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AN INTERNATIONAL FORUM FOR THE ADVANCEMENT OF MEDICAL SCIENCE BY STUDENTS

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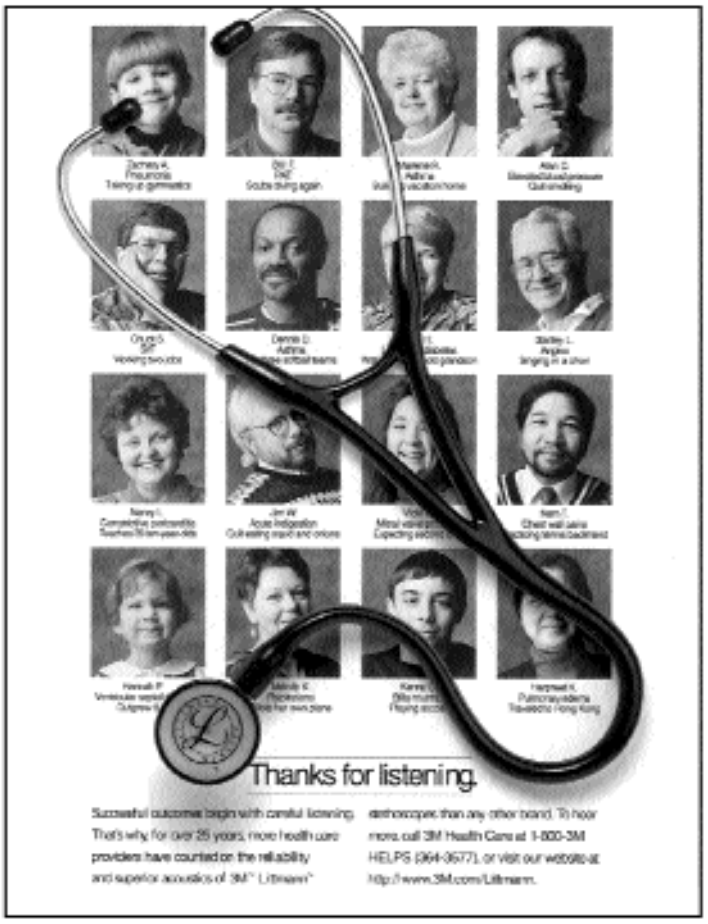

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EDITORIAL

STUDENT JOURNALS IN TODAY'S WORLD: DEFINING A NICHE

Some speakers like to use graphical terms such as "booming" or "exploding", to impress upon the audience that a particular field of medicine is progressing rapidly. Properly trained to avoid unsubstantiated claims, they will invariably toss in some astronomical number-the publications this field has produced in recent years-compare to another, numbler number of published works during a similar time frame only a decade or so ago, and hopefully mesmerize a room full of astonished people.

I urge you to try this at home. Log on to PubMed (1); then, with a few simple clicks, you will see that the field of angiogenesis has generated close to 9000 papers in the past four years, more than 8 times as many as a similar period between 1988 and 1992; you will also see how the field of apoptosis has indeed "exploded", publishing 58 times more papers as it did a decade ago; even my favorite family of cell-surface receptors, the integrins, are yielding their secrets, in published form, three times as fast-which translates into more choices for the Journal Club. Despite the excitement of being part of a vibrantly expanding scientific community, keeping up-to-date with scientific discoveries never seemed more intimidating. There is simply too much going on out there to read everything; a reader must be selective.

At first glance, this may seem to question the value of student-run journals such as the MJM. Completely reliant on student volunteers, these establishments are frequently understaffed, occasionally under funded, and are subjected to a yearly flux of its members (and consequently the continuous loss of hard-won experience). There would seem to be absolutely no reason for anyone to choose a student-run journal over Nature, or any one of its more than a dozen derivatives. Is there something that makes the MJM special?

The answer is a definitive and reassuring "yes". Being an "International Forum for the Advancement of Medical Science by Students", MJM looks at the medical world from the students' perspective-and this is our unique niche. MJM's focus on the student is reflected not only in the composition of our internal and external editorial staff, but also in the makeup of our contributing authors as well as our readership. In our Letters and Commentaries sections our readers discuss issues pertaining to students; clinical and/or basic science concepts are reviewed by students; student research is presented in MJM's Original Articles and Case Reports sections; even our Book Reviews, written by students, feature titles that may be particularly relevant and interesting to this group.

Furthermore, it has been our policy to extend MJM's vision to "help young, unknown scientists" (2) as they prepare and submit manuscripts, often for the first time. We are also upgrading MJM's peer-review system, by establishing an internet-based eMJM Forum, which will facilitate the participation of external student editors. I would like to encourage you to take advantage of these opportunities offered by MJM, and in turn help MJM serve as "an important forum for those who will be the leaders in the medical sciences" (2) in the near future.

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Alexander Zhai is the sixth Editor-in-Chief of the MJM. He has obtained a B.Sc. in Biochemistry at McGill university, and is currently in his fifth year in the M.D./Ph.D. Program. His current research is conducted in McGill Cancer Centre, and involves the interaction of cell surface glycoproteins. His research is funded by the CIHR studentship.

LETTERS TO THE MJM

PHYSICIAN-SCIENTIST TRAINING THROUGH MD/PHD PROGRAM

Dear *MJM*:

The physician-scientists, sometimes known as the clinical-investigators, have long been identified as an "endangered species" (1, 2, 3). In Canada, MD/PhD programs have been formalized in a number of medical schools since the 1980s (4). The Canadian Institutes of Health Research (CIHR) provides funding each year to support a limited number of students wishing to pursue a career that combines medicine with scientific research at one of nine institutes across the country (5). Students enrolled in these joint MD/PhD programs typically spend seven to nine years to complete it, during which they not only meet the requirements of the medical curriculum, but also defend their research work done at the PhD level. Funding offered by the CIHR, in the form of an annual stipend and research allowance, is available for up to six years.

The Canadian medical school that offers the joint MD/PhD degree run their programs differently. For example, some schools limit the length of time spent in the program, while other schools are less stringent. In addition, financial support available in addition to CIHR funding varies among schools. Furthermore, the manner in which the graduate research is integrated with the medical curriculum also differs with each school - some require that the PhD be completed all at once, while others allow students to alternate between programs in several month segments, until both programs are completed.

