of accountability in acute care. While on the other hand, there is a constant need to examine the education and training of healthcare professionals to ensure the delivery of patient-focused quality of care.

Aide Mémoire: important points to keep in mind

- Make sure that the medical staffing and human resources are aware of your on-call swaps
- Page yourself before starting on-call duty to double check that your pager is working
- Try to present as many cases as possible to the on-call consultant; it will improve your case summarising skills, which are invaluable for passing clinical examinations.
- Always look for any potentially treatable cause of confusion in the elderly before labelling them as demented
- The medical assessment unit is probably the best place to consolidate your communication skills with the patients and relatives, and to involve them at every stage of the management plan.
- Try not to discuss about patients and their illness when having your cup of tea during your breaks
- Think twice before sending a patient home from the medical assessment unit. Verify that you are not missing any serious underlying disease. If unsure, detain and always asks yourself: "what if?"
- Keep a diary/PDA with you to make note of any interesting cases or rare disease you have seen.
- Make every effort to publish rare and/or interesting casse in the case reports section of medical journals. It will be a positive addition to your CV and will help when you start applying for sought-after senior grade career positions
- The diagnosis often comes out of the patient's mouth; listen to them and focus on non-verbal clues
- Always think of common and potentially treatable illnesses when considering a diagnosis, but remember: rare is rare, but rare is always there

Naseem Naqvi (MBBS, MRCP 2) is second-year speciality trainee in acute medicine at the Aintree University Hospitals NHS Trust in the UK and a member of the Society for Acute Medicine.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Gerald M. Fried MD, FRCSC FACS, Heather Gill MD

Interventional Radiology: from idea to device to patient
Anthony Ciarallo MSc, Cam-Tu Emilie Nguyen, Lawrence Stein MD, FRCPC

Teaching technical skills using medical simulation: a new frontier
Kevin Lachapelle MD, MSc, FRCSC

Interventional Cardiology: the future beyond the coronaries
Eric Horlick MD, FRCPC

FOCUS REVIEW

Surgery through the keyhole: a new view of an old art

Gerald M. Fried, Heather Gill

Surgery is defined as the branch of medicine concerned with the treatment of disease, injury, and deformity by operation or manipulation. (*Stedman's Medical Dictionary*, 28th edition).

Surgery, like medicine in general, is a dynamic entity that changes and evolves with time. Surgeons themselves are a product of the society in which they live, and thus, the operations they perform are also a reflection of the times. What is interesting about the history of surgical practice is how it has managed to mold and change while still maintaining its core principles.

Classically, surgical procedures can be divided into four main categories: incisional, exemplified by the drainage of an abscess or the release of a carpel tunnel compression; excisional, utilized in most major cancer operations, from colectomies to lobectomies and gastrectomies; reparational, seen in the repair of an inguinal hernia or the re-implantation of a digit; and finally replacement, a technique used in transplantation and joint surgery. Despite our vast advances, these categories hold true to this day.

Another area of surgery that remains stable is that every operation has both risks and benefits. The benefits, which have not changed significantly with time, arise from the durable or permanent treatment of a condition and or the avoidance of the need to take chronic medication that may have potential side effects. The results of surgery are usually immediately obvious to both the patient and the treating surgeon. The risks on the other hand, are where the creative surgeon has the most power to affect change.

Until recently, a major risk or downside to surgery was related to the incision. The incision needs to be sufficiently large for the surgeon to palpate the tissues with his hands or instruments as well as to obtain adequate illumination of the internal structures. The deeper the target structure, the more shadowing there is, and generally the larger the incision has to be for adequate exposure. The cost of a large incision may range from immediate pain to a prolonged period of recovery before the tissues may be fully functional. Surgery thus may result in deformity or cosmetic scarring and there is often a substantial period before one is able to return to usual activities. Finally, another consideration regarding surgery is that surgery requires considerable technical skill, and there is a potential for large variation between outcomes of the identical procedure performed by different treating surgeons. Thus surgical outcomes may be less predictable than those related to pharmaceutical treatments.

Fortunately, surgery has evolved at an incredible rate, particularly over the past 30 or 40 years. Advances in imaging, electronics, and optics combined with an innovative spirit have led to vast changes. With the introduction of open-heart surgery, total parenteral nutrition, transplantation, biomaterials, minimally invasive and robotic surgery, surgery as a discipline has evolved in leaps and bounds. James C. Thompson, M.D., one of the giants of surgery over the past 25 years once said, "Without research, the surgery of today would be the surgery of yesterday, and the surgery of tomorrow would be the surgery of today" (personal communication). This spirit sums up the determination of academic surgeons to provide the benefits of durable and effective surgical care without the costs associated with the incision. As Yogi Berra has often been quoted as saying, "The future ain't what it used to be".

One of the most dramatic advances in surgical care is the development of minimally invasive surgery (MIS), also known as laparoscopy, arthroscopy, thoracoscopy, or minimal access surgery. MIS involves image-guided surgery. This utilizes small diameter telescopes connected to a miniature video camera to provide

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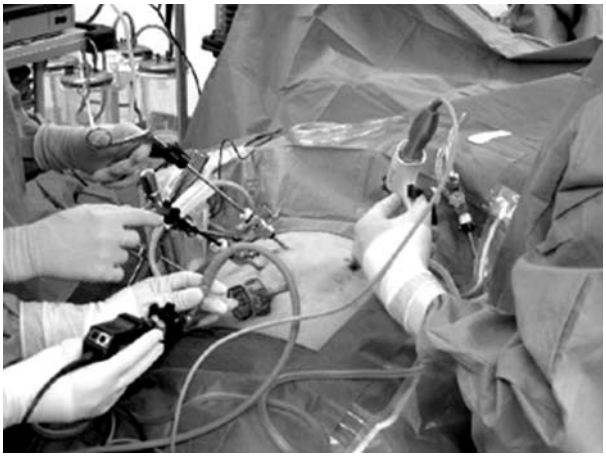

Figure 1. Minimally Invasive Surgery: The surgeon is manipulating instruments placed through ports (trocars) in the abdominal wall. The internal organs and instrument tips cannot be seen directly, but can be viewed on a monitor that displays the video image created by an endoscopic camera.

magnified, bright and shadow free visualization of internal structures. Instruments placed through small puncture-size incisions access the target organ without the need for a large incision (Figure 1). In order to create a working space, carbon dioxide is pumped into the cavity of choice using an electronic insufflator to a specified pressure, usually between 12 and 15 mmHg. In most relaxed and compliant abdominal cavities this allows an space of between 3 and 6 liters to be created, and allows one to visualize the abdomen quite clearly. The benefit of MIS is less pain, nearly invisible scars, and a markedly accelerated recovery. This is a dramatic improvement on the previous risks associated with most surgical operations. Many operations that required 7 to 14 days of hospitalization and several weeks of post hospital recovery in the past are now done as outpatient procedures, i.e. the patient is discharged within a few hours of surgery and is able to resume normal activities often within a week. MIS techniques have been applied in a large number of procedures in general surgical care. The most common is its use in gallbladder surgery. Today ~ 90% or more of elective cholecystectomies are carried out using laparoscopic techniques (1,2). MIS has also been applied to hernia surgery, colorectal surgery, repair of hiatus hernias, anti reflux surgery as well as radical prostatectomies, donor nephrectomies and a large number of other procedures (3).

However, MIS poses some very real challenges to the surgeon. First, internal structures are usually visualized through a two dimensional optical system which diminishes depth perception. The surgeon must acquire new cues to know where he or she is in three-dimensional space. As well, the tissues being operated on are accessed by long instruments interposed between the surgeon's hands and the target tissue, thus resulting in decreased tactile sensation and amplification of any tremor. Furthermore, trocars must be placed through the body wall to allow passage of instruments toward the target tissue. Because the instruments must be passed through these trocars, there is a limited range of motion of the surgeon's instruments and there is a lever like relationship whereby the surgeon's hands move in a direction 180 degrees opposite to the motion of the tip of the instrument (Figure 2). A fourth difference arises in the way a tissue appears during MIS. The endoscope provides a markedly sharper and magnified view of the target compared to what could be seen with the naked eye. When visualizing internal structures through an endoscope, a certain focal distance must be present to separate the tip of the scope from the target organs. Without this minimal focal distance red out occurs, and the lens may become coated with blood or mucous impairing the view. As evidenced by all these differences, MIS requires very unique skills that are not necessarily transferable from those skills acquired during traditional or open surgery.

A second congruent and equally important change in surgical practice is that simulators have been developed to teach surgeons in training, and surgeons wishing to learn minimal access techniques, how to use their instruments and a monocular optical system effectively. The McGill Inanimate System for Training and Evaluation of Laparoscopic Skills (MISTELS) is one of the most widely used such simulators internationally (4-7). Students are taken through a series of exercises in a trainer box under monocular optical guidance and their performance is measured according to efficiency and precision. Standards have been developed such that proficiency in the MISTELS system is highly predictive of a surgeon's skill in the operating room.

This concept of proficiency-based training is an important new paradigm in surgical education. It is based on the premise that surgeons learn better once they develop the fundamental skills outside of the stressful operating room. They can then apply their knowledge and skill to the surgical care of their patients in a much more productive and less stressful situation once they have the opportunity to assist in the operating room.

While advancement is important, it needs to be done in a controlled manner and the ultimate goal of surgical innovation should be to apply new technology to benefit the patient, to introduce innovation safely, and to ensure the competence of those adopting innovative techniques in their surgical practice. The use of simulation-based training is a better process than that of the traditional model of graded responsibility, and simulation-based training can be thought of as complimentary to on the job learning. A battery of physical and virtual reality

simulators are available to McGill medical students and residents to acquire that skill set necessary to perform this complex new type of surgery.

Another recent achievement is the concomitant use of other imaging modalities to further enhance the surgeon's capabilities, without requiring larger incisions or increasing the risks. At the surgeon's disposal are technologies such as ultrasound, fluoroscopy, and views via flexible endoscopes passed through natural orifices such as the mouth or rectum. By combining traditional laparoscopic visualization with these other modalities surgeons have an opportunity to see more than the surface of their target. In exchange for the loss of direct touch and the sensation of texture, a tissue's density and its characteristics can be evaluated by three-dimensional imaging such as ultrasound in conjunction with the surface view provided by the scopes.

Along with advancement in surgical technique, evolution in the surrounding conditions have helped to push this revolution even further. We have learned a great deal about creating an enabling environment for MIS. A surgeon requires multiple images of the surgical field. The use of multiple flat panel monitors and plasma displays allows a surgeon to have access to laparoscopic images, flexible endoscopic images as well as radiologic images. In addition, preoperative imaging studies can be viewed as required, and the vital signs of the patient can be seen without the surgeon's eyes ever leaving the surgical field. Further, operating rooms designed with ergonomics in mind have resulted in enhanced technical capabilities, less fatigue, and safer procedures. We have recently designed integrated platforms for performing MIS procedures that are optimal both from the perspective of the surgeon as well as the rest of the medical team. The operating room can now be a source of rich educational material. Whatever is displayed on the monitors can be recorded on digital media, which can then be used for teaching purposes, to document surgical findings, or provide feedback to the radiologist who provided pre-operative diagnoses. A complete video-editing suite is available at McGill to provide enduring educational materials based on images acquired in the operating rooms. Additionally, a videoconferencing link between the MIS suites and the outside world provides an opportunity for two-way communication, intra operative consultation, and a wonderful opportunity for teaching.

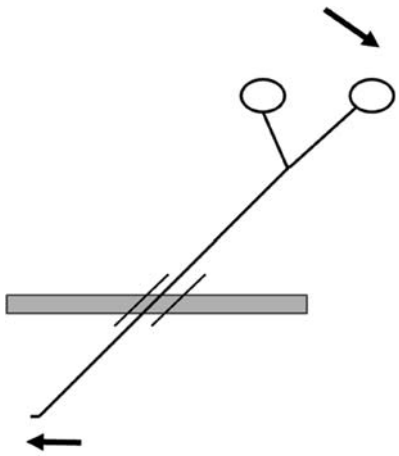

Figure 2. The Fulcrum Effect: The MIS instrument is inserted into the abdomen through a trocar. The trocar allows instruments to be inserted and withdrawn through a valve so that the positive intra-abdominal pressure can be maintained. To make the instrument tip go in one direction the handle of the instrument must be directed in the mirror-image direction.

In conclusion, MIS is an innovative application of surgical therapeutics that was developed to minimize patients' pain and suffering while improving surgical precision. Performing minimally invasive surgical procedures requires a new set of skills in addition to those required for traditional open surgery. The simulation center provides an environment to hone these skills and to verify the technical proficiency of surgeons before they apply MIS techniques to the care of their patients. Due to progress in engineering capabilities and optics, and as a result of surgical creativity, MIS principles are being applied to an increasing number of health care problems. The technology required to optimally incorporate MIS into clinical care requires a creative surgical environment that addresses issues of ergonomics and patient safety while providing the entire health care team access to a variety of digital data.

Looking to the future, an extension of MIS is robotic assisted surgery. This takes MIS a step further and the surgeon is physically detached from the patient. The surgeon sits at a console, which may be in the same room as the patient, or quite a distance away. By using his hands and feet the surgeon controls instruments that are being manipulated by a robot. Robotic surgery provides the capability of scaling, whereby the movement of the surgeon can be set to a particular ratio with respect to movement of the instruments inside the patient. Scaling allows a surgeon to produce very small and precise movements such as those required for microsurgery. The robot can also filter out human tremor, providing the potential to further improve the accuracy of very fine movements. Robotic technology is addressing the universal nursing shortage as well. A robotic scrub nurse, called Penelope (website: http://www.roboticsystech.com/), has been developed at Columbia University in New York and responds to voice commands from the surgeon with remarkable accuracy.

Surgery has dramatically evolved in the recent past as

a result of breakthroughs in engineering, optics, and the information age combined with human creativity and ingenuity. The benefits of durable and effective care with minimal pain and loss of productive time is the goal of every surgeon, and thus we continue to push surgical care further and further forward.

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FOCUS REVIEW

Interventional Radiology: From Idea to Device to Patient

Anthony Ciarallo, Cam-Tu Emilie Nguyen, Lawrence Stein

> "… the vascular catheter can be more than a tool for passive means for diagnostic observations: used with imagination it can become an important surgical instrument."(1)

This 1963 quote is from Dr. Charles Dotter, the man who is widely regarded as the father of interventional radiology. In addition to being used profusely in journals and textbooks of interventional medicine, this citation succeeds in capturing the essential elements that constitute modern interventional radiology (IR): firstly, the use of the words "vascular catheter" reaffirms IR as a technique-based specialty of medicine where instruments and new technologies play a crucial role; secondly, this quote foresees that angiographic catheterization will evolve from a purely diagnostic science towards a means of delivering treatment; lastly, the word "imagination" constitutes Dotter's appeal to physicians to find newer, more innovative ways of putting imaging and image-guided therapy at the service of the patient.

Over the past forty years, the scope of IR has grown incessantly to encompass all the major organ systems, while interventional radiologists continue to prove that they are among the most versatile and inventive medical specialists. Because they can be visualized precisely and instantly using modern imaging techniques and explored with increasingly sophisticated probes, blood vessels constitute ideal roads for interventionists to reach sites of pathology all over the human body. In the present article, we will try to illustrate some of the key procedures of IR through a rapid overview of angioplasty and embolotherapy. Moreover, the contribution of IR to patient management, treatment and care will be discussed through a case history.

ANGIOPLASTY

The first interventional procedure ever documented was a percutaneous transluminal angioplasty of the superficial femoral artery performed by Dr. Charles Dotter in 1964 on an 82-year-old woman with symptomatic leg ischemia and gangrene who declined amputation (2). The treatment was considered successful and percutaneous transluminal balloon angioplasty (PTA) has now evolved to become one of medicine's best known minimally invasive treatments. Using the example of peripheral vascular disease, we will see how interventional approaches can contribute to the salvage of an ischemic limb.

Patients with symptomatic peripheral vascular disease (PVD) of the lower extremities that is poorly controlled with pharmacological therapy and lifestyle changes are candidates for percutaneous or surgical revascularization. Lesions that are amenable to angioplasty of the lower extremities often have the following characteristics: short, concentric, noncalcified, solitary and nonocclusive (3). Conversely, PTA has limited benefit in patients with diffuse disease or a large, irregular atherosclerotic plaque, because of the increased risk of distal embolization (3). The choice of interventional rather than surgical treatment depends both on patient factors and disease characteristics and will not be further reviewed here.

After initiation of appropriate antiplatelet and anticoagulation therapy, and percutaneous arterial catherization using needle, guidewire and catheter, the balloon angioplasty catheter is advanced in the vessel harbouring the obstruction. Once it is positioned near the stenosis, a guidewire is used to traverse the lesion (3). The dimensions and mechanical characteristics of the balloon have previously been chosen according to the lesion and vessel being treated. The balloon is then inflated over the center of the stenosis using dilute contrast material with the goal of breaking the atherosclerotic plaque and increasing the luminal cross-sectional area (3). Moreover, catheter-delivered stents are mechanical devices that can be used to provide a rigid and permanent scaffold to the diseased blood

vessel segment, thereby maintain luminal patency and inhibiting remodelling, elastic recoil and neointimal hyperplasia, which are causes of restenosis (3). A wide variety of stents are now available on the market, including self-expanding, balloon-expandable and drug-eluting stents. The latter category of stents releases drugs such as sirolismus or paclitaxel which inhibit smooth muscle proliferation, with the objective of decreasing the incidence of restenosis (4). Their potential benefit in PVD is currently under study.

Other interventions aimed at restoring adequate blood flow include coronary artery angioplasty and stenting, carotid artery angioplasty and stenting to prevent ischemic stroke and catheter-directed enzymatic thrombolysis to remove blood clots.

EMBOLOTHERAPY

Transcatheter embolization or embolotherapy is commonly used by interventional specialists to treat vascular abnormalities (e.g., aneurysms, arteriovenous malformations, varicoceles), to eliminate unwanted tissue (e.g., neoplasms, uterine fibroids) and to control many different sources of bleeding.

For cases of acute gastrointestinal hemorrhage that do not resolve spontaneously and do not respond to fiberoptic endoscopic management, emergency angiographic intervention can be considered. The effectiveness of this method is greater when active bleeding is present, because the threshold for detection of hemorrhage using angiography is 0.5 ml/min (5). Therefore, angiographic intervention is indicated for patients with high transfusion requirements or signs of hemodynamic instability.

The primary goal of radiographic imaging is to locate the source of the hemorrhage. Visualization of contrast medium extravasation into the bowel is the only sure way to demonstrate that a blood vessel is leaking. Sometimes, specific angiographic findings can provide further clues about the etiology of the bleeding. When upper gastrointestinal bleeding is suspected, arteriographic evaluation of the celiac trunk, followed by the superior mesenteric artery is performed (5). For lower gastrointestinal bleeding, the superior mesenteric artery and the inferior mesenteric artery are assessed in this order (5). When no site of hemorrhage site is found, empiric embolization of the left gastric or gastroduodenal artery can sometimes halt a barely discernible bleed (5).

The rationale for using embolotherapy in acute gastrointestinal hemorrhage is that it is possible to selectively interrupt the blood flow to the bleeding artery while avoiding intestinal ischemia, because of the rich network of collaterals formed by the arterial arcades. Selective catheterization of the culprit vessel, the key step to successful embolotherapy, is now achieved using coaxial microcatheters inserted through the diagnostic catheter. “[D]istal embolization at the level of the vasa recta feeding the bleeding site” is the favoured technique, as it reduces to a minimum the length of bowel at risk of ischemia (3).

The choice of the embolic agent to be delivered through the end-hole catheter to the site of bleeding greatly depends on the accessibility of the vessel for selective catheterization, which in turn depends on the vascular anatomy. For instance, if the rupture site can be crossed, it is possible to perform the sandwich technique utilizing coils as embolic agents (5). Metallic coils placed both proximal and distal to the site of vessel rupture will mechanically obstruct blood flow as well as promote thrombosis, thus causing hemostasis. Furthermore, if the catheter cannot reach the lesion site, particulate embolization can be considered. The size of particles used is determined by the diameter of the vessel to occlude. The released particles are directed distally by the bloodstream and find themselves shunted towards the point of least resistance, usually the site of vessel breakage.

CASE REPORT: CESSATION OF A LOWER GASTROINTESTINAL HEMORRHAGE USING MICROCATHETER ANGIOEMBOLIZATION

Introduction

Lower gastrointestinal (GI) hemorrhage is a significant source of morbidity and mortality in the aging population (6). Diverticulosis, neoplasms and angiodysplasia are among the most common etiologies responsible for lower GI bleeding. Clinically, they share many similarities which render them virtually indistinguishable upon presentation. For this reason, the acuity of a GI hemorrhage ultimately determines the management. Small bleeds are mainly managed using a conservative medical approach. On the other hand, severe hemorrhages are handled mostly via interventional angiography, especially in hypotensive patients who need emergent treatment. Angiography provides an effective approach for localizing and embolizing the source of the GI bleed in a relatively short period of time with a minimal amount of preparation.

Angioembolization is establishing its place in the management of gastrointestinal hemorrhage largely due to the advent of microcatheter technology. Initially, its use was limited by procedural complications such as ischemic colitis and bowel infarction (7-9). The objective of the procedure is to adequately reduce the perfusion pressure in a hemorrhagic region such that it facilitates hemostasis without causing total

devascularization and infarction. Superselective embolization allows specific targeting of the distal marginal mesenteric arteries thereby achieving the desired degree of hemostasis within the affected territory while minimizing complications.

CASE REPORT

A 78 year-old male patient presented to emergency on the day of admission with diminished mental status and bradycardia of 38 bpm. His past medical history is significant for diabetes mellitus II, hypothermia secondary to autonomic dysfunction, anemia, chronic renal failure, and hypertension. A few days after admission, the patient was noted to have epistaxis with subsequent melena. His hemoglobin fell from 90 to 69g/l and he received two units of packed red blood cells. ENT was consulted and concluded that the epistaxis was unlikely to be the cause of the melena or the marked decline in hemoglobin. The esophagogastroduodenoscopy (EGD), performed to rule out an upper GI bleed, revealed multiple duodenal bulb ulcers but failed to demonstrate active bleeding.

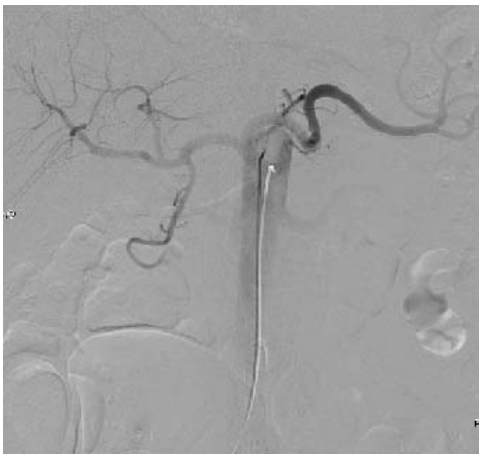

Figure 1. **Visualization of the celiac trunk**. The injection of contrast material demonstrates normal vascular integrity in the distribution of the celiac trunk.

The patient remained hemodynamically stable over the course of the following week until he passed new melena with fresh blood per rectum. His blood pressure dropped to 80/50 mm Hg and his hemoglobin level reached a nadir of 56g/l. The patient was transfused with a total of 6 units of pRBC, 10 units of platelets, and 2 units of fresh frozen plasma within the two days following the episode. A second EGD revealed no change from the previous. The patient was then sent for a colonoscopy in attempts to identify a source of bleeding from the lower GI tract. A very large amount of fresh blood was found in the rectum and sigmoid colon along with moderately severe diverticulosis in the sigmoid. The source of bleeding, however, could not be identified because the abundance of blood and clots precluded proper visualization of the intestinal mucosa. Therefore, the patient was sent for an emergent

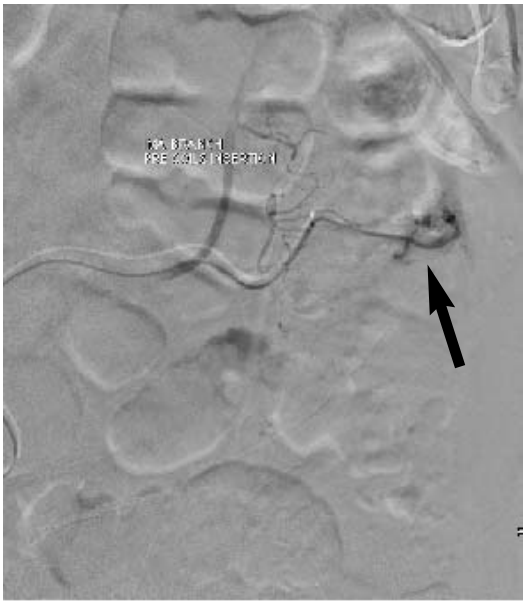

Figure 2. Left colic artery. The catheter was placed through the inferior mesenteric artery into the branch of the left colic. Injection of contrast material demonstrates extravasation into the lumen of the descending colon (arrow).

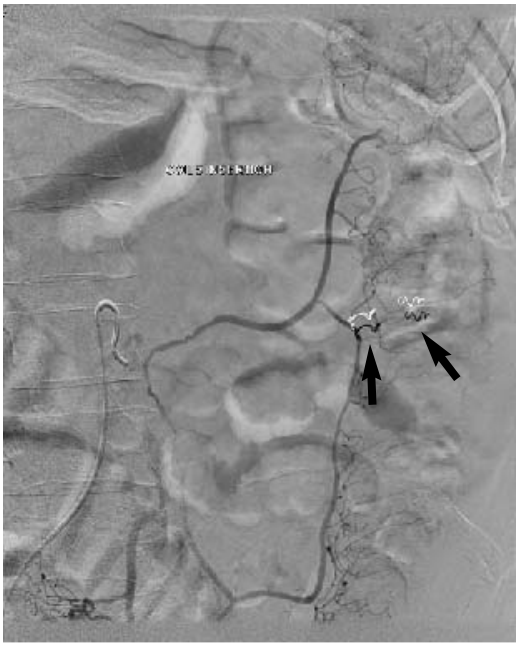

Figure 3. Coil insertion. The vascular distribution of the left colic artery is shown with contrast following placement of two 2mm by 4mm coils (arrows) in the affected marginal branch. The absence of extravasated contrast confirms that this was the source of the hemorrhage.

angiography.

Using the transfemoral approach, a 5F sheath was placed in the right femoral artery and 5F Cobra catheter was advanced to the origin of the celiac truck using a standard 0.035 in. Terumo guidewire. The celiac angiogram failed to show any abnormality in the gastroduodenal region (Figure 1). Catheterization of the inferior mesenteric artery demonstrated an extravasation of contrast material from the left colic artery into the colonic lumen indicating an active hemorrhage in the mid descending colon (Figure 2). A Renegade 3F microcatheter was negotiated through the notably tortuous terminal branches of the left colic artery. The affected branch was successfully embolized using two microcoils, 2mm in diameter and 4mm in length (Figure 3). The procedure was well tolerated by the patient despite suffering a right inguinal hematoma.

One week after the angioembolization, the patient underwent a repeat colonoscopy for the purpose of identifying a more specific source of bleeding. A complete colonoscopy was performed with visualization of the appendiceal aperture. With exception to diverticulosis of the sigmoid and descending colon, the examination was otherwise normal. During his admission at the hospital, the patient was subsequently diagnosed with a factor XII deficiency coagulopathy. Despite this, he has not experienced a recurrence of GI hemorrhage.

DISCUSSION

The inception of angioembolization in the 1970s was curtailed by its high complication rate. Bowel infarction was estimated to range from 13-33% and was largely attributed to embolization of proximal vessels (7-9). The catheter caliber was simply too large to selectively target distal mesenteric hemorrhages. Embolization of a proximal vessel involves a relatively large vascular territory and increases the risk of ischemic events due to inadequate collateral supply. Since then, the introduction of microcatheters has renewed interest in angioembolization. The improved precision of the procedure has been documented in numerous cases and the incidence of major ischemic complications has declined to 0-5.9% (10-15). It should be noted that the rate of overall complications is higher, but the vast majority are not clinically significant.

Angioembolization has been reported to effectively stop GI bleeding in 70-100% of patients (10, 12, 13, 16-20). However, the outcome of the procedure may depend on the etiology and location of the GI hemorrhage. It has been demonstrated that right-sided colonic bleeds are more likely to recur compared to those on the left side (15, 16). Incidentally, the observation that angiodysplasias are more common on the right side whereas diverticulosis is more common on the left may provide a reason as to why that is (21, 22). According to the natural history, angiodysplastic lesions have a propensity to rebleed 85% of the time versus 10-20% in diverticular disease (23-25). Moreover, the risk of recurrent bleeding following embolization is intuitively higher in patients with extensive or multifocal disease of the bowel since they are more likely to bleed from multiple sites.

CONCLUSION

Advances in microtechnology over the last 20 years have paved the way for superselective microcatheter embolization of end arteries. The management of lower gastrointestinal hemorrhage has especially benefitted from this achievement as it is becoming more widely used in various centers. This targeted approach has shown to reduce the risk of ischemic bowel complications and has resulted in improved safety as well as efficacy in the management of gastrointestinal hemorrhages.

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FOCUS REVIEW

Teaching technical skills using medical simulation: a new frontier

Kevin Lachapelle

As a medical student, I remember clearly the first time a surgeon gave me a needle driver and forceps and asked me to "sew" the fasciae of an abdominal incision. The closure was clumsy, tentative, and being left-handed, I could not easily open the driver which is designed for a right-handed person. The surgeon summarily removed the instruments from my hand and commented that it was good I wanted to be a surgeon because I could use the training! He said I needed to practice. This proved to be somewhat of a problem since I could not improve without being allowed to stumble through some portion of an operation.

Medical education and certainly the teaching of technical skills have essentially not evolved over the last 100 years. In the same period, our medical profession has exploded in terms of knowledge of disease, diagnostic tools, pharmacotherapy, and therapeutic interventions. However, we continue to teach clinical medicine through the apprenticeship model of "see one, do one, teach one". The way in which we learn is through the sheer volume of clinical exposure and practice on real patients.

The present health care system, with its emphasis on efficiency, time management, patient satisfaction, and outcome measures while delivering increasingly complex multidisciplinary care, can no longer rely solely on this model of clinical training. With further reduction in operating time, the "see one, do one, teach one" approach will no longer "cut it" for technical training. The problem is even more acute among junior house staff and medical students as they are seldom asked, allowed, or even required to perform technical skills during their training.

Teaching technical skills and interventions outside of the operating room using models and simulators specially intended for basic technical skills, may better prepare trainees for a positive and enriching educational experience in the technical or interventional platform. The recent advent of medical simulation centres such as the McGill Medical Simulation Centre is an attempt to improve the skills of all healthcare providers within a "patient safe" environment. The transfer of these newly acquired skills to the clinical arena is the ultimate goal. Osler brought the students to the bedside to learn first-hand from the patients. Simulation furthers this Oslerian doctrine by bringing the learner to the patient prepared, able, and engaged for the encounter.