The Faculty of Medicine at McGill University in Montreal, Quebec, has maintained an active MD/PhD program for more than 10 years. The program typically accepts three MD/PhD students into the first-year medical class (6), but also accepts application from second-year medical students who are in the process of completing their pre-clinical training. On top of the annual stipend and research allowance, the program also pays for the students' graduate school tuition, and have additional funding that supports the students' travel to select scientific conferences. Additional scholarships are also available to certain students at the discretion of the MD/PhD advisory committee. The MD/PhD program coordinator, Dr. Jacquetta Trasler, herself a clinician-scientist, organizes weekly meetings for MD/PhD students in all years to give informal talks about their research. These meetings primarily provide the opportunity for students to stay scientifically well rounded, by learning about topics outside their field of study, but also serve to ensure that the students stay in touch with one another, as well as members of the MD/PhD advisory committee. McGill's MD/PhD program requires that each student complete his or her PhD in less than four years; and to ensure that this requirement is met, the MD/PhD advisory committee maintains close contact with each student, identifying issues that may hinder a student's progress through the chosen research project, and helping to provide solutions to problems that may arise during their course of study.

During informal conversations that I had with other McGill MD/PhD students, it was apparent that most of us felt positively about McGill's MD/PhD program. However, we do share some common concerns about the program - concerns that may reflect those of MD/PhD students at other universities. At the Canadian Society for Clinical Investigation annual conference, in September 2002, I will present these issues in the MD/PhD town hall discussion. The first area of concern was funding. We thought that better funding could be provided either by CIHR or by the faculty's MD/PhD program during the pre-research years. Furthermore, the travel allowance currently provided by CIHR should be increased to allow students more freedom in experiencing an integral and essential part of their research training. The second area of concern was that the provincial quota for out-of-province students was impeding our program from accepting the best MD/PhD candidates, as well as possibly preventing students from working with their preferred supervisors and/or research topics. Elimination of such quotas for the MD/PhD program would probably be helpful in solving this problem, especially since CIHR, the main funding source for an MD/PhD student during his or her research years, is a federal agency. The third area of concern was the lack of knowledge regarding career options after obtaining the MD/PhD degrees. Trainees were unclear about which residency training programs are supportive toward residents who want to continue doing both medicine and research. We think that there should be a central database, or source where MD/PhD trainees could find out this type of information. This would be much better than what some of us currently rely on - namely, anecdotes by past MD/PhD students.

Sincerely,

Anna Lee
Student Representative to MD/PhD Committee
Faculty of Medicine
McGill University

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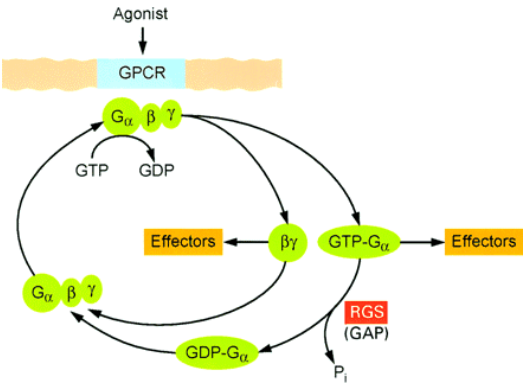

Figure 1. The GTPase cycle and role of RGS proteins in limiting the life span of GTP-bound Gα subunits.

RESEARCH LETTER

MOLECULAR CLONING AND EXPRESSION OF HUMAN REGULATORS OF G-PROTEIN SIGNALING 4, 5, AND 16

Dear *MJM*,

It has become clear, particularly over the past decade, that a relatively large family of heterotrimeric GTP-binding and hydrolyzing proteins play an essential transducing role in linking hundreds of cell surface receptors to effector proteins at the plasma membrane. These systems are widely utilized in nature, controlling processes ranging from mating in yeast to cognition in man. Receptors that activate G proteins are correspondingly diverse and encompass proteins that interact with hormones neurotransmitters, autacoids, odarants, tastants, pheromones, and photons. G-Protein Coupled Receptors (GPCRs) are septahelical integral membrane proteins that link to downstream signaling pathways through activation of heterotrimeric G-proteins; Gabg. Upon agonist binding, the receptor causes the associated Ga subunit to exchange GDP for GTP, thus activating the G-protein and causing dissociation into Ga and bg subunits. The Ga subunit has an inherent GTPase activity, which causes hydrolysis of GTP, reassociation with bg, and a return to the inactive state. The family of proteins known as Regulators of G-protein Signaling (RGS) accelerate the slow inherent GTPase activity on the Ga subunit, favoring the return of the G-protein to the inactive form (1 , Figure 1). The RGS proteins provide a mechanism by which cells can regulate both the duration and the magnitude of a signal generated through a heterotrimeric G protein. Such fine-tuning is undoubtedly essential for the orchestrated events that occur in response to chemokines, hormones and neuropeptides which signal through GPCRs.

Through understanding the biology of the signaling system, a number of diseases have been linked to altered expression of RGS proteins, including sepsis and cardiac hypertrophy (2,4,9). Sepsis and septic shock are systemic responses to infection, and afflicts more than half a million people annually in North America. Sepsis is characterized by severe systemic hypotension, peripheral vasodilation, and decreased tissue perfusion, despite elevated circulatory levels of vasoconstricting agents such as angiotensin II (AngII) and catecholamines. Since these vasoactive agonists function via GPCRs, hypotension induced by sepsis may partly be due to an increase in RGS gene expression. In fact, Panetta *et al*. (1999) have demonstrated that RGS1 and RGS16 were upregulated in the heart and aorta of septic pigs (2), while Grant *et al.* (2000) have identified that Ang II mediates RGS2 up-regulation (3). The discovery that RGS16 induction is mediated by PMA and TNF-a, a cytokine released in response to inflammation, further implicates the involvement of RGS in the pathophysiology of sepsis (4). In addition, it has been shown that RGS5 mRNA is expressed abundantly in the heart (5).

Heart failure is a condition that affects nearly five million North Americans of all ages and is responsible for more hospitalizations than all forms of cancer combined. Cardiac hypertrophy is an important adaptation in response to chronic heart failure, which in the longer term, leads to thickening of ventricular chambers and impaired contractility (6). Since decreased responsiveness of the β-adrenergic receptor (βAR) has been implicated in heart failure, it is possible that the elevated circulating levels of catecholamines that occur during heart failure may trigger an RGS mediated GPCR