The development of motor skills and the acquisition of technical skills are thought to occur in three stages according to Fitts and Posner. In the first stage (cognitive), the learner tries to understand and conceptualize the procedure (indications, contraindications, tools needed) and the mechanics of a technical skill and the steps involved. In the second stage (integrative), the learner translates the knowledge of the steps into action. Movements are clumsy and successes sporadic. In the third stage (autonomous), the learner, through practice, is able to perform in a competent manner the specific skill or procedure. The insertion of a central jugular line, which is taught at the Simulation Centre, highlights well how these three stages of learning frame teaching at the Centre. First, the learner must have knowledge about the procedure, the tools used, the appropriate anatomy, and an idea of the steps. A video is helpful. This preparatory phase is crucial and is known to accelerate learning. Second, the learner will actually use similar hospital equipment and attempt multiple times line placement on special task trainers using the same sterile technique used in the hospital setting. Feedback from instructors and peers is coupled with self-reflection. Appropriate feedback is the key to learning the right steps to avoid negative training. Third, (ideally) the learner schedules regular practice time on task trainers until the insertion is

performed to a level of proficiency as determined by a panel of experts. The learner, armed with a basic level of competency and some confidence, performs a jugular line insertion on a real patient, while the bulk of the problems and difficulties with this procedure where dealt with in the simulation centre. There are a number of potential advantages to this type of training. The learner can focus on more important issues in the care of the patient. The patient is treated by a competent individual and suffers fewer complications. The hospital may save money by an overall reduction in central line infection. A whole list of technical skills, especially basic ones, such as knot-tying, suturing, chest tube insertion, biopsy, etc, can be taught using this staged approach to learning, which can be done outside the clinical arena. Unfortunately, none of theoretical advantages have been proven.

Similarly, more complex procedures and ones that involve many steps such as laparoscopic cholecystectomy may be taught and learned by breaking down the whole procedure into its component parts or individual skill set. In order to perform a laparoscopic cholecystectomy, the learner must be able to insert instruments safely into the abdomen, obtain a 3-D perception from a 2-D image, grasp, move, cut tissue, and clip the appropriate arteries and ducts. Each of these component tasks needs mastery, and all potentially can be accomplished outside the operating room using basic models. As a further help, one can bring all the components together: laparoscopic simulators mimic, albeit at a reduced level of realism, the whole procedure. It is theoretically possible to perform your first procedure without ever having "practiced" on a patient. There are 29 randomized trials comparing traditional technical training to simulation-based training. Although these studies are plagued by low number of participants and difficulty in measuring appropriate outcomes, some have demonstrated the value of simulation. In a study of laparoscopic cholecystectomy among junior residents, those trained using simulators completed the procedure 30% faster and made 6 times fewer errors than those trained using traditional methods. Many more studies are needed which help guide teaching of simulation and the impact it will will have on learning and eventually, patient outcome.

One area of growing interest and research in simulation and skill acquisition is how to tell if the learner, after all this training, is now competent to treat patients? Until now, we have relied on our own gestalt as teachers by essentially observing learners working in a clinical environment. In the assessment of a particular skill, it is crucially important to know what to measure and ensure that the measure is valid and reliable. The identification of the important metrics is not easy. The time to completion of a task is frequently used and certainly an experienced operator will be faster than a novice, but when is it that speed is less of a factor? In the jugular line example, a speedy operator may finish the procedure quickly but this speed should not come at the expense of a complication such as a tension pneumothorax. In general, experts decide which measures are important and then rigorously test them on novices, competent operators, and experts. This will be laborious as each skill must be dissected, understood, and tagged with appropriate valid and reliable performance criteria. Dr Fried's group at McGill has been able to demonstrate through the MISTELS (McGill Inanimate System for Training and Evaluation of Laparoscopic Skills) program that simulation can appropriately assess laparoscopic skills. Using this paradigm, courses are given worldwide using these criteria of performance developed locally. The Toronto group use OSATS (Objective Structured Assessment of Technical Skills) in which the learner performs a standardized technical task under the observation of an expert.

The Fitts and Posner construct of motor skill acquisition may be well suited to developing competency using simulation training but will probably not lead to expertise. There is a difference in skill level required to place a jugular line in a thin, cooperative, stable patient who can lie in the Trendelenburg position versus a patient who is in full cardiac arrest and is having chest compressions. Ericsson has suggested that the difference between the competent individual and the expert is the amount of time devoted to deliberate practice. The term "deliberate practice" refers to highly focused training on defined tasks in which there is coaching or feedback and self-reflection. In comparing the average piano player and the concert pianist, the major difference is the amount of hours devoted to focused practice. The expert is born through tailored training and hours of repetition. However, the expert interventionalist is not only a technician. The patient–doctor interface is always in the forefront and, with increasingly complex patients and procedures, the treating physician must be an expert communicator, team player, and be able to decide the best treatment option for any individual patient from a number of different decision trees. Expert judgment becomes as important as expert technical ability in treating patients. The learner needs to acquire all to become an expert; knowledge, skill, and judgment. There is new interest in understanding how one gains judgment, and whether it can be taught.

While adapting these concepts to simulation-based medical education, it is apparent that the best way to use

this tool is to integrate it within a well-designed training curriculum, with simulation sessions that are learner and task specific. A layered approach to simulation with increasing complex situations over a course of training will complement the clinical exposure. For example, the first simulation-based training for jugular line insertion would be simply procedural; perform the line insertion on the inanimate task trainer. The second would be on a high fidelity mannequin which can react physiologically and unexpectedly to the procedure, mimicking real complications, a more involved situation where one must recognize the complication and treat it accordingly. The third would integrate performing the procedure on a high fidelity mannequin in the context of a multiple injury trauma victim attended to by a whole team of doctors, nurses, and therapists. This is a life-like situation where communication, prioritization, decision-making, teamwork, and technical skill all come to bear on the successful jugular line insertion and patient outcome. This type of training can also be applied to the maintenance of skills of professionals and to those all ready in practice who wish to learn new techniques and procedures.

Simulation is new to healthcare, and unlike aviation, we are just starting to learn how to use it. We are not yet experts in this new field. Through experience and research, we will understand how to best implement this important tool. It is safe to say that a new generation of doctors will be trained using simulation–based education and that a whole new group of medical educators will be born.

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FOCUS REVIEW

Interventional cardiology: the future beyond the coronaries

Eric Horlick

It was not long ago that I was a medical student at McGill. I graduated in the class of 1996.

When I started to do interventional work as a Cardiology Resident at McGill, we were well into the era of coronary intervention. Almost every balloon angioplasty was followed by the implantation of a stent, which had been shown to improve the immediate and long-term results of interventional therapy. Life was becoming easier, dual antiplatelet therapy had emerged and replaced heparin, dextran, and warfarin which were initially used to treat every patient with a stent to prevent acute thrombosis. Our equipment had improved with better, less bulky balloons that allowed for smaller caliber guiding catheters and arterial access. This allowed vascular complications to diminish significantly. Stents were now being manufactured attached to balloon catheters decreasing the risk of stent embolization. There were also a number of pharmacologic strategies including 2B3A inhibitors which significantly reduced morbidity in high risk patients.

It has been about 8 years since I first scrubbed for an angioplasty and much has changed. We now know quite a bit about patient selection, risk management, and the outcomes after coronary interventions. Regular stents are still widely in use but drug eluting stents are being implanted in great numbers. The albatross around the neck of the stent era of coronary intervention was in-stent restenosis, an aggressive healing response to the arterial injury which occurs with both balloon angioplasty and stent implantation. This process results in renarrowing of the stented segment over the course of the first 6 to 9 months of follow up. Restenosis generally presents as recurrent angina. The risk of restenosis is related primarily to the presence of diabetes, and the length and diameter of the stent implanted. The arrival of drug eluting stents has greatly diminished the risk of patients developing restenosis and thereby requiring repeat procedures and suffering recurrent symptoms. Drug eluting stents have encouraged a more aggressive percutaneous approach to the treatment of coronary artery disease in patients who would have previously been directed toward surgical revascularizton. When only bare metal stents were available, it was hard to justify pursuing an angioplasty that would almost certainly result in restensosis.

Drug eluting stents have been implicated in an increased risk of stent thrombosis (a much more deadly acute occlusion of a stented segment) late after the index procedure. Drugs are likely to delay endothelialization of the stents by blocking the intense healing response which causes restenosis. A prolonged duration of dual antiplatelet therapy with ASA and Clopidogrel, longer than the 3-6 months recommended in the initial trials of these therapies, has been suggested by most interventional practitioners and is thought to be protective. The most recent analyses have suggested no increased risk of drug eluting vs bare metal stents in up to 4 years of follow up after a coronary intervention. They have also shown no difference in the rate of death or death/ myocardial infarction in these 2 groups calling into question the cost effectiveness of drug eluting stents, which are three to four times the cost of bare metal stents. Despite the above, I am unaware of any cardiologist who would not want a DES implanted at the time of their own angioplasty.

Coronary intervention has been compared to coronary artery bypass surgery in many populations both with bare metal stents and with drug eluting stents. In

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summary, there is really no evidence of mortality benefit of one of these treatments versus the other. The angioplasty patients generally require repeat procedures versus the surgical patients. Despite an apparent advantage of surgery, most patients who have been given a full informed consent choose angioplasty for its less invasive properties and the shorter recovery times required.

A discussion of coronary disease could easily fill volumes with data, conjecture and opinion. Although coronary artery disease by far occupies the most time of any cardiac interventional program, many other interventions are currently being performed to treat cardiac disease and will be the focus of the remainder of this discussion.

Structural heart disease is an explosive area of cardiovascular interventional cardiology and can be alternatively defined as non-coronary cardiac intervention. Interventions in structural heart disease occupy increasing time at many scientific meetings and are seeing a rise in the number of practitioners carrying out these interventions.

ASD CLOSURE

The skills to perform structural procedures are based in pediatric cardiology and interventional radiology. By far the most common intervention is atrial septal defect closure. Patients born with a significant atrial septal defect go on to develop right ventricular dilatation and manifest symptoms or exercise intolerance and cardiac rhythm disturbances. As decades go by, the pulmonary pressure may rise to moderate levels. Symptoms worsen over time in relation to the stiffening of the left ventricle with age which promotes increased left to right flow through these defects. Probably the best marker of a significant defect is right ventricular enlargement. Surgery for atrial septal defects has been carried out since the 1950's. The catheter-based solutions came into common usage in the mid to late 1990's.

The method of closing any intracardiac defect with any device is similar. A device, usually with 2 discs or umbrellas is attached to a delivery cable. By virtue of the design or mechanical properties of the device it can be advanced through a narrow tube or catheter, usually less than 3 mm in diameter. The catheter is advanced across the defect and one side of the device is released. The catheter and exposed disc or umbrella are withdrawn until they make contact with the defect and then the other disk is exposed on the opposite side of the hole providing closure. The device can then be released from the delivery system. Using this method, atrial septal defects up to 40 mm in diameter can be closed.

Transcatheter ASD closure offers benefits over surgery. In an elegant study by Dhillon, decreased right ventricular total excursion and peak lengthening rate was noted in patients undergoing surgery but not in those undergoing transcatheter closure. It is thought that this effect was related to poor myocardial preservation in the operating room, selective damage to longitudinal muscle fibers which compose the majority of the right ventricle and poor temperature regulation of the right ventricle intraoperatively. In our institution catheter based ASD closure is the preferred method of ASD closure.

PFO CLOSURE

About thirty percent of the population has a residual embryologic defect known as a patent foramen ovale. This connection between the right and left atrium served to shunt blood between the right atrium and left during fetal life in utero. During that period, the lungs were filled with fluid and oxygenated maternal blood would bypass the right-sided circulation to reach the fetal organs. PFOs have been implicated in a number of disease states including cryptogenic stroke, migraine headache, platypnea orthodeoxia, altitude sickness, and obstructive sleep apnea. Cryptogenic stroke i.e. stroke in the absence of classical vascular or cardioembolic sources, is usually implicated when patients under the age of 55 years present with prolonged or permanent neurologic dysfunction. The mechanism has been attributed to paradoxical embolism through this residual embryologic intratrial communication in some patients. There are currently several large randomized trials underway comparing medical therapy in the form of ASA or warfarin and percutaneous device closure for the prevention of recurrent cryptogenic stroke. The jury is clearly out in this particular patient population although there is a suggestion from single centre experiences and meta-analyses that device closure may offer some protection from recurrent events. Our practice is to offer patients a complete stroke investigation including MRI/MRA of the brain, thrombophilia screening, holter monitoring, and transesophageal echocardiography when the etiology of a stroke is unclear. Those patients with cryptogenic stroke after full investigation will be offered entry into a randomized trial. Should they not be candidates, individual consideration of device closure will be offered after informed consent.

PDA CLOSURE

Patent ductus arteriosus (PDA) is another residual embryologic structure that can persist in adults. Large defects usually present in childhood with heart failure. A PDA in adults usually manifests as a continuous murmur; i.e. a murmur which passes through the second heart sound unchanged. It is usually heard best in the

left infraclavicular space and can be missed if not specifically listened for. The indication for PDA closure is the presence of an audible murmur and the reason for closure is to prevent endarteritis which results in a systemic bacteremeia. The transcatheter procedure is technically uncomplicated and involves crossing over the defect from the pulmonary artery and implanting either a coil or device in the ductus to promote thrombosis and occlusion.

PROCEDURAL IMAGING

The procedure for closure of ASDs, PFO and PDAs are well established and can usually be accomplished in well less than an hour. ASD closure procedures in our institution are performed with the use of fluoroscopy as well as intracardiac echo (ICE) guidance. Many sites around the world continue to close ASDs with patients under general anesthesia and transesophageal echo guidance. The advantages of intracardiac echo (a ten French or 3.33 mm diameter catheter) include: increased patient comfort, freedom from general anesthesia and the required recovery period, independence from busy echocardiographers, and unique views of the septum not available with TEE. Most importantly, the use of ICE requires and promotes the development of the echo skills of the operator who is ultimately responsible. Knowledge of echo anatomy allows translation to other structural heart disease interventions. PFO closure is generally performed with the use of fluoroscopy alone as is PDA closure.

There are a number of devices available for intracardiac use each with their own advantages and disadvantages. Probably the most relevant factor in device selection is operator comfort and knowledge of the system in question. There are particular anatomic situations where one device's features are clearly advantageous, arguing for operators to have a working knowledge of multiple devices or systems. Low volume operators in structural heart disease intervention are clearly a liability and while the optimal number of annual procedures is not well defined, it is quite clear that high volume operators are likely to have better outcomes and broader experience than low volume operators.

OTHER INTERVENTIONS

While the three lesions described above constitute the bulk of adult structural heart disease practice, there are countless lesions that require treatment in this patient population on a less frequent basis. They include stent placement for coarctation of the aorta, stenting of stenotic pulmonary arteries, pulmonary or systemic veins, surgically created baffles used to treat transposition of the great arteries, or conduits from the ventricles to the great arteries to bypass inoperable subvalvular obstruction. We are often called upon to occlude venous or arterial collateral vessels from the aorta or great veins as well as pulmonary arteriovenous malformations. Coronary fistula closure is a regularly performed procedure. Pulmonary and aortic balloon valvuloplasty are not infrequently performed procedures for stenotic valves. With experience in device use and sophisticated techniques, closure of perivalvular leaks around mechanical valves, aortoatrial fistulas, aortic pseudoaneurysms, and ventricular septal defects of both congenital and post myocardial infarction etiology is possible.

TRANSCATHETER VALVE THERAPIES

Whereas as recently as five years ago, catheter-based treatment of valvular heart disease consisted only of balloon valvuloplasty, our present armamentarium of therapies allows treatment of pulmonary, aortic and mitral valve disease.

TRANSCATHETER PULMONARY VALVE REPLACEMENT

The earliest clinical experience with percutaneous heart valve therapy was for the treatment of pulmonary regurgitation. In certain patients with congenital heart disease, particularly those with Tetralogy of Fallot, a valved conduit from the right ventricle has been used as part of the definitive repair in patients with inoperable right ventricular outflow tract disease. Unfortunately, the natural history of these conduits is one of progressive regurgitation or stenosis or both. Conduit regurgitation/stenosis leads to right ventricular dilation and dysfunction, resulting in progressive symptoms of exercise intolerance, arrhythmia and right sided heart failure. Philip Bonhoeffer pioneered a technique which involved harvesting a bovine jugular venous valve and suturing it into a stent that could be placed into a surgical conduit to eliminate conduit regurgitation and improve stenosis. This therapy has the advantage of increasing the time between major cardiac operations in children with certain forms of congenital heart disease. The Medtronic Melody™ pulmonary valve is the product of this endeavour and has been implanted in well over 200 patients. I was fortunate to implant the first of these valves in North America with Dr. Lee Benson at the Hospital for Sick Children several years ago.

The challenge at present is to be able to offer percutaneous pulmonary valve therapy not only to the relatively few patients with conduits, but to the broader population of patients with congenital heart disease who have severe native pulmonary regurgitation, a common lesion. The challenge with the present technology is that

the largest bovine jugular venous valves are 22mm in diameter when fully expanded. Many native outflow tracts are significantly larger than this. There are a variety of strategies being considered to deal with this problem. For example, a "docking station" has been proposed: a larger "reducing" stent would be implanted first to decrease the diameter of a large native outflow tract. A smaller valve could then be implanted within. Only time will tell whether this will be successful. A second manufacturer has also produced a bioprosthetic valve that has been implanted in humans. Edwards Lifesciences has implanted the Harmony™ valve in the pulmonic position in 4 patients. This valve is a bovine pericardial bioprosthesis that at present comes in 2 sizes measuring 23 and 26 mm.

TRANSCATHETER AORTIC VALVE SOLUTIONS

Potentially the largest group of patients in need of a valvular heart disease solution is those with severe aortic stenosis. Aortic stenosis is generally a disease of the elderly. Concomitant coronary artery disease, pulmonary, renal, hepatic and cerebrovascular disease are also prevalent in this population. When expected surgical mortality exceeds 15%, both patients and surgeons are hesitant to accept and offer therapy respectively. The possibility of deploying a valve in a stent that could displace the patient's native valve is an attractive alternative that has been hypothesized since the early 1980s. There are 2 potential routes to deliver an aortic valve on a stent. The antegrade approach, which involves a transeptal puncture and passage of the valve from the right atrium to the left atrium and through the left ventricle, has been abandoned. This route has been implicated in injury to the mitral valve and is technically demanding. A potentially less complicated approach is to deliver the valve via a retrograde approach, which involves passing the valve from the femoral artery around the aortic arch and into position.

At present there are 2 protheses that have been implanted in humans; Edwards' Sapien ™ valve and Corevalve's Revalving™ system are two different approaches to the challenge of aortic stenosis. The Sapien ™ valve is mounted on a balloon, and deployed with a single balloon inflation during rapid pacing of the right ventricle to reduce cardiac output. The limitation of this valve at present is its large profile, a 23 mm valve and a 26 mm valve are available and require a 22 french and 26 french sheath to deliver respectively. In the elderly with aortic stenosis, it is a challenge to find patients with iliac arteries of between 8 and 9 mm required to deliver this valve. A novel alternative, using a minimally invasive surgical technique, involves the performance of a left mini thoracotomy for delivery of the valve through the apex of a beating heart. Several hundred of these transapical procedures have been performed with quite reasonable results allowing for the infirmity of those patients who require this approach.

The Corevalve Revalving™ prosthesis is now approved in Europe with an 18 french system. The lower profile is achieved primarily through the use of a self-expanding stent platform as opposed to a balloon expandable system.

Over 20 other companies are working on the development of percutaneous aortic valve technologies. Key features of the next generation of technologies include the ability to reposition, remove, and exchange a new valve and the ability to deploy them through an arterial access that allows the majority of eligible patients to be treated. The ideal valve would have a low risk of infection, be easy to use, and not require anticoagulation. The future is bright and many new therapies are near human implantation.

TRANSCATHETER MITRAL VALVE SOLUTIONS

The mitral valve is by far the most difficult valve to treat percutaneously. While the aortic and pulmonary systems are fairly simple consisting of a relatively fixed annulus with attached leaflets, the mitral valve is of far greater complexity. For the mitral valve to function correctly, a synchronized effort on behalf of the mitral leaflets, annulus, chordae, papillary muscles and the walls of the left ventricle is required.

There are a number of strategies hypothesized to impact the regurgitant mitral valve. The most mature technology already in a randomized trial vs surgery is the Evalve MitraClip™. This technology is implanted using a specialized delivery catheter via a transeptal approach using primarily transesophageal echo for guidance. The clip is implanted on the A2 and P2 leaflets (mid portion of each mitral leaflet) for pathology ranging from functional, prolapse, flail and ischemic mitral insufficiency. The initial results are promising, demonstrating the ability to reduce mitral insufficiency from 4+ to <2+ in a majority of patients. The procedure is technically demanding but with experience, procedure times are falling as results improve.

The "leaflet" approach to mitral repair is but a single avenue of therapy. Several companies have utilized the relationship of the coronary sinus to the mitral valve annulus to advantage. By implanting a device which cinches the coronary sinus, it is possible to reduce the size of the annulus of the mitral valve. This mechanism for valve dysfunction occurs primarily in those patients with congestive heart failure and a dilated mitral valve

annulus. Early results are promising but whether or not sufficient MR reduction will occur with this type of therapy remains to be seen. A second obstacle is the variable relationship of the coronary sinus to the annulus and circumflex coronary artery.

Mitralign™ is a therapy designed to be delivered from a retrograde access. This technology employs a direct suture annuloplasty delivered from the inside of the ventricle. The first human procedure is expected soon.

Reshaping of the heart muscle into a more favorable geometric configuration for the mitral valve is the aim of the Myocor Coapsys system. This therapy, which has been used surgically, is being adapted as a percutaneous procedure. Access is obtained to the pericardial space, and two anchors, joined by a tether which runs through the ventricle, are implanted on the outer surface of the anterior and posterior surface of the left ventricle. The system aims to reduce the antero-posterior dimension of the ventricle to reapproximate mitral valve leaflets that no longer coapt because of annular dilation. Another innovative technology created by Ample Medical™ uses a novel technique to deliver a suture from the coronary sinus to an atrial septal occlusion device. Under TEE guidance, the AP diameter of the annulus can be reduced by tightening the suture and reapproximating mitral valve leaflets in a dilated annulus.

A NOTE OF CAUTION

The success of transcatheter structural heart disease therapies has been the ability to repair intracardiac defects that once required surgery in a minimally invasive fashion. In high-risk patients these therapies are readily accepted. In patients of low operative risk we must constantly re-examine and be critical of transcatheter therapies with the availability of excellent surgical therapies with long-term track records. Although device closure of atrial septal defects is the standard of care, we have learned the importance of patient selection to avoid device erosion which almost uniformly leads to tamponade or important complications. Similarly, although the closure of perimembranous VSDs in children is almost uniformly successful, there is a small, but present, risk of heart block requiring permanent pacing. A pacemaker implant in a small child will require multiple revisions with attendant morbidity. A healthy respect for the complications of new and innovative therapy is critical for interventionalists. Careful evaluation, monitoring and reporting of outcomes of these new therapies are critical for further advancement and insight into ideal patient selection. A referral to a structural heart disease interventionalist should be a true consulting process where the relative indications for therapy are explored and the merits of conventional surgery vs a catheter intervention are discussed overtly with the patient.

CONCLUSION

The future of transcatheter therapies for structural heart disease is bright. There are a paucity of practitioners at present who are able to deliver these therapies in adult patients. As this subspecialty of cardiology evolves and matures, it will have a profound impact on the natural history of our patients and their families. It will only be through partnering with our surgical colleagues that we will optimize outcomes and improve decision-making. Collaboration with industry to modify, improve and develop future therapies is a critical part of the future. In 1996, when I graduated from medical school, many of the therapies I have described had not been conceived.

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MJM Special Abstract Edition

The MJM is proud to present
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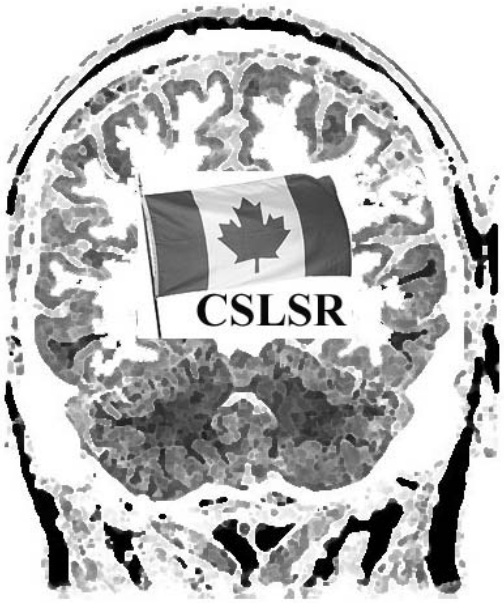

CANADIAN SOCIETY FOR LIFE SCIENCE RESEARCH

LETTER FROM THE CSLSR PRESIDENT

It is with great pleasure that I announce the collaboration of the Canadian Society for Life Science Research (CSLSR) and the *McGill Journal of Medicine* for the publication of the student abstracts from the CSLSR's 2nd Annual Conference held at McGill University on July 13th and 14th, 2007.

The CSLSR is a nationally registered non-profit organization created by life science students for fellow student researchers. We are dedicated to bringing together young student researchers at the undergraduate, graduate, post-graduate and professional levels to share upcoming scientific/health research and discoveries, and allow for furthering the knowledge amongst these future academics, clinicians, clinician-scientists and industry professionals in the life science fields.

During the conference, we were honoured to welcome renowned scientist keynote speakers from both academia and industry. For a synopsis of the conference, and for more information on our upcoming events and student opportunities, please visit our website at www.cslsr.ca.

On behalf of the executive and chapter representatives of the CSLSR, I would like to thank the Canadian Institutes of Health Research (CIHR)'s Institute of Infection and Immunity and Merck-Frosst for their support of this year's conference. We gratefully acknowledge McGill University for their support in our hosting of this year's conference at their institution. I would also like to personally congratulate the student oral and poster presentation award winners:

Poster Presentations:
1. Rozanne Arulanandam - Queen's University
2. William Montgomery McKillop - University of Western Ontario
3. Reva Vidia Mohan - Queen's University

Oral Presentations:
1. Safaa Sebak - McGill University
2. Stephen Andrews - McGill University
3. Dominic Paquin Proulx - Universite Laval

Finally, we would like to thank all attendees, speakers, affiliates and sponsors from across the country for making this conference a great success. We look forward to seeing you in 2008.

Thank-you for your support of young researchers at McGill and across Canada!

Philippe Rizek
President
Canadian Society for Life Science Research/
Société Canadienne de Recherche des Sciences de la Vie
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The Soldier and the Animal: metaphor in maintenance of antiretroviral adherence in Ghana's Eastern Region

Matthew J. Akiyama and David Napier
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Background: Adherence is of the utmost importance in human immunodeficiency virus (HIV) antiretroviral therapy (ART). Poor adherence leads to viral resistance and increases the likelihood of treatment failure. As ART becomes increasingly available in the developing world, differences in cultural understandings of illness and language pose specific challenges to maintaining adherence. **Objective:** To identify cultural and linguistic barriers to treatment comprehension and the successful strategies used to achieve adherence in a population of people living with HIV/AIDS (PLWHA) in rural Ghana. **Methods:** Fieldwork was carried out in a Krobo community in Ghana's Eastern Region. In depth, semi-structured, and open-ended interviews were conducted with PLWHA in the early stages of ART. Observations during clinic visits and support group sessions for PLWHA were made. Focus group discussions were held with clinical staff, community leaders, and PLWHA representatives. **Results:** An understanding of the relationship between the immune system, HIV, and ART was crucial for establishing adherence in PLWHA. This relationship was negotiated organically by PLWHA and health care providers in the early stages of treatment and resulted in a set of mutually agreed upon metaphors to draw from during the therapeutic process. For example, the globally utilized metaphor of 'soldiers' as white blood cells defending the body against foreign invaders was used to illustrate the role of the immune system. The local term 'lowii' - the Krobo word for animal – was used to represent the virus. Metaphors used to characterize the role of ART in viral suppression were perhaps the most important. Many believe they are not sick in the latent phase of the illness since symptoms are not yet present. Therefore adherence was promoted using metaphorical scenarios such as applying constant pressure on a bowl to prevent the escape of a snake from beneath it. **Conclusions:** Metaphoric amalgamations of local cosmology with biomedical representations of the immune system, HIV and ART are instrumental to the way in which complex ART regimens are grappled with and ART adherence is maintained. The descriptive findings in this study may be of use in other treatment settings where cultural and language barriers persist.
Keywords: HIV, antiretroviral therapy, adherence, Ghana, Krobo, illness narrative

Lipid metabolism in peroxisomes, endoplasmic reticulum and lipid bodies controls chronological aging in yeast

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The diet known as calorie restriction (CR) extends life span and delays diseases of aging. It seems that the fundamental mechanisms of aging and the stimulatory effect of CR on life span are conserved from yeast to humans. We use yeast as a model organism for studying the molecular and cellular mechanisms of aging. Our research is aimed at understanding how defects in the biogenesis and function of the peroxisome, an organelle known for its essential role in lipid metabolism, affect the life span of yeast placed on the CR diet. We found that CR promotes the lipolysis of neutral lipids (NL) stored in lipid bodies (LB). In addition, the CR diet stimulates the synthesis of peroxisomal enzymes involved in oxidation of the LB-derived CoA esters of fatty acids (FA-CoA), thereby leading to the rapid consumption of free fatty acids (FFA) in chronologically aging cells. Using lipidomics, we monitored the dynamics of the age-related changes in the intracellular levels of NL, FFA, diacylglycerols (DAG) and cardiolipins (CL) in numerous long- and short-lived mutants. Our findings imply that the mobilization of FA-CoA from LB and their subsequent oxidation in peroxisomes play a key role in regulating chronological life span and in protecting CR yeast from caspase- and mitochondria-dependent apoptosis and various stresses. Moreover, our data provide evidence that the steady-state levels of the LB-derived FA-CoA and of the LB- and endoplasmic reticulum (ER)-derived DAG control the rate of chronological aging. Furthermore, our findings suggest that lipid metabolism in peroxisomes of CR yeast modulates the steady-state levels of CL in the inner mitochondrial membrane. This, in turn, regulates a distinct set of processes that take place in mitochondria. Our data suggest a mechanism by which the remodeling of lipid metabolism in peroxisomes, ER and LB of CR yeast extends their life span by regulating the apoptosis- and stress response-related mitochondrial functions.