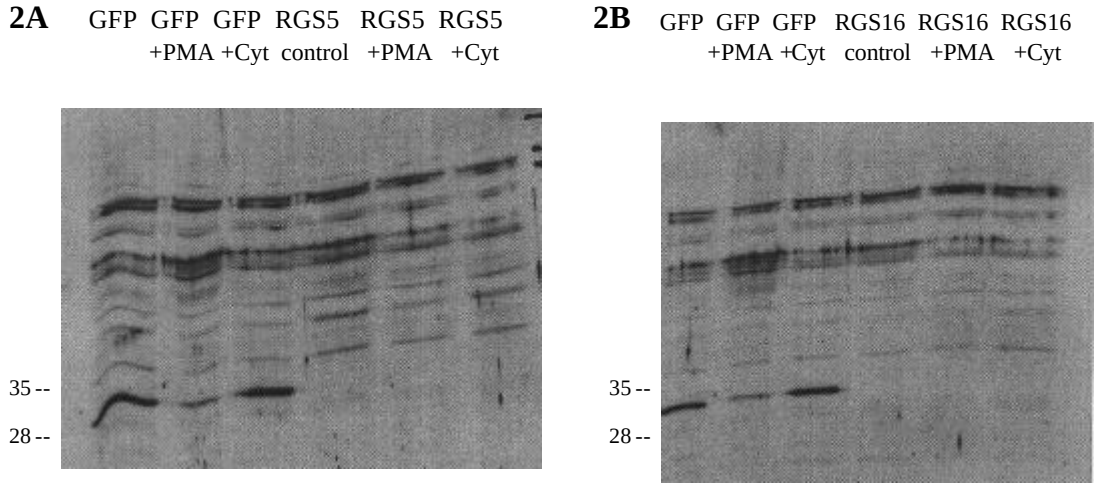

Figure 2. .Western Blot Analysis of RGS 5 (A) and RGS 16 (B) GFP fusion protein expression in SVEC-40 mammalian endothelial cells. Using anti-GFP antibody, it was determined that the RGS fusion proteins did not accumulate under both control (RGS control) and sepsis-like conditions (RGS+PMA, RGS+Cyt). As a control for transfection, the cells were transformed with the pGFP-N3 vector alone and expression of the GFP protein was measured under each of the three conditions (GFP, GFP +PMA, GFP + Cyt)

desensitization (7). Recent studies have demonstrated that RGS4 gene expression is up-regulated in cardiomyocyte hypertrophy (8), and in acutely failing donor hearts and end-stage heart failure (9).

In view of the fact that certain RGS proteins may be implicated in the inflammatory response that occurs during sepsis, the expression of green fluorescent protein (GFP) tagged RGS5 and RGS16 proteins was studied under normal and sepsis-like conditions in mammalian endothelial cells. PCR was used to amplify both RGS5 and RGS16, and primers were designed based on the coding sequences found at GenBank (10). For both RGS5 and RGS16, forward oligonucleotides contained a HindIII restriction endonuclease site and a consensus mammalian Kozak sequence 5' to the translational initiation codon. For reverse primers, a BamHI restriction endonuclease site was added to the 3'end of the RGS coding sequence, which lacked a stop codon. PCR using Taq polymerase was allowed to proceed for 20 cycles. RGS5 and RGS16 were subcloned into the pGFP-N3 vector to express the RGS proteins as GFP fusions in mammalian cells. Restriction mapping of pGFP-N3-RGS5 with HindIII/PstI and BamHI/PstI, and pGFP-N3-RGS16 with BglII and BamHI/ScaI restriction endonucleases produced bands of the expected size (not shown).

The mouse lymph node derived endothelial cell line SVEC-40 (ATCC no. CRL-2161) was transfected with the pGFP-N3-RGS5 and pGFP-N3-RGS16 plasmids using lipofectamine (Life Technnologies), and stably transfected cells were selected with 400µg/ml G418 (11). The SVEC-40 cells were grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. Sepsis-like conditions were induced by stimulating the cells with a cytokine mix (4 hours) consisting of TNFa (10 ng/ml), IFN-g (100 µm/ml), LPS (100 µg/ml), and IL-1b (50 µm/ml). The expression of RGS was also verified under PMA treatment (0.1 mm/ml, 2 hours), which is an important mediator of the inflammatory response. Transfected cells were lysed in SDS sample buffer. Proteins were extracted by sonication and separated on 12% SDS-polyacrylimide gels. After transferring to nitrocellulose membranes, the expression of GFP tagged proteins was examined using anti-GFP monoclonal antibody as previously described (12). Western blot analysis revealed that the RGS-GFP proteins did not accumulate under either of these conditions (Figure 2). As a control for transfection, the cells were transformed with the pGFP-N3 vector alone and expression of the GFP protein was measured under each condition.

In addition, to investigate the role of RGS4 in the β-adrenergic receptor desensitization observed during cardiac hypertrophy, full length human RGS4 (618 bp) was isolated from a human fetal brain cDNA library (Clontech). The forward primer contained a BamHI restriction endonuclease site and a consensus yeast Kozak sequence linked 5' to the translation initiation codon. The reverse primers added a XhoI recognition site 3' to the translational stop codon. PCR using Taq polymerase was carried out for 35 cycles. RGS4 was subcloned as a BamHI/XhoI fragment into the polylinker of p423GAL1, placing its expression under the control of the GAL1 promoter (13).

Since a number of the mammalian RGS genes can functionally replace the endogenous SST2 gene (5,16),

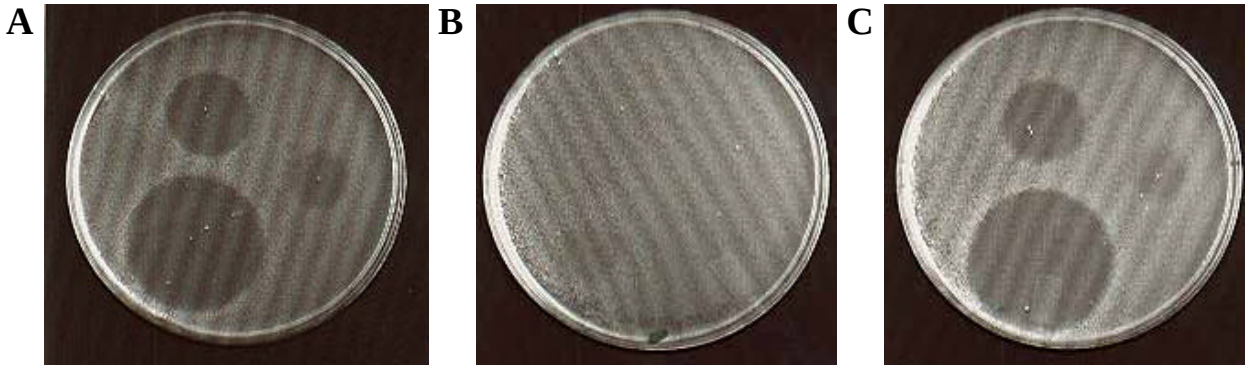

Figure 3. Yeast pheromone response halo assays. A p423GAL1construct containing full length RGS4 was transformed into BC-180 yeast cells and assessed for its ability to attenuate response to pheromone. A) p423GAL1 control plasmid. B) p423GAL1-RGS1, positive control plasmid. C) p423GAL1-RGS4. The assay was perfomed with 3 different concentrations of a-factor (10, 100, 1000 pmol, counterclockwise from right), Relative pheromone sensitivity is measure by the size of the clear area elicited in response to a given dose of α-factor.