Activation of global DNA demethylation by MBD2 converts non-transformed cells into highly invasive and metastatic cancer cells; MBD2 mediates RAS transforming activity

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Introduction: Cancer cells are hallmarked by global DNA hypomethylation and regional promoter hypermethylation. The latter has been extensively studied while insufficient attention has been directed at global DNA hypomethylation. MBD2 is the only protein that has been implicated in removal of methyl groups from CG di-nucleotides. Inhibition of MBD2 attenuates tumorigenesis, metastasis, and reverses the hypomethylation of metastatic genes. Additionally, germ line deletion of the mbd2 gene protects mice from intestinal tumors. Therefore, we tested the hypothesis that oncogenic signals induce MBD2 resulting in metastatic transformation of cells through the triggering of global DNA hypomethylation. **Results:** Activated HA-RAS expression induced MBD2 protein levels and induced global DNA hypomethylation. Overexpression of MBD2, but not a mutant MBD2, converted untransformed mouse fibroblast and human epithelial cells into highly transformed metastatic cells with hypomethylated genomes. Cells overexpressing MBD2 form tumors in Nude mice and invade and degrade bone in SCID mice. Consistent with the hypothesis that MBD2 plays a critical role in oncogenic transformation, MBD2 knockdown in HA-RAS transformed cells attenuates transformation and increases DNA methylation. Remarkably, this phenotypical reversal by MBD2 knock down was rescued by ectopic expression of wt MBD2 but not mutant MBD2. Thus, our data suggests that MBD2 mediates part of the oncogenic and prometastatic activity of Ras. To determine if MBD2 induces oncogenic and metastatic transformation by inducing gene expression through hypomethylation, Affymetrix expression arrays were used and revealed that MBD2 induced a panel of genes involved in metastatic transformation. ChIP assays and methylation analyses confirmed that several of these genes were induced through MBD2 binding and demethylation. Finally, knockdown of HA-RAS in a human cancer cell line with an activated RAS mutation, T24, resulted in decreased: MBD2, DNA demethylation, cellular invasion, and gene expression of metastatic genes. The same effect was shown when MBD2 was decreased in T24 cells but not when only the metastatic genes were knocked down. **Summary:** Our results have unraveled a critical transformation pathway leading from RAS through MBD2 to global hypomethylation and activation of metastatic genes. These data support a new therapeutic approach to metastasis, which would involve targeting MBD2 and DNA demethylation.
This work was supported by a grant from the National Cancer Institute of Canada to MS.

Keywords: metastasis, epigenetics, demethylation, RAS activation, oncogenic signaling

Influence of oral and subcutaneous bisphenol-A on intrauterine implantation of fertilized ova in inseminated female mice

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Intrauterine implantation of fertilized ova in inseminated females is sensitive to minute levels of natural estrogens. Bisphenol-A (BPA), a widely used chemical in the production of polycarbonate plastics and epoxy resins, can be estrogenic. Here we administered BPA during the period of implantation to determine levels of exposure required to terminate pregnancy in mice. Varied doses were given through either injection of ingestion. Subcutaneous injections during days 1-4 of gestation significantly reduced litter size at 3.375 mg/day and substantially reduced the proportion of females that were parturient at 10.125 mg/day. Uterine implantation sites were significantly reduced at a dose of 10.125 mg/day whereas a dose of 6.75mg/day completely inhibited implantation. Exposure to lower doses was without significant effect. When inseminated females' diets were supplemented on days 1-5 with peanut butter contaminated by 0.11-9.0% BPA, litter size and percent parturient were not affected. However, when the animals' diet was exclusively comprised of mixture of BPA, peanut butter, and powdered chow during days 1-4, an average daily intake of 68.84mg BPA terminated all pregnancies. No significant effects at lower doses of BPA were seen in number of births or other measures through either mode of administration.
Keywords: Bisphenol-A, implantation, estrogens, pregnancy, uterus

Regulation of the Breast Cancer Susceptibility Gene 1 (BRCA1) by the Stress Hormone Hydrocortisone

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Psychological stress has been correlated with breast cancer development in numerous epidemiological studies. However, few physiological and molecular models which may account for this association are available. We have found that the stress hormone hydrocortisone down-regulates the expression of the breast cancer susceptibility gene BRCA1 in non-malignant mouse and human mammary cells. Since low levels of BRCA1 have been implicated in the development of sporadic breast cancer, this may represent a novel molecular mechanism through which stress signaling disrupts intracellular pathways involved in the prevention of tumorigenesis. The hydrocortisone effect on BRCA1 was observed at both the promoter and mRNA levels, and was found to be concentration-dependent and reliant on continuous hydrocortisone presence. Also, it is unchanged by the addition of lactogenic hormones, or growth conditions. Hydrocortisone was also found to negate a known positive effect of estrogen on BRCA1 expression and therefore, may interfere with estrogen-related signaling in mammary epithelial cells. The repressive effect of hydrocortisone is lost in malignant mouse and human mammary cells, suggesting alteration of signaling to the BRCA1 promoter in the course of cell transformation. We have uncovered two promoter regulatory sites, which are involved in BRCA1 regulation by hydrocortisone, the BRIBS and UP regulatory elements. Binding of the transcription factor GABPα/β to both sites and binding of the transcription factor USF2 to the UP site are lost upon hydrocortisone addition, suggesting the involvement of MAPK signaling in hydrocortisone-induced repression. Interestingly, we have identified a direct role of the glucocorticoid receptor (GR) in BRCA1 regulation in the absence of hydrocortisone which is independent of GR DNA binding ability. Thus, GR may act as an activator of BRCA1 expression, which is recruited away from the BRCA1 promoter along with its protein binding partners in periods of stress.

Breast cancer cells inhibit osteoblast differentiation

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Skeletal metastasis is a major complication of advanced breast cancer which results in bone fractures and considerable pain burden. Osteoclasts recruited to the site of metastasis lead to the destruction of bone and formation of osteolytic lesions. Osteoblasts are known to contribute to osteoclast activation by producing a key pro-resorptive cytokine RANKL, however the effects of cancer cells on osteoblasts are not fully understood. We have studied the effects of MDA-MB-231 human breast carcinoma cells on proliferation and differentiation of primary osteoblast precursors. MDA-MB-231 cells were cultured until 80% confluent, and conditioned medium was collected after 24h of culturing. Bone marrow cells were isolated from the long bones of C57BL/6J mice, and treated for 5 days with ascorbic acid (50 mg/ml) in the presence or absence of MDA-MB-231 conditioned medium (10%). As a control, we used medium conditioned by MC3T3-E1 cells. Cell numbers were assessed in samples labeled with nuclear stain DAPI, and processed using image analysis. Treatment with ascorbic acid alone led to formation of 390 +/- 90 cells/mm2. Addition of MDA-MB-231 conditioned medium to osteoblastic cultures led to a 2-3 fold increase in cell number, whereas treatment of cultures with MC3T3 conditioned medium had no effect, indicating that MDA-MB-231 cells produce factors that stimulate proliferation of osteoblastic cells. To assess osteoblast differentiation, the cultures were stained for alkaline phosphatase (ALP) and the labeled area was quantified using image analysis. Even at this early cultures, treatment with ascorbic acid alone led to significant increase in APL-positive cells, compared to untreated cultures. Addition of MDA-MB-231 conditioned medium to ascorbic acid-treated cultures resulted in a 5-fold decrease in ALP expression. Thus, our data indicate that soluble factors produced by breast cancer cells stimulate proliferation and inhibit differentiation of native osteoblasts. Since it has been previously shown that immature osteoblast precursors represent a major source of RANKL, compared to mature osteoblasts, our findings suggest a novel mechanism for osteoblast-mediated stimulation of osteoclasts that potentially contributes to the establishment and progression of metastatic lesions in bone.

Heats shock cognate protein 70 accumulates in the nucleolus of HeLa cells during heat stress recovery

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Heat shock cognate protein 70 is a constitutively synthesized chaperone involved in many cellular functions, such as in the proper folding of proteins, protein targeting to various organelles, refolding of damaged proteins and in the targeting of severely damaged proteins to the degradation pathway. It therefore prevents the formation of dangerous protein aggregates and is necessary to cell survival. Hsc70s are involved in multiple physiological mechanisms including aging, cancer development, ischemia of the heart and brain, in the response to stress such as heat shock and other environmental changes.

Under normal growth conditions, hsc70 is present throughout the cell. Following heat exposure, hsc70s accumulate in the nucleoplasm and concentrate transiently in nucleoli when cells recover from stress through unknown mechanisms. To define those mechanisms, I determined the kinetics of hsc70 nucleolar accumulation in human culture cells that recover from stress. The molecular signaling events that play a role in hsc70 nucleolar accumulation have yet to be defined. My results demonstrate that several kinase cascades and phosphatases contribute to the control of hsc70 nucleolar accumulation in cells recovering from stress. Furthermore, I have shown that hsc70s are present in high molecular mass complexes in nucleoli of both heat-shocked and control cells. However, the size and possibly composition of these complexes changes in response to stress; the binding partners that associate with hsc70 in nucleoli are currently being identified.

To identify regions of hsc70 that are necessary and sufficient for transport to the nucleolus, constructs were generated that contain defined portions of hsc70 fused to EGFP. Using confocal microscopy, I identified a short segment of hsc70 that is sufficient for targeting a non-nucleolar passenger protein to the nucleolus of stressed cells. My research is now defining the minimal region sufficient for stress-induced nucleolar targeting.

I expect my studies to contribute to the understanding of the dynamic localization of hsc70s and the signaling events that control these reactions. Using a combination of biochemistry, cell and molecular biology, my research will provide new insights into the stress response and, in particular, the physiological roles of hsc70s in nucleoli of stressed cells.

Keywords: Hsc70, nucleoli, stress, accumulation, nucleolar targeting

Probing into the GTP specificity of an mRNA capping enzyme

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Most cellular and some viral mRNAs contain at their 5' ends a cap structure, which is essential for the mRNA stability, its nucleo-cytoplasmic transport and its translation. The synthesis of this cap structure, a 7-methyl guanosine residue linked via a 5'-5' triphosphate bridge to the RNA transcript, is carried out co-transcriptionally via three sequential enzymatic reactions, the first one involving the hydrolysis of the RNA 5' triphosphate terminus by an RNA triphosphatase to form a diphosphate end, the second step being the transfer of a GMP moiety to the diphosphate end of the RNA by an RNA guanylyltransferase activity which is followed by the methylation of the GpppN cap at position N7. The RNA guanylyltransferase belongs to the nucleotidyltransferase superfamily, which also includes ATP dependent DNA ligases. Despite sharing the same conserved motifs, each enzyme uses different substrates for its activity. The aim of this research is to probe into the molecular determinants for the GTP specificity of the model RNA guanylytransferase from PBCV-1 (the smallest known enzyme of this family) by gaging its effectivemess to use various purine analogs as substrate. In addition to providing an insight into the capping mechanism this approach will also enable the determination of molecular specificities for the methylation of the cap structure.

Analysis of DAF-18/PTEN in VAB-1 Eph RTK Signaling

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The elucidation of signaling pathways involved in cell movements is critical for understanding complex developmental processes. Accordingly, consideration must be given to the function of the individual components of such signaling cascades. DAF-18, a C. elegans homolog of the PTEN human tumor suppressor, is a component of the DAF-2 Insulin Receptor-like signaling pathway involved in dauer formation, longevity and metabolism. Our lab has shown that DAF-18 can physically bind VAB-1, an Eph Receptor Tyrosine Kinase with roles in axon guidance and embryonic morphogenesis. To corroborate these varied functions as well as explore additional roles of DAF-18 in the worm, affinity purified DAF-18 antibodies were used for in situ detection of DAF-18 as well as Western blotting. Results of Western blotting confirm that DAF-18 is present in N2 as well as our over-expressing lines at the expected molecular weight of 110 kDa but is absent in daf-18 (ok480), suggesting this represents a molecular null allele. Here we show that DAF-18 is expressed in amphid neurons, ventral nerve cord, nerve ring and the germline precursor cells, Z2 and Z3. The presence of DAF-18 in neuronal tissues is consistent with the known expression pattern of VAB-1, further substantiating an in vivo interaction. Additionally, we have shown endogenous VAB-1 is also expressed in the Z2 and Z3 germline precursors. Previous literature implicates VAB-1 in regulating oocyte maturation, suggesting expression in the germline. DAF-18 has recently been implicated in regulating germline proliferation in L1 arrest, and our results are consistent with DAF-18 functioning in the germline. While the exact mechanism of the DAF-18/VAB-1 interaction requires further investigation, preliminary molecular evidence proposes VAB-1 may be a negative regulator of DAF-18. DAF-18 has been shown to exhibit PIP3 lipid phosphatase activity and we are currently testing whether DAF-18 has protein phosphatase activity on VAB-1. Taken together these results support the in vivo interaction of DAF-18 and VAB-1 which we will be exploring further with 4D analysis to ascertain the role of daf-18 in embryonic morphogenesis, double mutation analysis, aging and dauer studies.

Keywords: VAB-1 Eph Receptor, DAF-18/PTEN, C. elegans, tumour suppressor, antibodies

Megakaryocytic cells expressing a peptide derived from a protein regulating the actin cytoskeleton, MTPG-24, exhibit an increased cell size

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Megakaryocytes (MKs) are specialized hematopoietic cells that produce blood platelets as a result of the fragmentation of their cytoplasm during the last phase of their life. Prior to this event, MKs undergo a complex maturing process during which they accumulate large nuclear mass and cytoplasmic cellular volume. The large size of the MKs is associated with extensive polyploidization, involving multiple rounds of incomplete mitosis termed endomitosis. There is now accumulating evidence that endomitotic MKs develop as a result of aberrant regulation of cleavage furrow formation, to which is normally associated a localized production of phosphatidylinositol 4,5-biphosphate (PI(4,5)P2) during the creation of membrane barriers between two daughter cells. Here we report that human megakaryocytic cell lines over-expressing transgenic constructs encoding the MTPG-24 peptide undergo remarkable changes in cellular size, resulting in cells with the appearance of mature MKs. MTPG-24 is a 24mer-peptide with (PI(4,5)P2) binding affinity which derives from a protein involved in regulating actin-based structures and motility. MTPG-24 tagged with fluorescent proteins was detected closely associated to the plasma membrane where it accumulated in a patchy pattern, in addition to strongly co-localizing with a Golgi marker. Since no significant differences in cellular proliferation nor in DNA content were observed between control cells and those expressing the peptide, the increased cell size exhibited by MTPG-24-expressing cells, in contrast to MKs, does not result from endomitosis. Because it likely interferes with actin cytoskeleton regulatory protein by masking membrane (PI(4,5)P2), MTPG-24 may thus provide an interesting tool to modulate cell shape and size. We are currently testing whether this peptide could be used to increase the in vitro platelet production of human cord blood-derived MKs which have a low propensity to become polyploid.

Keywords: hematopoiesis, blood platelet production, megakaryocytes, polyploidy, cell division

Effect of sensori-motor interventions on the oral feeding performance of preterm infants

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Background: Up to 30-40% of preterm infants may encounter oral feeding difficulties. Successful oral feeding is a complex process necessitating the appropriate function and integration of multiple systems, including oral, cardio-respiratory, gastrointestinal and neurological. Oral feeding difficulties may ensue from oral and non-oral origins. At present, most sensori-motor interventions are aimed at improving oral-motor skills only. Limited information is available on the effect of non-oral sensori-motor interventions on preterm infants' oral feeding performance. **Objective:** To assess whether oral (O), tactile/kinesthetic (T/K), and combined (O+T/K) stimulations enhance the oral feeding performance of preterm infants, and to elucidate the impact of multiple sensori-motor stimulations on oral feeding outcomes. **Methods:** Seventy-five preterm infants (< 32 weeks gestation) were randomly assigned to an O, T/K, combined (O+T/K), or sham (S) group. The O intervention consisted of stroking the lips, gums, tongue and sucking on a pacifier. The T/K intervention involved stroking the head, trunk, limbs and passive range of motion to limbs. The combined (O+T/K) was similar to the above. The S intervention involved observing the infant only. Interventions were administered for 10 days, 2X/day, for 15 minutes. The outcome measures included 1) number of days from introduction to independent oral feeding, 2) volume transfer (%volume taken/prescribed volume), and 3) rate of transfer (ml/min). Outcome measures were monitored when infants were taking 1-2, 3-5, and 6-8 oral feedings/day. One-way ANOVA and repeated measures 2-way ANOVAs were applied. **Results:** Independent oral feeding was achieved significantly earlier by the O (11.1±3.5), T/K (11.4±3.3), and combined (O + T/K) (10±3.5) groups than the control group (20.7±6.6), $p<0.000$. Volume transfer ($p=0.000$) and rate of transfer ($p=0.003$) were significantly greater in the 3 experimental vs. control group. The 3 sensori-motor interventions had similar oral feeding outcomes. **Conclusion:** The O, T/K, and combined (O+T/K) interventions accelerated the transition from tube to independent oral feeding to the same degree. This was associated with enhanced volume transfer and rate of transfer observed in all 3 experimental groups. The results support the concept that development of oral feeding skills may be influenced by early sensori-motor experiences beyond the boundaries of the oral system.