the GAP activity of RGS4 was assessed using a yeast mutant lacking this RGS containing gene. These cells are hypersensitive to GPCR stimulation, and thus are hyper-responsive to growth inhibitory effects of a-factor pheromone (14). Thus, the Saccharomyces cerevisiae strain BC-180 (MATa, ade2-1, his 3-1D, leu 2-3, 112 ura 3-52, sst2-Δ2) was used to assess the ability of hRGS4 to complement its sst2 defect (14). Yeast cells were routinely grown on synthetic medium consisting of Yeast Nitrogen Base (YNB) containing 2% glucose supplemented with the appropriate nitrogen bases and amino acids. The construct (p423GAL1-RGS4) was introduced into yeast using lithium acetate as described ( ). The resulting transformants were selected by the omission of histidine. Negative control cells were transformed with p423GAL1 vector alone, whereas positive control cells were transformed with a p423GAL1-RGS1 construct that had been previously made (2). Replacing glucose with 2% galactose and 2% raffinose induced GAL1 dependant RGS expression. The halo assay was carried out essentially as described (16). Two μl of each of 3 concentrations of α-factor (10, 100, 1000 pmol) were spotted onto a lawn of yeast cells. The plates were incubated at 30^{o}C for 4 days. Results from the halo assay indicate that cells transformed with the plasmid p423GAL1 (negative control) showed a significant zone of no growth around the 3 concentrations of a-factor, whereas overexpression of RGS1, the positive control, significantly attenuated the pheromone response (Figure 3). However, the expression of RGS4 had little effect on the pheromone response, since p423GAL1-RGS4 transformed cells were phenotypically indistinguishable from negative control cells.

Heterotrimeric G-proteins are components of a complex membrane signaling system designed to transduce extracellular ligands into intracellular signals. RGS proteins increase the GTPase activity of Gα, thereby inhibiting its function. The expression of GFP tagged RGS proteins should permit studies of subcellular distribution in mammalian cells, since it has been demonstrated that RGS-GFP fusions maintain their GAP activity (17). Here, expression of RGS5-GFP and RGS16-GFP fusion proteins was studied in SVEC-40 cells under control and sepsis-like conditions. Since our experimental data suggests that fusion proteins did not accumulate in these cells, it is possible that the GFP-RGS fusion proteins are unstable. To address this issue, RGS5 and RGS16 will be tagged with the much smaller HA tag (9 aa), which may increase protein stability. Monoclonal anti-HA antibodies will be used to determine expression of the RGS-HA fusion proteins.

RGS4 was cloned into the yeast expression vector p423GAL1 and a pheromone response halo assay was performed as a functional assay for RGS GAP activity. Results indicated that RGS4 did not inhibit the pheromone response pathway. It is possible that the RGS4 protein may be unstable and rapidly degraded in yeast, or that the protein does not function with endogenous yeast G-proteins. However, western blot analysis to measure the level of RGS4 expression must await the availability of suitable antibodies directed to this protein. In order to generate specific antibodies directed against the RGS4 protein, an RGS4 cDNA was cloned into the TrcHisA vector. This will allow us to isolate milligrams of pure RGS4 protein that can be used as an antigen to generate RGS4 anti-sera in rabbits.

Through further studies, we would like to examine the possibility that RGS4 expression may be part of a negative feedback loop for the long-term regulation of cardiac hypertrophy. In fact, Rogers *et al*. have recently described that cardiac-specific co-expression of RGS4

in Gαq overexpressing mice, delays the cardiomyocyte hypertrophy of mice that overexpress Gαq alone (17). In addition, the laboratory of Dr. Greenwood has recently discovered that acute administration of the βAR agonist isoproterenol induces RGS5 expression in the heart.

There is increasing evidence that RGS plays a central role in the pathophysiology of sepsis and cardiac hypertrophy. Future studies examining the differential up-regulation of RGS proteins in a rodent model of catecholamine-induced myocardial hypertrophy, should clarify the mechanism that triggers βAR desensitization. It is clear that the RGS proteins will become the target for more intense investigation and pharmacological manipulation to treat critical illness.

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COMMENTARIES

ANTHRAX: AN OLD DISEASE RAISING NEW FEARS

Anthrax is a potentially fatal bacterial infection caused by the aerobic, Gram-positive rod *Bacillus anthracis*. It is a disease of great historical interest, which has recently been the subject of many headlines as a result of its potential use as a biological weapon. Anthrax is primarily a disease of herbivores, which are exposed to spores in the soil while grazing. The disease is most prevalent among domestic herbivores such as cattle, sheep, horses, and goats. The distribution of anthrax is worldwide. In Canada, "anthrax zones" include the western Prairies, northern Alberta, and the Northwest Territories (1). In North America, veterinary vaccination programs have drastically reduced the number of outbreaks. Anthrax continues to be endemic however, in regions of Africa, Central Asia, Spain, Greece, Turkey, Albania, Romania (2). Infection in humans develops when spores of *B. anthracis* enter the body through a skin abrasion, via ingestion, or inhalation. Ninety-five percent of human cases of anthrax are the cutaneous form and are the most often the result of contact with infected animals or animal products in an agricultural or industrial setting. Gastrointestinal anthrax, resulting from the ingestion of viable spores, is exceedingly rare. Approximately 5% of human anthrax cases are of the inhalational type.

History

Anthrax was first described over 3500 years ago. It is believed to have been responsible for one of the great plagues in ancient Egypt and cases were recorded by the ancient Romans (3). The anthrax bacillus was the organism used by Robert Koch in the development of his postulates and is considered the first "germ" to be proven to cause human disease (4). More recently, *B. anthracis* has been the organism of choice experiments of biological weapons. In 1941 the British released anthrax spores on Gruinard Island off the coast of Scotland. Spores capable of infection survived for 45 years until the island was decontaminated with formaldehyde and seawater in 1986 (5). The United States experimented with anthrax in the 1950s and 1960s until the program was stopped by Richard Nixon in the 1970 (6). In 1979, a large outbreak of anthrax in the former Soviet Union at Sverdlovsk resulted in the deaths of dozens of people. Though autopsy results were confiscated by the government, the source of the infections is thought to have been a nearby military microbiology plant (7).