Complementary role for TC-PTP and PTP-1B in interferon-gamma signalling

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The control of tyrosine phosphorylation depends on the fine balance between kinase and phosphatase activities. Protein tyrosine phosphatase 1B (PTP-1B) and T cell protein tyrosine phosphatase (TC-PTP) are two closely related phosphatases that are known to control cytokine signalling, for example, in macrophages. We wished to study the redundancy of PTP-1B and TC-PTP on interferon signalling by deleting one or both copies of PTP-1B in tcptp-/- and tcptp+/- mice by interbreeding. Our results indicated that the double mutant was lethal at a relatively early stage of embryonic development. Mice heterozygous for TC-PTP on the ptp1b-/- background developed symptoms similar to a chronic inflammatory disease, and their macrophages were highly sensitive to interferon-γ as shown by increased Stat1 phosphorylation and nitric oxide production. Together, these data indicate a nonredundant role for PTP-1B and TC-PTP in the regulation of interferon signalling.
Keywords: T cell protein tyrosine phosphatase, protein tyrosine phosphatase-1B, interferon-gamma, inflammation, cell signalling

Inhibition of hsc70s shuttling upon stress, import, export, and beyond?

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Heat shock proteins are molecular chaperones that are involved in multiple cellular processes. This includes repair of stress induced damage and targeting of proteins to different organelles in unstressed cells. Under normal growth conditions cytoplasmic members of the hsp/hsc70 family shuttle between nuclei and cytoplasm and they are found in both compartments. Upon exposure to stress, hsc70s accumulate in nuclei of stressed cells and heat shock is the most efficient treatment to induce their nuclear accumulation. Although heat increases the steady-state concentration of hsc70s in nuclei, it is not known whether stress also controls their ability to shuttle between nuclei and cytoplasm. To gain more insight into the effect of stress on hsc70s shuttling, and the molecular mechanisms which control this process, we have analyzed hsc70s trafficking in heat–stressed human/mouse heterokaryons. We found that heat shock inhibits hsc70s shuttling and sequesters the chaperone in nuclei. However this stress-induced shuttling inhibition is transient only as hsc70s were able to resume shuttling when cells recover from heat. We have defined nuclear retention as one of the mechanisms which prevent hsc70s exit from nuclei, and leads to shuttling inhibition in stressed cells. This retention depends on two forms of hsc70 interaction with nuclear anchors, ATP-sensitive binding of hsc70s to chaperone substrates and ATP-insensitive association with nucleoli. Our results showed increase in the association between hsc70s and nucleolar proteins fibrillarin and rpS6 upon stress, in line with the idea that hsp/hsc70s protect the nucleoli from stress induced damage. Taken together, our studies show that heat stress increases the retention of hsc70s in nuclei which prevents the exit of the chaperone to the cytoplasm and therefore inhibits shuttling. We propose that upon recovery, hsc70 is liberated from nuclear and nucleolar anchors and this is a prerequisite to resume shuttling.
Keywords: Hsc70s, nucleus, shuttling, stress, heterokaryons

Spotlights on the DNA repair system in late spermatogenesis: stage-specific DNA fragmentation and activation of H2AX

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Introduction: The histone variant H2AX is involved in early DNA damage response and in the recruitment of repair proteins at sites of double-strand breaks. In testis, the active form of H2AX (gH2AX) is thought to be involved in two processes namely the inactivation of sex chromosomes and the control of genome integrity during meiotic recombination. We hypothesized that H2AX is also activated during spermiogenesis, when 100% of elongating spermatids (ES) harbor transient DNA breaks. **Material and methods:** We used confocal and epifluorescence microscopy applied to both immunofluorescence and Terminal deoxynucleotidyl transferase dUTP nick-end labeling on squash preparations of seminiferous tubules and fixed mouse testis sections. **Results:** We demonstrate the presence gH2AX foci in ES, coincident with the onset of transient DNA strand breakage and chromatin remodeling as shown by the hyperacetylation of histone H4. Topoisomerase IIb is also present in foci in ES and may be involved in the DNA fragmentation and activation of H2AX. Futhermore, we were found the tyrosyl DNA phosphodiesterase 1 (TDP1), an enzyme known to remove topoisomerase adducts. In addition, in-situ incorporation of dUTP-FITC clearly shows that an active DNA polymerase is present. **Conclusions**: These results demonstrate for the first time that a complex DNA repair system is required during the chromatin remodeling in ES and likely essential to the genomic integrity of male gametes. Given the haploid character of ES that cannot rely on homologous recombination for DNA repair, we hypothesize that the error-prone non-homologous end-joining (NHEJ) mechanism is present in late spermiogenesis. Faulty repair of the NHEJ may be involved in male infertility and bear dramatic consequences for offspring. Not surprisingly, alterations in the nuclear integrity of the male gametes have been associated with de novo genetic disorders, developmental and morphological defects, cancer and miscarriage. Funded by the Canadian Institutes of Health Research (grant #MOP-74500)
Keywords: spermiogenesis, chromatin, DNA repair, genomic integrity, topoisomerase

Murine model for implant osseo-integration

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Rationale: Bone regeneration decreases with advancing age in humans. Consequently, there is an increased risk of failure to achieve cementless implant fixation by bone attachment in the elderly. Individual and age-related differences in bone regeneration are believed to arise in large part from genetically programmed pathways that regulate the response of osteogenic cells to growth factors and to surface topography. We used mice deficient in growth factor signaling as a model to study bone regeneration in response to different surface textures. **Methods:** Femoral implants with smooth (Sm) or micro-textured (μTx) surfaces were fabricated and coated with titanium to generate a bio-inert surface. Mice deficient in FGF (FGFR3-/-) and PTHrP (PTHrP+/-) signaling were used as in vivo models to examine bone regeneration. Bone was quantified in vivo using micro-CT and classic histology. **Results:** Bone regeneration was enhanced around Tx femoral implants compared with Sm implants and was decreased in PTHrP+/- and FGFR3-/- mice compared with wild type littermates. An extensive layer of fibrous tissue was seen apposed to the Sm implant in FGFR3-/- mice. **Conclusion:** Impaired growth factor signaling in vivo resulted in decreased bone formation and fibrous tissue formation, similar to that commonly seen in aged humans who have undergone total joint arthroplasty. Combined with results from in vitro assays, this approach represents a simple, biologically relevant model to screen for combinations of surface texture and biologic agents to promote bone regeneration in the elderly bone regeneration decreases with advancing age in humans.
Funding : CIHR, RRBO, RRTQ
Keywords: osteoporosis, bone regeneration, tissue engineering, implant osseo-integration

Interleukin 33 (IL-33) in severe asthma and modulation of its expression in airway smooth muscle cell (ASMC)

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Rationale: IL-33, a recently described IL-1 cytokine family member, promotes Th2 inflammation but evidence on implications of this cytokine in asthma is lacking. IL-33 is mainly expressed by structural cells including ASMC, but whether pro-inflammatory cytokines modulate its expression in ASMC is unknown. **Methods:** RNA was extracted from endobronchial biopsies collected from adults with mild (n = 8), moderate (8), severe (9) asthma and from control subjects (5). Reverse transcriptase and real-time quantitative PCR were used for determining IL-33 transcript levels. ASMC isolated from pathologically uninvolved bronchial airway segments of resected lung specimens were cultured with or without TNFα, and the effects of IFNγ, Dexamethasone (DEX) or Mithramycin A (MMA) additions were investigated. **Results:** Higher levels of IL-33 transcripts were detected in biopsies from asthmatic patients (mild, moderate and severe) compared to control subjects ($p = 0.932$, 0.0186 and 0.002, respectively). In ASMC, TNFα upregulated IL-33 mRNA in a time- and dose-dependent manner. IFNγ synergized with TNFα-induced upregulation of IL-33. MMA reduced the TNFα-induced IL-33 upregulation, whereas DEX did not display significant effect. **Conclusion:** IL-33 expression was shown to increase with asthma severity in bronchial biopsies. In cultured ASMC, MMA reduced the TNFα-induced IL-33 upregulation, whereas DEX had not effect. Although IL-33 was shown to promote eosinophilia, Th2-type phenotype and cytokines, our data suggest that IFNγ induces IL-33 expression in ASMC.
Funding: Richard & Edith Strauss Canada Foundation.
Keywords: asthma, inflammation, interleukin 33, bronchial biopsies, airway smooth muscle cells

Characterization of CD11d leukocyte integrin surface expression

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Primary spinal cord injury (SCI), a mechanical trauma to the spinal cord, results in death to neurons and activation of various cellular responses that contribute to secondary SCI and further neuronal loss. The most potent of these cellular responses is the inflammatory response at the site of injury involving cells resident in the spinal cord, as well as infiltrating leukocytes. As leukocytes leave blood vessels they release pro-inflammatory molecules, including free radicals in the form of an oxidative burst. These free radicals cleanse the area of potential infectious agents, but also contribute to cell death. Previous studies have shown that this cellular infiltration of leukocytes into a site of inflammation involves the CD11d/CD18 integrin as monoclonal antibodies to CD11d block this process and result in reduced secondary SCI and improved neurological recovery. Very little is known about the CD11d/CD18 integrin itself other than that extensive surface expression of the protein is limited to leukocytes at regions of local inflammation. Our study focuses on identifying the mechanisms that regulate CD11d surface expression on leukocytes. CD11d is a member of the leukocyte-specific β2 family of integrins. Other members of this family (CD11a,b,c) require heterodimerization with CD18 for surface expression. Thus it is expected that CD11d will also be expressed on the surface of leukocytes expressing both CD11d and CD18. An analysis of the human CD11d amino acid sequence revealed a potential Casein Kinase II (CKII) phosphorylation motif. Numerous proteins have their cellular localization controlled by CKII phosphorylation, but no integrin has previously been reported to contain an active CKII phosphorylation site. We hypothesize that CKII phosphorylates CD11d, and that this phosphorylation contributes to the complex regulation of the intracellular distribution of the CD11d/CD18 integrin. Our specific aims are to determine if prior formation of a CD11d/CD18 heterodimer is required for CD11d to localize to the cell surface, if CKII phosphorylates CD11d, and to elucidate how the interplay between these two factors relates to CD11d surface expression.

When HEK293 cells stably expressing a CD11d-YFP fusion protein alone are visualized by confocal microscopy CD11d-YFP appears to be confined to the Golgi apparatus. When HEK293 cells stably expressing a CD18-mRFP fusion protein alone are visualized by confocal microscopy CD18-mRFP appears to be confined to the Endoplasmic Reticulum/Golgi complex with no significant surface expression. Transient transfection of CD18-mRFP into CD11d-YFP stably expressing HEK293 cells, or CD11d-YFP into CD18-mRFP stably expressing HEK293 cells, causes relocalization of CD11d-YFP from the Golgi apparatus to the cell surface. CD18-mRFP surface expression was also increased, and colocalized with CD11d-YFP at the cell surface, in those cells that co-expressed both proteins. Flow Cytometry confirmed surface expression of CD11d and CD18 in those cells that co-expressed both proteins.

The C-terminus of CD11d contains the purported CKII phosphorylation site. A GST-CD11d-C-terminal fusion protein was constructed. An In vitro kinase assay confirmed the ability of CKII to phosphorylate purified GST-CD11d-C-terminal fusion. However, flow cytometry comparing the surface expression of CD11d to a mutant of CD11d that cannot be phosphorylated by CKII showed no significant effect on the surface expression of CD11d in the presence of CD18. A chimeric fusion protein replacing the C-terminus of CD25 with that of CD11d has been constructed. Recombinant CD25 proteins have been used in the past to study plasma membrane protein trafficking, and we are adapting the system to compare the surface expression of wild type CD25 to the surface expression of chimeric CD25 with its C-terminal domain swapped for the C-terminal of CD11d. Flow cytometry conducted on the constructs have shown the CD25-CD11d C-terminal fusion to have enhanced expression on the cell surface compared to the wild type CD25, suggesting that the CKII site may not be a regulatory region in the C-terminal of CD11d responsible for restricting its movement to the plasma membrane when not heterodimerized with CD18.
Future studies will expand the chimeric domain swapping experiments to investigate if regulatory motifs controlling CD11d surface expression exist in the N-terminal and transmembrane domains of CD11d and will also determine if CKII phosphorylates CD11d in tissue culture and in vivo. Furthermore, we propose to study the effects of mutating this CKII phosphorylation site on CD11d sub-cellular localization. A furthered understanding of CD11d biology gained from this study may lead to improved neuroprotective strategies for therapy of secondary SCI.

The role of CnABP in urogenital system development and cancer

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Amongst the most important genes controlling urogenital system development are the Pax transcription factors (Pax2/8). Pax2 homozygous mutants lack kidneys and genital tracts and die within 24 hrs after birth. Pax8 homozygous mutants do not display urogenital defects but Pax8 is found to cooperate with Pax2 in early kidney development. In an effort to isolate genes regulated by Pax2 in the kidney, CnABP was identified as a target gene of Pax2. CnABP is located in the shortest region of overlap at the Wilms' tumor locus 16q24. Further, we showed that CnABP expresses in the condensing mesenchyme of the kidney overlapping with Pax2 and Wt1, a known Wilms' tumor gene. Based on these observations, we hypothesize CnABP is involved urogenital system development and Wilms tumor, a kidney cancer of mesenchymal origin. From screening of 50 Wilms tumors, we identified two mutations in CnABP. To understand the function of CnABP, we expressed CnABP in embryonic kidney cell line HEK293. We showed that CnABP localizes to the membrane through an N-terminus myristoylation signal. Through two independent approaches, we demonstrated that CnABP interacts with Calcineurin A beta. We further showed that CnABP colocalizes with Calcineurin A beta and modulates the phosphatase activities of Calcineurin. Calcineurin is a calcium-dependent phosphatase that signals through the NFAT transcription factor to turn on target genes, some of which are known to be involved in cell cycle regulation. To better assess the role of CnABP in vivo, we generated mice from ES-cells containing a genetrap (gt, beta-gal and neomycin fusion) insertion within the CnABP locus. From heterozygous matings, it can be determined that CnABPgt/gt homozygous are not embryonic lethal. However, CnABPgt/gt males are subfertile. Pax2 is necessary for the formation of the nephric ducts, the primordium of the male genital tracts (efferent ducts, epididymis, vas deferens). In addition, Pax2 and Pax8 double heterozygous males are subfertile. We plan to characterize the fertility phenotype of CnABPgt/gt and how this correlates to Pax2 and Pax8 double heterozygous phenotype. To further assess the role of CnABP in any late onset disease or tumor formation, we have generated a cohort of 20 mice of each genotype (CnABP+/+, CnABP+/gt, and CnABPgt/gt) that will be followed up to 2 year for any signs of disease.
Key words: CnABP, Pax2, Wilms' tumor, Calcineurin, fertility

Ameliorating benefit assessment procedures for genetically modified organisms

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While government regulators have devoted many resources towards assessing the risks of Genetically Modified Organisms (GMOs) in agriculture, they have done little to assess the multifaceted aspects of the benefits society can accrue from such agricultural products. We seek to examine structures in government policy making and suggest places for innovation that will better assess the risks, as well as the benefits, of GMOs so that the commercialization of future products will be based more on factors that aim to provide the greatest good to society, than simply avoiding known or potential risks. For instance, we argue for more input from the general public in the assessment of GMOs, primarily from farmers, who are a key but largely ignored stakeholder in this important public policy issue. Currently, GMOs are assessed by scientific protocols that do not incorporate the fact that farmers often optimize their methods of farming such crops, in ways not predicted by scientists/technologists or the agrochemical companies providing the seed stock. For example, many farmers know to plant crops that are resistant to certain pests in areas that have the greatest level of pest infestation in order to obtain the best control of this problem and greatly reduce their need for additional pesticides. In scientific trials studying pesticide requirements of pest-resistant crops, crops are planted in random fields independent of infestation levels. Thus, scientific trials may underestimate the possible decrease in pesticides needed for these crops in comparison to practical farming procedures. Accurate assessments of pesticide use are essential for the development of relevant and effective health policies that aim to reduce toxic exposure to farmers or the broader build-up of such chemicals in our environment. We aim to clarify additional faults in existing assessment procedures, similar to the example just presented, in order to contribute to the development of more accurate public health policies in relation to the use of GMOs in agriculture in Canada and internationally.