Pathogenesis

Capsular polypeptide and anthrax toxin are the primary virulence factors of *B. anthracis.* Anthrax toxin consists of three proteins (8) including protective antigen (PA), edema factor (EF), and lethal factor (LF). PA binds to plasma membranes of target cells where it is cleaved by a cellular protease into two fragments. The larger of the two fragments remains bound to the cell surface and serves as a receptor that mediates endocytic enter of EF and LF into the cell. EF serves to increase intracellular cyclic adenosine monophosphate (cAMP), which, ultimately results in the massive edema seen in anthrax patients. At high doses LF causes lysis of macrophages. At lower doses it serves to increase expression of tumour necrosis factor (TNF) and interleukin-1 (IL-1). It is believed as infection progresses, the threshold for lysis is reached causing a massive release of inflammatory mediators leading to shock and death (6).

Clinical Manifestations

Cutaneous anthrax occurs when spores of *B. anthracis* are introduced into the skin. Within hours, the spores begin to germinate and release anthrax toxin. Soon thereafter, a small red macule appears at the site of inoculation. The lesion progresses to a papular stage and within 24 to 48 hours, the papules enlarge and become vesicular. The lesion ruptures during the first week and forms an ulcer encircled by a black eschar and surrounded by edema out of proportion to the size of the ulcer. The fully developed lesion is usually painless. In mild cases, the patient is afebrile with no constitutional symptoms, however, in more severe infections, associated features include fever, malaise, and regional adenopathy (9). Eighty to ninety percent of untreated cases undergo spontaneous healing. Ten to twenty percent of untreated cases lead to a bacteremia and death.(8) If recognized and treated promptly, cutaneous anthrax is very rarely fatal.

Gastrointestinal anthrax usually results from the ingestion of undercooked meat of infected animals. Bacteria are transported to mesenteric and regional lymph nodes leading to hemorrhagic adenitis, ascites,

and bacteremia. The patient presents with variable symptoms including severe abdominal pain, fever, nausea and vomiting, and bloody diarrhea. The pharynx may also be infected causing ulcers and edema of the neck, occasionally leading to airway compromise. Early diagnosis of gastrointestinal anthrax is difficult resulting in a high mortality rate (9).

Inhalational anthrax has historically occurred among wool workers and those closely associated with infected animals. It is aerosolized anthrax that is most lethal and has potential use as a biological weapon. Initial symptoms may present up to 6 weeks after exposure and closely resemble a severe viral respiratory disease, making early detection difficult. Accordingly, a high degree of suspicion is required to correctly diagnose this condition. Inhalational anthrax is not considered a true pneumonia. Though the 1 to 2 mm spores are an ideal size for alveolar deposition, the spores are engulfed by alveolar macrophages and transported to the mediastinal and peribronchial lymph nodes. The anthrax bacilli multiply in the lymph nodes and spread throughout the body and blood (10). The infected patient initially presents with fever, non-productive cough, myalgia, and malaise. Radiographs show a classic widened mediastinum, which is evidence of hemorrhagic mediastinitis and pleural effusions. One to three days after the onset of symptoms, the disease enters a rapid, fulminant course consisting of dyspnea, strident cough and chills, and culminates in death (10). The mortality of inhalational anthrax approaches 100% and treatment is rarely successful (8). The number of spores required for infection is not known. The U.S. Department of Defence estimates the number to be between 8000 and 10000 spores (11).

Diagnosis

In cutaneous anthrax, the ulcerative eschar must be differentiated from other causes of papular lesions causing lymphadenopathy. The most likely cause of such lesions is staphylococcal lymphadenitis (10). When in an endemic anthrax region, and the ingestion of contaminated meat is suspected, symptoms of an acute abdomen should be considered to possibly be the early signs of intestinal anthrax infection (10). Diagnosis of inhalational anthrax in the early stage is difficult because of the similarity of the presenting symptoms to a viral respiratory tract infection. The classic widened mediastinum on radiograph may help in diagnosis but must be differentiated from non-infectious causes such as superior vena cava syndrome or a dissecting or ruptured aortic aneurysm. The widened mediastinum may also be seen in an acute bacterial mediastinitis and fibrous mediastinitis due to Histoplasma capsulatum (10).

Regardless of the suspected route of infection, Gram staining of pertinent body fluids should be done to determine the causative organism. Serologic diagnosis may be made through a microhemagglutination test for the PA component of the anthrax toxin. Diagnosis may also be made by specific enzyme-linked immuno-sorbent assays (ELISA).

Prevention and Treatment

The anthrax vaccine is an inactivated, cell free product given in 6 doses (12). It is recommended for military personnel, individuals closely associated with potentially infected animals, and researchers who may be in contact with anthrax spores. It is not currently licensed for use in Canada but may be obtained through Health Canada's special access program (12). There are safety issues that have limited the use of the vaccine in humans. It has been associated with edema and necrosis at the site of injection and rarely death (6). Few comparative studies have examined the efficacy of human anthrax vaccines. A review of two studies (a 1962 US study using an inactivated vaccine similar to the US vaccine of today and a 1976 Russian study using a live attenuated vaccine) concluded that it carries an overall efficacy of 84% in preventing anthrax infection (13). A newer examination of the efficacy of the current vaccine is required. The ability of the vaccine to provide protection from aerosolized anthrax, such as in the form of a biological weapon, has not been tested and remains unknown (10).

B. anthracis is susceptible to penicillin, amoxicillin, chloramphenicol, doxycycline, erythromycin, strepto-mycin, and ciprofloxacin. Intravenous administration is recommended in cases of inhalational and gastro-intestinal anthrax as well as in severe cutaneous infections (10). It should be considered resistant to third generation cephalosporins, cefuroxime, sulfameth-oxazole, and trimethoprim. Of particular concern in bioterrorism is the fact that it is possible to manufacture anthrax to be resistant to penicillin. When biological warfare is suspected, ciprofloxacin is the drug of choice (14).

Summary

The history of anthrax and that of human agricultural development have been intertwined for thousands of years. In the present time, new fears have arisen of the use of *B. anthracis* as a biological weapon. Rapid recognition of the signs and symptoms of an anthrax infection is essential if antibiotic treatment is to be effective. This is especially true with inhalational anthrax. Rapid diagnosis of inhalational anthrax is made difficult by the non-specific nature of the initial presenting illness. A high degree of suspicion is

required to make a timely diagnosis. Health care professionals must have an understanding of the clinical presentation, the pathophysiology, and treatment of anthrax infection.