A pediatric genome-wide association study identifies two novel Type 1 diabetes loci

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Introduction: Type 1 diabetes (T1D) results from autoimmune destruction of pancreatic beta cells. A number of genetic determinants of T1D have already been established through candidate gene studies. These genetic associations with T1D explain little more than half of the genetic risk for T1D. To identify novel genetic factors that confer risk to the pathogenesis of T1D, we performed a genome-wide association (GWA) study. **Methods:** A two-stage design was adapted in this study. (1) In Stage 1, two cohorts of European population were studied, i.e. a case-control study including 499 T1D probands and 1,058 controls, and a family-based study including 483 nuclear families with at least one diabetic child and both parents. We genotyped 550,000 single nucleotide polymorphisms (SNPs) using the Illumina Human Hap550 Genotyping BeadChip. (2) In Stage 2, the 60 most highly significant SNPs from 30 loci were fast-tracked using the SNPlex platform from Sequenom in two other cohorts of T1D families, i.e. 549 nuclear families with 1,333 affected offspring from the Type 1 Diabetes Genetics Consortium (T1DGC), and an additional 390 Canadian families. **Results:** Besides confirming previously identified associations, one locus reached genome-wide significance for T1D association ($P \leq 1.03 \times 10^{-8}$) in Stage 1, and was confirmed in Stage 2. This novel T1D locus involves the gene KIAA0350 on Chr16p13, predicted to be a sugar binding C-type lectin. The gene is expressed in B lymphocytes, dendritic antigen presenting cells and T cells, including NKT-cells, which is in keeping with a function relevant to an immune-mediated disease such as T1D. Of the remaining findings, an additional locus at Chr12q13 which came within an order of magnitude of Bonferroni correction for 550,000 tests was also replicated in Stage 2. The combined analysis of the T1D association with the minor allele of rs1701704 has $p=2.13 \times 10^{-9}$. **Conclusion:** This study provides proof of principle for the genome-wide association approach and evidence for two novel T1D loci, pointing to previously unknown pathways in the etiology of T1D.

Keywords: Type 1 diabetes, genome-wide association, single-nucleotide polymorphism.

Biological activity of cross-linked intravenous immunoglobulins (IVIg) on human B cells

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Therapeutic preparations of intravenous immunoglobulins (IVIg) are derived from the plasma of thousands of blood donors. IVIg were initially used as a supportive therapy for primary or secondary immunodeficiences. However, IVIg have also been used at very high doses (2g/Kg of body weight) for more than 20 years to treat a significant number of autoimmune or inflammatory diseases, making IVIg the mostly used blood-derived product. The need for high doses of IVIg, combined to the increasing number of diseases treated with IVIg and the limitation of plasma available for fractionation could lead to product shortages in a near future.

One example of an autoimmune disease treated with IVIg is the immune thrombocytopenic purpura (ITP). ITP patients produce autoantibodies against their own platelets, leading to their destruction and resulting in bleeding problems. In ITP patients treated with IVIg, short and long term effects are observed. Short term effects include a rapid inhibition of platelet phagocytosis while long term effects include a reduction of autoantibody titers in the patient's serum. We recently showed that small size immune complexes prepared by cross-linking IVIg with a mouse monoclonal anti-human IgG (C5-1) were about 10 times more efficient that IVIg to prevent platelet destruction in a mouse model of ITP, suggesting that the same therapeutic efficacy could be achieved with less IVIg using this cross-linking strategy.

In this work, we studied whether the long term effects of IVIg treatment observed in ITP patients (reduction of autoantibody titers) could also be obtained with cross-linked IVIg. We have previously reported that IVIg induced the differentiation of human B cells using an in vitro culture system. The increase in differentiation was caracterized by a decrease in proliferation of about 50% and an increase in IgG secretion. We show here that cross-linked IVIg are about 30 times more potent than IVIg in inducing human B cell differentiation. Furthermore, our results show that this effect is not dependent on the classical receptor for immunoglobulins expressed on B cells (FcγRIIb) and suggest that it may involve a new class of immunoglobulin receptors (FcRL).

Apoptosis in epithelial fetal lung cells exposed to stretching as a result of positive ventilation

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The use of positive mechanical ventilation and oxygen in pre-term infants who have respiratory distress syndrome (RDS) can lead to Bronchopulmonary Dysplasia (BDP). Lungs that develop BDP are at risk of developing inflammation, alveolar arrest, pulmonary hypertension, and fibroblast hyperplasia. In addition, high levels of apoptosis have been found in the lungs of patients who suffer from RDS.
Apoptosis is controlled cell death that is characterized by cell condensation, nuclear fragmentation and changes to the morphology of the cell membrane. Unlike cell necrosis which results in the release of all the components of the cell, apoptosis culminates in cell death without inflammation. Two caspase-dependent pathways are implicated in apoptosis, namely the intrinsic and extrinsic pathways. The intrinsic pathway is characterized by the release of cytochrome C and subsequent caspase 9 activation while the extrinsic pathway is characterized by the activation of the Fas (CD95/APO1) receptor and caspase 8 cleavage. Both pathways lead to the production of cleaved caspase 3, the effector caspase. *We hypothesize that both pathways are involved in the overdistension-induced apoptosis off lung epithelial cells.* To test the hypothesis, freshly isolated fetal day 19 rat lung epithelial cells were seeded onto Bioflex culture plates (Flexercell International) and subjected to a continuous 20% stretch for 6 hours. Afterwards, cells were lysed and samples were analyzed by Western blotting. Our results indicate that un-stretched samples have lower levels of cytochrome C and cleaved caspase-3 than stretched samples. Future experiments will include Western Blots using caspase-8, Fas and Apaf-1 antibodies to confirm the presence of these pathways.
Keywords: apoptosis, caspase-3, cytochrome C, stretch

Idiopathic toe walking: a marker for developmental delay

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Background: Idiopathic Toe Walking (ITW) is a diagnosis of exclusion in children who display this gait pattern without associated neuromuscular disease. Previous smaller studies have found an association of ITW with autism and various developmental delays, specifically in speech, language and learning. **Objectives:** The aim of this study is to re-examine the association between ITW and developmental delay by conducting a larger trial of children seen in an orthopaedic clinic. **Design/Method:** Parents or guardians were contacted by telephone and were asked to complete a survey, which was designed by two developmental paediatricians. Results were analysed by a separate data analyst at Kingston General Hospital. **Results:** Forty three parents completed the survey. The mean age of children was 8.0+2.4 years (range of 3.5 to 12.3), and 58% of the sample was male. Diagnosed developmental delays included speech/language delay in 15 (35%) children, motor delay in 5 (12%) children, and learning disabilities in 8 (19%) children. ADHD and autism were diagnosed in 9 (21%) and 5 (12%) children, respectively. In those without a diagnosis of autism, an additional 29% displayed one or more red flags of behaviour known to be exhibited by children with autism. **Conclusion:** These results support previous research and suggest that developmental delay, autism and ADHD have a higher prevalence in children with ITW. Hence, a diagnosis of ITW should be considered a red flag for potential neurodevelopmental issues and warrants developmental screening.
Keywords: idiopathic toe walking, developmental delay, autism, ADHD

Optimization of human serum albumin nanocapsules for drug delivery applications

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Introduction: Despite impressive results available chemotherapeutic drugs have severe symptoms associated with them including gastrointestinal toxicity, susceptibility to the development of drug resistance, poor bioavailability that results in a need for prolonged intravenous infusions, and the use of toxic solubility agents. Thus, new methods of drug delivery are being explored to minimize harmful side–effects through the use of nano-scaled drug delivery systems. The objective of this study is to design albumin nanocapsules of controllable size and investigate their properties for the optimal, safe and effective deliverance of anti-cancer drugs. **Methods:** Novel albumin nanocapsules were designed, prepared and characterized in-vitro. 50-200mg of human serum albumin (HSA) was dissolved in 10mM NaCl. The pH was adjusted to values 7-11 by adding NaOH. Ethanol was added dropwise at a defined rate (ml/min) using a peristaltic pump to form uniform nanocapsules. The nanocapsules were stabilized by crosslinking with 8% and 25%grade glutaraldehyde. Particles were purified by a 4-fold centrifugation (50,000 rpm, 15min) to remove free albumin and excess glutaraldehyde. Centrifuged particles were resuspended in PBS, and lyophilized. The nanocapsules were studied under scanning electron microscope (SEM) and particle size, stability, and zetapotential were analyzed and compared to determine optimal conditions in order to achieve a colloidal system with well-defined physicochemical characteristics. **Results:** The albumin nanocapsules were prepared varying the HSA concentration, pH, and crosslinker in order to optimize the preparation procedure with respect to a defined particle size and distribution. The optimal preparation conditions resulted in controllable particle size between 150-500nm. The first study was the effect of HSA concentration on particle size as the HSA concentration increased, the particle diameter decreased. The pH value of the HSA prior to ethanol addition strongly influenced the resulting particle size. At pH>7 the particle size was significantly reduced with increasing pH value. At pH 8, the particles were stable with the size increasing by less then 20nm. No influence of crosslinking conditions on the resulting particle size was observed. Considering the results on the influence of different parameters under evaluation, a standard protocol for the preparation of HSA nanocapsules was established. **Discussion:** TheseThese findings reveal a correlation between preparation conditions of nanocapsules and physiochemical characteristics. Literature suggests that this method of preparation of nanocapsules has potential resulting in nanocapsules with the required pharmacokinetics to selectively target cancerous cells, improve drug stability, and increase bioavailability. Surface modification procedures aiming at specific drug binding and targeting have yet to be investigated.

Mitochondrial targeting and folding functions of chaperones

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Mitochondrial preproteins that are imported via the Tom70 receptor are complexed with the cytosolic chaperones Hsp90 and Hsc70 before targeting to the mitochondrial outer membrane. Dissection of the pathway with Hsp90 inhibitors found that the chaperone helped to maintain preproteins in the cytosol before import, to target preproteins onto the Tom70 receptor, and to promote translocation across the mitochondrial outer membrane. To identify other proteins involved, a purified mitochondrial preprotein was reconstituted with chaperones in reticulocyte lysate, and bound proteins were identified by mass spectrometry. In addition to Hsc70 and Hsp90, a specific subset of co-chaperones was found, but no mitochondria-specific targeting factors. Interestingly, three different Hsp40-related J-domain proteins were identified: DJA1, DJA2 and DJA4. The DJAs bound preproteins to different extents through their C-terminal regions. DJA dominant negative mutants lacking the N-terminal J-domains impaired mitochondrial import. The DJAs also showed significant differences in activation of the Hsc70 ATPase and Hsc70-dependent protein refolding. In HeLa cells, the DJAs increased the activity of newly synthesized luciferase and mitochondrial accumulation of a preprotein, although to different extents. No single DJA was superior to the others in all aspects, but each had a profile of partial specialization. We suggest that multiple co-chaperones with similar yet partially specialized properties cooperate in optimal chaperone-preprotein complexes.
Keywords: protein folding, mitochondrial import, chaperones, Hsp70, Hsp90

A novel pathway of cadherin, Rac1/Cdc42 and Stat3 interaction

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Cellular interactions with neighboring cells profoundly influence a variety of signalling events including those involved in mitogenesis, survival and differentiation. Unlike cultured cells, cells in a tumor have extensive opportunities for adhesion to their neighbors in a three-dimensional structure, therefore in the study of signal transduction it is important to take into account the effect of neighboring cells. The Signal Transducer and Activator of Transcription-3 (Stat3) has emerged as an important signaling molecule with a role in the etiology of many cancers, such as breast cancer, and is required for transformation by a number of oncogenes.

Our lab has demonstrated that cell-to-cell adhesion, as observed in confluent cultured cells, can lead to a dramatic increase in Stat3 activity, which peaks at 2-4 days post-confluence. Most importantly, this Stat3 activation required calcium but was resistant to inhibition of a number of tyrosine kinases, often activated in a variety of cancers. Downregulation of Stat3 induced apoptosis, which was most prominent in post-confluent cell cultures, indicating that Stat3 plays a central role in regulating cell survival (Oncogene 2004). Cadherins have recently emerged as a group of cell-cell adhesion molecules involved in the regulation of signalling events, as well as the maintenance of tissue architecture. It has been shown that cadherin engagement can result in the rapid activation of the Rac1 and Cdc42, Rho family GTPases. Furthermore, expression of activated forms of the Rho GTPases can lead to Stat3 phosphorylation and activation. In this report we demonstrate that E-cadherin engagement can activate Stat3. Stat3 activation is preceded by a dramatic increase in the activity as well as the levels of Rac and Cdc42 and is followed by potent survival signalling. Moreover, downregulation of Rac and Cdc42 through expression of dominant-negative mutants or pharmacological inhibitors caused a dramatic reduction in Stat3-ptyr705 levels, indicating that their activation may be part of the pathway whereby E-cadherin engagement leads to Stat3 activation and survival.

Keywords: Stat3, cadherin, Rho GTPases, cell-cell adhesion, confluence

ADAM12 effects on Dupuytren's Disease cell morphology and cytoplasmic beta catenin accumulation require Type I IGF receptor tyrosine kinase activity

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Background: Dupuytren's disease (DD) is a debilitating fibroproliferative disease of the palmar fascia in the hand that results in the formation of a collagenous disease cord and permanent contraction of affected fingers. Our laboratory has previously documented increased levels of cytoplasmic beta-catenin, a signalling molecule involved in cell proliferation, in DD. The signalling pathway regulating beta-catenin accumulation in DD is not known. Affymetrix microarray analysis of surgically resected DD tissue performed in our laboratory indicates that A Disintegrin And Metalloprotease (ADAM) 12 is highly expressed in this fibrosis. This extracellular matrix-associated protease has been shown to increase Type I Insulin-like Growth Factor Receptor (IGFRI) tyrosine kinase activity, promote adherens junction disruption, and increase cytoplasmic levels of beta-catenin in other systems. The purpose of this study was to determine if ADAM12 affects beta-catenin accumulation in DD and, if so, whether this was dependent on IGFRI tyrosine kinase activity. **Methods:** Cells derived from DD patients were cultured on Type-1 collagen and treated with exogenous ADAM12 / vehicle in the presence or absence of NVP-ADW742-NX-7, a specific inhibitor of IGFRI tyrosine kinase activity. To more closely replicate the in vivo disease environment, a collagen co-culture method was employed. Briefly, primary DD cells were cultured on insert wells coated with collagen containing ADAM12 / vehicle, in co-culture with normal PF or DD cells cultured on untreated collagen. Immunofluorescence microscopy and Western blotting were used to analyze cell morphology and beta-catenin levels. **Results:** The addition of exogenous ADAM12 to DD cells, but not normal PF cells, resulted in marked changes in cellular morphology including condensed actin stress fibres and cytoplasmic beta-catenin accumulation. These effects of ADAM12 on DD cells were strongly inhibited by NVP-ADW742-NX-7, an IGFRI-specific tyrosine kinase inhibitor. Further, DD cells grown on collagen in co-culture with DD cells grown on collagen containing ADAM12 displayed increased beta-catenin levels relative to controls. **Conclusions:** This study demonstrates for the first time that inhibition of IGFRI tyrosine kinase activity markedly inhibits changes in cell morphology and that beta-catenin accumulation is induced by ADAM12, consistent with IGFRI signalling being an essential component of beta-catenin accumulation in DD.
Keywords: Dupuytren's Disease, ADAM12, IGF, tyrosine kinase

Caspase substrates screening by diagonal gel approach and study on caspase-1 substrates on glycolytic pathway

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Apoptosis is executed by caspase-mediated cleavage of various proteins. Elucidating the consequence of substrate cleavage provides us insight into cell death and other biological processes. In this study, we applied the diagonal gel approach, a proteomic strategy, to screen for caspase-1 and –3 substrates. Our results showed significant overlap between caspase-1 and –3 substrates obtained by the diagonal gel approach. Substrates for both caspase-1 and caspase-3 are implicated in common cellular functions, such as maintenance of the cytoskeleton, chaperons, translation, glycolysis, bioenergetics, signaling and trafficking. An important finding is that many glycolysis enzymes are targeted by caspase-1 in the diagonal gel approach. Cleavage of these glycolysis enzymes was confirmed by cleaving in vitro transcribed and translated substrates with recombinant caspase-1. Point mutation of GAPDH blocks its cleavage by caspase-1. This provides a direct link between apoptosis and glycolysis.
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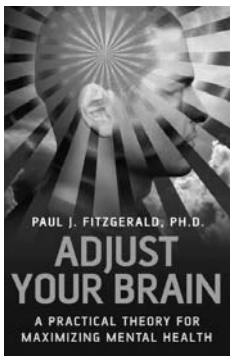
BOOK REVIEW



Book Review by Garrett R. Bird, University of California San Francisco, by Paul J. Fitzgerald PhD

O Books, Winchester, United Kingdom, October 26, 2007 planned release, 224 pages.
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I do anatomize and cut up these poor beasts, he said to Hippocrates, to see the cause of these distempers, vanities, and follies, which are the burden of all creatures.

- Democritus (1)

Dualism, a belief in a fundamental opposition in all things like the body and the mind, good and evil, right and wrong, is found throughout ancient civilization. Dualism was expanded by John Cottingham(2) to include sensation, and the theory of trialism, or three opposing forces, evolved.

Paul Fitzgerald Ph.D. presents his contemporary version of trialism "The Big Three" (Norepinephrine, Serotonin, Dopamine) in his new book "Adjust Your Brain" which will be released in October 2007.

Dr. Fitzgerald is a native of Lafayette, Indiana. He attended Indiana University on the Wells Scholarship and graduated with highest distinction and a B.S. in biology in 1995. He attended neuroscience graduate school at Johns Hopkins, eventually finishing his Ph.D. in 2005. His brain mapping research has been published in several peer reviewed journals, including Nature and The Journal of Neuroscience. He was diagnosed with bipolar disorder in 2000.

Dr. Fitzgerald writes of his struggle to find an effective combination of available therapies. His goal: to allow him to continue his life's pursuits and contribute to society. If his narrative had merely described the plight of a well educated man coping with a debilitating disease it would have been noteworthy. Dr. Fitzgerald took it one step further by attempting to turn psychiatry, as it is practiced now, on its head.

The book is an interesting read on several levels. His depiction of personal struggles with mental illness will educate clinicians and students alike. Sad, yet inspiring, his ability to find a treatment that allowed some normalcy gives hope to sufferers of mental disease. More interesting, and far more controversial, is his assertion that everyone can do the same thing by bucking conventional psychiatry and "Adjusting Your Brain."

Dr. Fitzgerald proposes that the current version of the major psychiatric text, the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR) is merely a "caricature of the truth(3)." His theory is that, like the four humors of Hippocrates, the brain, and indeed all mental illness, is derived from specific quantities of three main neurotransmitters (Norepinephrine, Serotonin, Dopamine) and their adjustment.

Dr. Fitzgerald draws on his own experience, the writings of Michael Nordin(4) and Peter Kramer(5), and from the literature. He uses analogies and simple examples to try and make this complicated topic more approachable for the general public.

It is, however, far from a simplistic theory. Dr. Fitzgerald spends a large proportion of his book explaining the complex interactions that exist within neurons, the brain, other organs, as well as receptor and reuptake modification of these signals. In the end, however, he proposes that all overt mental disease can be cured, or dramatically improved, by balancing these three neurotransmitters(3).

Unlike authors like David Healy(6) and Elliot Valenstein(7) who railed against the use of antidepressants and against pharmaceutical companies in general. Paul Fitzgerald is sure to win some friends in the industry. He proposes giving specific multi-drug regimens to every mental illness sufferer to alleviate their symptoms and lead a more normal life. He even suggests that at least fifty percent of the regular population would benefit from drug therapy(3).

He also names specific individuals (living and dead) who fit his criteria for distinct neurotransmitter levels. The entertainment and shock of some of these comparisons can only be experienced by reading the book. Dr. Fitzgerald asks some bold questions—were Hitler and Abraham Lincoln cut from the same neurotransmitter cloth? Is the author really Clint Eastwood in a smaller frame?

Not famous? Do not feel left out. Are you a strong norepinephrine or a weak dopamine? Should you have more serotonin or less to make life better for you? Dr. Fitzgerald gives you the guide to discover, according to his theory, where you lie on the spectrum of these three neurotransmitters. Armed with this knowledge you can approach your favorite medical professional for a

prescription to raise and lower these levels as needed and voila, life is better.

Perhaps this is the biggest reason for clinicians, medical professionals, and students to read this book. You will likely disagree with some of the assertions, but you should be aware of them. Patients will undoubtedly study this text for clues into their own illness and will expect their physicians to at least be aware of the implications.

Like mixing ingredients to make a cake, Dr. Fitzgerald gives lists of drugs which either raise or lower specific neurotransmitters and states that clinicians should give two or three at the same time to adjust the brain back to normalcy. This flies in the face of established practice where clinicians are careful to slowly add and titrate psychoactive medications one at a time. It also decries the practice of switching drugs when one is not giving the desired effect—instead add another drug to the mix.

This is not to say that the theory cannot be carefully applied, but it does raise an interesting point about the safety of the interactions of these drugs. His own description of the side effects of commonly used medications and his persistent "brain freeze" should make clinicians pause a moment longer before prescribing. Their side effects can be complex and dangerous and merely mixing them into a therapeutic regimen based solely on their perceived neurotransmitter properties might be dangerous.

Dr. Fitzgerald does acknowledge that randomized control trials are needed to establish his theory, which is commendable. He readily admits that the available evidence does not entirely support (or denounce) this "Big Three" theory. If his purpose is to cause the reader to question the validity of the status quo, he succeeded. If he hopes to inspire researchers to create new drug combinations for mental disease treatment, he has at least opened the door. If he wanted to change the treatment practice of the current day, time will have to be the judge.

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MJM

AN INTERNATIONAL FORUM FOR THE ADVANCEMENT OF MEDICAL SCIENCE BY STUDENTS

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The *McGill Journal of Medicine* (*MJM*) provides an international forum for student contributions to the field of medicine. The scope of *MJM* articles encompasses clinical medicine (clinical trials, case reports, surgery, epidemiology, etc.), basic science (biochemistry, cell biology, physiology, cancer research, etc.), and the humanities (ethics, health policy, history of medicine, etc.). Submissions of original research articles, review articles, and articles relating medicine to the humanities ("Crossroads" articles) are encouraged. Submissions of single clinical case reports and special features are also welcome, as are letters and commentaries on previously published articles, discussing various medical issues, or communicating preliminary research results (see below).

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For all peer reviewed articles, each required component should begin on a new page and appear sequentially, as follows. Original articles: title page, abstract, introduction, methods, results, discussion, acknowledgements, references, tables, and illustrations. Case report: title page, introduction, the case, discussion, acknowledgments, references, tables, and illustrations. Review and "Crossroads" articles: title page, introduction, body of text, conclusion, acknowledgments, references, tables, and illustrations.

Tables

Tables must be submitted typewritten in the order corresponding to their first citation in the text and accompanied by brief titles. All non-standard abbreviations must appear below the table with an accompanying definition.

Illustrations

Figures and legends should contain sufficient information such that each figure is intelligible without reference to the text. Figures must be printed on high-quality paper. Photographs should be glossy in black and white. Authors may submit color prints but, if published, must bear the extra printing costs. Each figure legend (with figure title and corresponding figure number) should be submitted on a separate page.

REFERENCES

Reference citations should appear in numerical order in parentheses throughout the text and be listed in their order of appearance. References should be formatted according to the examples below.

Journal articles

1. Bunny B, Coyote WE, Le Pew P, et al. Impact trauma Caused by Descending GPI Anchors. McGill Journal of Medicine 2003; 7: 1-2.

Books

2. Bunny B, Coyote WE, Le Pew P. Subdural Hematomas. In: Jones J, ed. Head Injuries. New York: Acme Publishers; 1994: 249-260.

Internet

3. Bunny B. Computer-induced psychosis. Society for Cartoon-Computer Interactions. http://www.SCarComI.com/psychosis.html. 1999.

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All submissions of "peer reviewed articles" undergo a formal and anonymous evaluation process conducted by the *MJM* Editorial Board, comprised of trained students with diverse areas of expertise. This peer review process is performed in two stages. During the first stage, the manuscript is evaluated individually by each member of the Editorial Board. For submissions requiring revision, a detailed manuscript evaluation summary is returned to the author with suggestions for improvement. Manuscripts of high caliber proceed to the second stage, in which a review is performed by a McGill medical faculty member whose field of expertise correlates with the subject matter of the paper. Comments from the faculty expert are synthesized with the evaluations of the Editor-in-Chief, Executive and Senior editors, and members of the Editorial Board to achieve a decision to accept, defer, or reject the paper for publication. In all cases, a detailed manuscript evaluation summary is returned to the author along with any suggestions for revision.

After appropriate revision, the manuscript text must be resubmitted on diskette and a copyright transfer agreement signed. Prior to publication, authors will be required to review edited proofs without delay.

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Church & Dwight Canada Corp., the distributors of Trojan brand condoms in Canada, is committed to increasing the rate of consistent condom use by being the leaders in product innovation, consumer education and sampling to encourage responsible sex.

Recent statistics show that while the number of teen pregnancies is decreasing, the number of Sexually Transmitted Infections (STIs) among 15-24 year-olds is increasing substantially. In fact, the majority (65%) of STIs today occur in people under the age of 25.[2]

Over the past 15 years, a significant amount of education regarding safe sex has been directed at 15-34 year-olds; yet despite the fact that they are aware of the risks associated with unprotected sex, the rate of consistent condom use remains low.[6]

The truth about STIs

- 1 in 6 people will have an STI by age 25[1]
- Approximately 1.5 million people have an incurable STI[1]
- The highest rates of STIs in Canada occur amongst young adults between the ages of 15 and 24[2]

Condom Use

- 29% of males 15-19[3]
  44% of males 20-24
  do not use condoms
- 51% of females 15-19[3]
  53% of females 20-24
  do not use condoms

Sexually active adults who reported having sex without a condom:

- 44% of those aged 20-24[1]
- 33% of those aged 18-19[1]
- 22% of those aged 15-17[1]

Human Immunodeficiency Virus (HIV)
In Canada, 30% of people with living with HIV don't tell their partners because they don't know they have HIV[4]

Gonorrhea
Reported rates have close to doubled from 14.9 per 100,000 in 1997 to 28.9 per 100,000 in 2004[5]

Chlamydia
Close to 63,000 cases of Chlamydia were reported in 2004, the highest number of cases since the disease became reportable in 1990[5]

Syphilis
Syphilis is escalating in both males and females, but more so in males. 2004 rates for men were 15 times higher than in 1997 (6.3 vs. 0.4 per 10,000)[5]

1 Statistics Canada, data from the 1996/97, 1998/99, 2000/01 and 2003 National Longitudinal Survey of Children and Youth (NLSCY).

2 Public Health Agency of Canada. Canadian Communicable Disease Report, June 2005, 2002 Canadian Sexually Transmitted Infections Surveillance Report

3 Condom use, by age group and sex, household population aged 15 to 59, selected provinces, territories and health regions (January 2002 boundaries). http://cansim2.statcan.ca/cgi-win/cnsmcgi.exe?Lang=E&RootDir=CII/&ResultTemplate=CII/CII___&Array_Pick=1&ArrayId=1050048

4 Health Canada, Sexual Health and Sexually Transmitted Infections, http://www.hc-sc.gc.ca/iyh-vsv/diseases-maladies/hiv-vih_e.html

5 Public Health Agency of Canada. Canadian Communicable Disease Report. 2004 Canadian Sexually Transmitted Infections Surveillance Report: Pre-Release, May 2006

6 http://www.sieccan.org/pdf/sexual_health_qs.pdf & http://www.sexualityandu.ca/teachers/data%2D5.aspx

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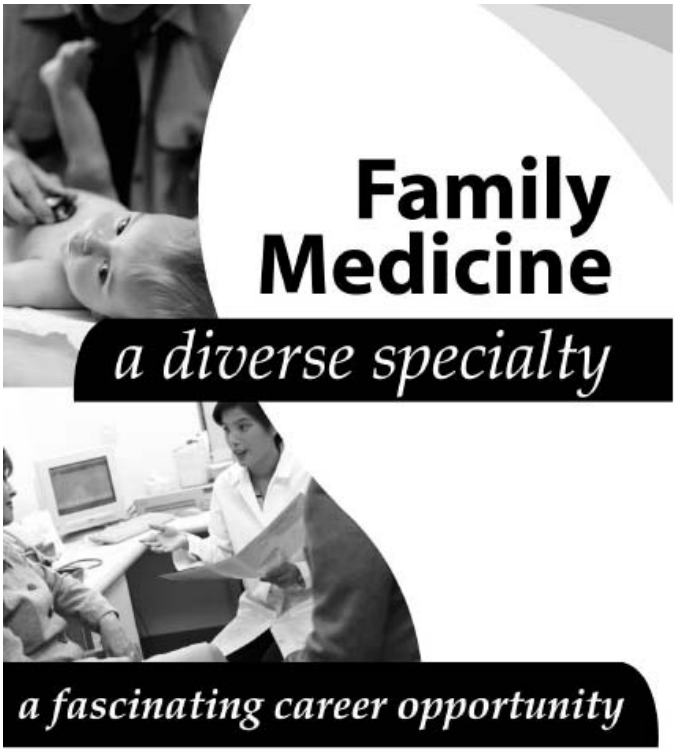

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To the right: **Hope**

Sheila Huang (MDCM 2008) is a 4th-year medical student at McGill University. This picture was inspired by an autoportrait of a cancer survivor. Sheila has been passionate about art and drawing since she was a child.

To the left: **The Naked Back**

Lubaba T. Shahrin is a 2nd-year student at Concordia University majoring in English Literature and studying a minor in Etudes Françaises. Despite her passion for art, over the years it only remained leisurely. Nevertheless, throughout her school career, art classes have always been a part. The figures shown here are taken from her classes on live figure drawing from her days at Marianopolis College. Aside from the human figures, she also enjoys drawing landscapes. Lubaba foresees a career as an English professor and a writer some day. But for now she hopes to finish her degree in English and meanwhile to carry on in taking art classes.