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ORIGINAL ARTICLE

A Comparison of Control Populations in Quebec Using the Short Musculoskeletal Function Assessment

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ABSTRACT The Short Musculoskeletal Function Assessment (SMFA) questionnaire is a health status instrument validated in the United States for use with patients with musculoskeletal impairments. It allows patients to self-rate their level of disability and impairment. This questionnaire has never been validated for Canada's English and French speaking populations. The first objective of this study was to determine the baseline responses of a healthy (i.e., without orthopaedic pathology) population representative of English and French speaking Canadians. The second objective was to compare the results of the Short Musculoskeletal Function Assessment to see if language or gender had any significant effect on the responses. A sample population (n=144) of Quebec Francophone and Anglophone subjects was interviewed using the Short Musculoskeletal Function Assessment questionnaire over the course of five weeks. All subjects were obtained from the orthopaedic clinic of the Montreal General Hospital in Montreal, Quebec. All subjects were self-reported as not being orthopaedic patients themselves in the past or present, and were merely accompanying patients of the clinic. Results were analyzed for differences between four groups using the ANOVA statistical test: Francophone females, Francophone males, Anglophone females, and Anglophone males. No statistically significant differences were detected between the four groups. Results were also analyzed for any differences between three age groups using the ANOVA statistical test, (15-35 years, 36-55 years, and 56 and greater years), with no significant differences detected. The overall Short Musculoskeletal Function Assessment values obtained for the four patient populations were: Francophone males = 5.41; Francophone females = 5.40; Anglophone males = 6.41; and Anglophone females males = 5.33. For the three age groups, the results were: 15 - 35 years = 6.87; 36 - 55 years = 5.22; 56 years and greater = 5.18. These results provide an initial baseline to which future orthopaedic patients can be compared, and suggest that analysis of the Short Musculoskeletal Function Assessment results may be compared across gender and language lines within Canada..

INTRODUCTION

Musculoskeletal disease is very common in North America and imposes a large direct and indirect economic cost. Arthritis alone accounted for 42.7 million patients and cost $US 65 billion in the United States in 1992, with the number of patients in 2020 being projected at 60 million. (1) In comparing the direct and indirect costs of various illnesses on the Canadian economy, musculoskeletal disease ranks third, behind only cardiovascular disease and cancer. (2) While no such projection data was available for Quebec or Canada at the time of publication, it can be inferred that musculoskeletal disease will also become more common in Canada.

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The use of functional status instruments plays an important role in the assessment of patients. These instruments are typically a series of graded questions answered by patients with regard to their levels of ability and impairment. They allow a rapid, cost-effective, and objective means of measuring the health level of patients. (3, 4) Such instruments are often designed for specific medical impairments of patient populations, and a variety of such instruments have been developed for patients with musculoskeletal impairments, such as the Arthritis Impact Measurement Scales (AIMS) (5), the Short Form-36 (SF-36) (6), and the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) (7). The Musculoskeletal Function Assessment questionnaire is a tool with which patients can self-rate their current levels of disability and impairment. It is intended for use with adult patients presenting with general musculoskeletal disease. Previously, it has been evaluated for use in the United States, and its validity and reliability have been studied, proving it to be consistent across gender and age categories. (8) The Short Musculoskeletal Function Assessment (SMFA) is a modified version (46 questions) of the 101-question Musculoskeletal Function Assessment, whose validity, reliability and consistency have also been established. (9, 10) To date, no studies have been done to validate a translation of the Short Musculoskeletal Function Assessment in a language other than English.

A validated version of the Short Musculoskeletal Function Assessment for use with Quebec's population would be useful in managing this group of patients. Such a tool would allow a means of comparing the efficacy and cost-effectiveness of various treatments for musculoskeletal disease, allowing for a more efficient allocation of health-care resources. It would also enable health-care workers to see if certain patient population subsets respond to treatments in a different fashion. However, any possible differences due to language or culture between the U.S. and Quebec populations must be taken into account. (11) Validating the pre-existing Short Musculoskeletal Function Assessment for the Quebec population would be more economical than developing a similar instrument de novo. In addition to validating the current Short Musculoskeletal Function Assessment for Quebec's Anglophone population, this would entail translating the Short Musculoskeletal Function Assessment into French for use with Quebec's Francophone population and validating this instrument as well. This has been performed with previous functional status instruments with favorable results. (12) For these purposes, a French translation of the Short Musculoskeletal Function Assessment was developed by the Department of Orthopaedic Surgery of the McGill University health Center (see Methods).

Table 1. Demographic characteristics of the healthy subjects administered the Short Musculoskeletal Function Assessment

Characteristics	Francophone		Anglophone	
	Women (n=40)	Men (n=30)	Women (n=34)	Men (n=40)
Age in Years, n (%)				
15-35	10 (25)	6 (15)	13 (32.5)	7 (17.5)
36-55	22 (55)	12 (30)	14 (35)	19 (47.5)
56+	8 (20)	12 (30)	7 (7)	14 (35)
Education, n (%)				
< high school	9 (22.5)	5 (12.5)	4 (10)	3 (7.5)
High school	16 (40)	14 (35)	16 (40)	20 (50)
University	11 (27.5)	10 (25)	7 (17.5)	7 (17.5)
> University	4 (10)	1 (2.5)	7 (17.5)	10 (25)
Race, n (%)				
White 38 (95)	27 (90)	32 (94.12)	27 (67.5)	
Hispanic	1 (2.5)	0 (0)	0 (0)	1 (2.5)
African-American	0 (0)	2 (6.67)	0 (0)	1 (2.5)
Asian/Pacific Isl.	0 (0)	0 (0)	1 (2.94)	5 (12.5)
Other	1 (2.5)	1 (3.33)	1 (2.94)	6 (15)
Marital Status, n (%)				
Married or living together	26 (65)	19 (63.33)	22 (64.71)	27 (67.5)
Widowed	2 (5)	0 (0)	2 (5.88)	1 (2.5)
Divorced or Separated	6 (15)	5 (16.66)	2 (5.88)	6 (15)
Never married	5 (12.5)	5 (16.67)	8 (23.53)	6 (15)
Missing 1 (2.5)	1 (3.3)	0 (0)	0 (0)	
Employment, n (%)				
Full-time	17 (42.5)	17 (56.67)	15 (44.12)	17 (42.5)
Part-time	3 (7.5)	0 (0)	4 (11.76)	3 (7.5)
Retired or unemployed	14 (35)	10 (33.33)	11 (32.36)	16 (.40)
Other / Missing	6 (15)	3 ()	4 (11.76)	4 (10)
Income, n (%)				
<$20,000	9 (22.5)	5 (16.67)	1 (8.82)	5 (12.5)
$20,001 - $70,000	18 (45)	20 (66.67)	16 (47.06	18 (45)
> $70,001	8 (20)	4 (13.33)	8 (23.53)	12 (30)
Missing	5 (12.5)	0 (0)	7 (20.59)	5 (12.5)

The purpose of this study is twofold. Firstly, the study attempts to establish base-line values for subjects without musculoskeletal problems within the overall Canadian population. These values are necessary to provide comparison with the results obtained from patients with musculoskeletal conditions in the future. Secondly, this study should help to determine whether or not the Short Musculoskeletal Function Assessment scores may be compared between Canada's Francophone and Anglophone men and women, and between patients of different age groups. This would also allow future investigators to determine to what extent reported

Table 2. Language and gender demographic comparison with the Short Musculoskeletal Function Assessment

	DIS	BIS	Short MFAIS
Francophone Women (n=40)	5.37±5.55	5.52±7.33	5.41±5.71
Francophone Men (n=30)	5.12±4.35	6.18±7.29	5.40±4.93
Anglophone Women (n=40)	6.40±5.33	6.43±9.75	6.41±6.32
Anglophone Men (n=40)	5.42±5.86	5.05±7.44	5.33±5.97
Inter-group difference	p=0.77	p=0.88	p=0.86

Table 3. Age demographic comparison with the Short Musculoskeletal Function Assessment

	DIS	BIS	Short MFAIS
Age 15-35 (n=36)	6.88±6.13	6.83±10.12	6.87±7.02
Age 36-55 (n=67)	5.32±5.47	5.66±7.65	5.22±5.64
Age 56+ (n=41)	5.27±4.31	4.93±5.94	5.18±4.49
Inter-group difference	p=0.31	p=0.58	p=0.32

DIS: Dysfunction Index score; BIS: Bother Index score; Short MFAIS: Short Musculoskeletal Function Assessment Index score. All scores are between 0 and 100, and are expressed here as mean ± SD.

scores are dependant on actual musculoskeletal disease, and to what extent they are dependant on language and/or gender among the Quebec population. It would also allow data regarding Anglophone and Francophone patients and male and female patients to be combined and compared without the fear of confounding variables. It would also help to determine the effectiveness of the translation of the Short Musculoskeletal Functional Assessment being used. By using non-musculoskeletal patients, any inter-group differences detected due to disease can be minimized.

METHODS

Translation of Short Musculoskeletal Function Assessment

The French translation of the Short Musculoskeletal Functional Assessment was produced using a translation/back translation technique. (13,14) This technique involved three translators who working as a group, translated the Short Musculoskeletal Function Assessment from English into French. Though not expressly trained in translation, the translators were all researchers in the Department of Orthopaedic Surgery at McGill University Health Center who were fluent in French and English. The newly produced French Short Musculoskeletal Function Assessment was then provided to a second similar group of three translators who had no knowledge of the wording of the original English Short Musculoskeletal Function Assessment. This second group translated the French Short Musculoskeletal Function Assessment back into English. The two groups then met to compare the original English Short Musculoskeletal Function Assessment and the reproduced English Short Musculoskeletal Function Assessment. Using the differences between the original and reproduced versions, the two groups then made adjustments upon the final wording of the French Short Musculoskeletal Function Assessment, in order to provide an accurate translation. Final changes to the French translation were made by consensus decision between the translators. This technique has been used previously to translate functional outcome tools with satisfactory results. (13, 14)

Subject selection

144 subjects were selected over the course of five weeks in the Montreal General Hospital's orthopaedic clinic. Subjects were chosen consecutively from persons that had come to the clinic accompanying patients, but who were not present as patients themselves. The writer, a third-year medical student, briefly interviewed subjects in French or English regarding their past musculoskeletal and general health history. Criteria for exclusion were: subjects currently receiving treatment for musculoskeletal problems; subjects having received treatment for musculoskeletal problems in the past with ongoing symptoms; non-residents of Canada; questionnaires in which greater than 50% of questions in any section were left unanswered; and inability to speak and read either English or French. Musculoskeletal problems were defined as arthritis (including but not limited to osteoarthritis), fractures, ligament injuries, musculoskeletal neoplasia, infections of the musculoskeletal system, congenital defects of the musculoskeletal system, and osteoporosis. Subjects were given either a French or an English Short Musculoskeletal Functional Assessment depending on which language they indicated a preference for, or which of the two that they used during a majority of their daily routine.

Scoring of the Short Musculoskeletal Functional Assessment

The Short Musculoskeletal Functional Assessment is composed of two parts: a 34-question Dysfunction Index and a 12-question Bother Index. The Dysfunction Index inquires into the amount of

difficulty the subject has performing tasks, as well as the frequency with which the patient experiences difficulty. The Bother Index inquires into the magnitude of intrusion imposed by the symptoms on various aspects of the patient's life. Each question allows the subject to respond on a 5 point scale, with a response of 1 indicating excellent function/no impairment and a response of 5 indication poor function/maximal impairment. (8) The scores for each index are calculating by summing the point values of the total responses (raw score) and subtracting from this value the minimum possible score for the Index (one point for each question, i.e. 12 points being the minimum possible score for the 12-question Bother Index), then dividing the total by the range of possible raw scores and multiplying by 100.

$$\frac{\text{(raw score-minimum score possible)}}{\text{range of possible scores}} \times 100$$

This formula allows both the dysfunction index and the bother index to be expressed on a 100-point scale, with a score of 0 indicating minimal disability and score of 100 indicating maximal disability. The overall Short Musculoskeletal Function Assessment Index score is similarly calculated from a raw score that is the sum of the dysfunction index and bother index raw scores. Questions left unanswered for each index were answered with the mean of the patient's answered questions. Questionnaires with more than 50% of the questions unanswered were discarded.

Score Analysis

The mean Dysfunction Index score, Bother Index score, and total Short Musculoskeletal Function Assessment Index score for each gender and language group (Francophones and Anglophones) were compared using an ANOVA statistical test. Similarly, the three Index scores were compared between three age groups (15-35 years, 36-55 years, and 56 years and over) using the ANOVA statistical test. The test was used to analyze the differences between the mean Index scores (dependent variable) of the various groups divided by the dependent variables of language and gender (Francophone men, Francophone women, Anglophone men, Anglophone women) or by age.

RESULTS

Demographic Characteristics of Subjects

The description of the subject population, grouped by language and gender, is presented in Table 1. The average age of the total subject population was 46.62 + 14.96 years, with a range of 15-81 years. 74 (51%) of the subjects were female, and 70 (49%) were Francophone. 20 subjects (14%) were non-Caucasian, 65 subjects (45%) were working full-time (more than 35 hours per week), and 94 subjects (65%) were married. The mother tongue of subjects was not recorded. The relationship between subjects interviewed and the patient(s) that they were accompanying to the orthopaedic clinic was also not recorded.

Comparison by gender and language

The comparison between the four subject subpopulations (Francophone women, Francophone men, Anglophone women, and Anglophone men) is presented in Table 2. Comparisons were made between total Dysfunction, Bother, and Short Musculoskeletal Function Assessment Index scores, but not by individual question. The null hypothesis for each comparison was that there was no significant difference between the four groups. The P-value for the dysfunction index is 0.77. The P-value for the bother index was 0.88. The P-value for the overall Short Musculoskeletal Function Assessment index was 0.86.

Comparison by age

The subject population was divided into three age groups, which were based upon previous studies using the Short Musculoskeletal Function Assessment. (6) The dysfunction, bother, and Short Musculoskeletal Function Assessment index scores for each group were compared with each other. Again, comparisons were made between total Dysfunction, Bother, and Short Musculoskeletal Function Assessment Index scores, but not by individual question. The results are presented in Table 3, with the null hypothesis again being that there was no significant difference between the age groups. The P-value for the dysfunction index is 0.31. The P-value for the bother index was 0.58. The P-value for the overall Short Musculoskeletal Function Assessment index was 0.32.

DISCUSSION

The findings of this paper support the hypothesis that the Short Musculoskeletal Functional Assessment is equally applicable for both men and women of Quebec's French and English speaking populations. No statistically significant differences were detected between control (non-patient) sample groups of Francophone women, Francophone men, Anglophone women, and Anglophone men for Short Musculoskeletal Function Assessment's dysfunction index, bother index, or total Short Musculoskeletal Function Assessment index scores. This indicated that the Short Musculoskeletal Function Assessment might be useful in making comparisons between

patients from among these groups in the future, and that any inter-patient difference is due to factors other than language or gender. It is possible that a difference in Short Musculoskeletal Function Assessment scoring between these groups might emerge among patients with musculoskeletal disease. This may be due to differences in patient perception of illness due to gender, language or culture. One way of testing this hypothesis may be to have patients rated in terms of disability by physicians and comparing physician-generated scores with patient-generated Short Musculoskeletal Function Assessment scores.

The Short Musculoskeletal Function Assessment also showed no significant differences among the three age groups examined. This indicates that Short Musculoskeletal Function Assessment scores may be compared between patients of these age groups without age having an effect on the score. However, there was more inter-group difference than expected, with age showing a statistically insignificant but potentially clinically significant inverse relationship to the dysfunction, bother, and Short Musculoskeletal Function Assessment index scores. This differs with the expected increase in Short Musculoskeletal Function Assessment score with age as predicted by the longer Musculoskeletal Function Assessment. (14) This may be an artifact due to small sample size, or may be due to increased frequency of sub-acute musculoskeletal injury in younger populations due to a more active lifestyle. Future studies of the Short Musculoskeletal Function Assessment in Quebec may be useful in clarifying this issue.

Other Short Musculoskeletal Function Assessment studies may wish to focus their attention on subsets of the Quebec population underrepresented in this study. For example, the subject population was derived from a single urban environment. A sample population from across Quebec would be of use in verifying the results of this study. In addition, the large majority of the subjects involved in this study were self-identified as Caucasian; future studies including a higher percentage of other ethnic groups would be useful. Related to this problem is the large immigrant population in Montreal whose mother tongue is a language other than French or English. This study did not differentiate between native French speakers and French-speaking immigrants whose first language was not French, or native English speakers and English-speaking immigrants whose first language was not English. While the results of this study seem to suggest that this should not be a confounding variable, larger studies evaluating the usage of the Short Musculoskeletal Function Assessment may address this issue. Finally, a comparison of Short Musculoskeletal Function Assessment scores by income was problematic due to a large number of subjects interviewed refusing to indicate their annual income. It would be of great interest to determine if there is a relationship between income and Short Musculoskeletal Function Assessment results.

In conclusion, the use of the Short Musculoskeletal Function Assessment in Quebec appears to be a useful tool for the self-evaluation of musculoskeletal patients in the future. Because of Quebec's unique linguistic populations, and due to the fact that the Short Musculoskeletal Function Assessment was developed in the United States, further testing of it will be required. However, it appears that the Short Musculoskeletal Function Assessment will be able to be used in Quebec in place of having to develop a separate functional assessment instrument for musculoskeletal disease.

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CASE REPORT

Hailey-Hailey Disease (Benign Familial Pemphigus): Carbon Dioxide Laser Therapy

Anatoli Freiman, BSc*†, Miriam D. Hakim MD†, Robin C. Billick, MD†.

INTRODUCTION

Familial benign chronic pemphigus (Hailey-Hailey disease) is a rare autosomal-dominant genodermatosis, characterized by recurrent skin eruptions mostly in the intertriginous areas. The clinical manifestations consist of closely grouped small vesicles with predilection for neck, axillae and groin areas. The vesicles usually progress to vegetating fissured plaques with bullae and erosions. Familial benign pemphigus differs from other forms of pemphigus in its genetic pattern, as well as by itsabsence of mouth lesions and absence of intercellular antibodies (1). Hailey-Hailey disease can be a chronic, debilitating condition, both physically and psychologically. Despite a wide variety of topical and systemic medical treatments, this illness presents a major therapeutic challenge due to a high recurrence rate. Review of the recent literature demonstrates that surgical modalities may offer the benefit of clearing active lesions with possible eradication of the disease in the treated areas. We present the successful management of a patient with Hailey-Hailey disease, unresponsive to conventional treatment, with a short pulse carbon dioxide laser therapy.

CASE REPORT

Our patient is a 34 y.o. white male with a longstanding history of vesicles, bullae and erosions in both axillae. The lesions would appear in crops and last for several weeks, aggravating during the summer months. His symptoms of pruritus, burning, pain, and malodor in the axillae caused significant discomfort, and made his occupation as a cleaner more difficult. The patient's family history was remarkable for his mother having similar eruptions.

Physical examination in our dermatology clinic revealed a young man in mild distress. Involving the right and left axillae, he had dry, crusted, scaling, rust-color plaques and erosions, with erythema more pronounced at the borders (Fig 1). The nape of the neck and the groin area were also mildly involved. The rest of cutaneous examination was unremarkable.

A 4-0mm punch biopsy specimen was taken from the affected axillar tissue. It demonstrated epidermal hyperplasia with presence of multifocal suprabasilar and intraepidermal acantholytic vesicles. The acantholytic cells had a "dilapidated brick wall" appearance. Histhopathologic diagnosis was consistent with Hailey-Hailey disease, otherwise known as benign familial pemphigus.

Prior to our treatment with the CO_2 laser, the patient had received conventional therapy, including topical antibiotics, antifungals and high potency steroids. Despite the best medical management, the patient continued experiencing major exacerbations without significant time duration between disease flares. Because of the refractory disease, the patient decided to pursue CO_2 laser therapy of his axillae, which were the main sites of discomfort. We went on to utilize the UltraPulse 5000 carbon dioxide laser and treated both axillae with four passes (300mJ, 60 watts, 200 pulses/sec settings). The surgery was successful, and there was a dramatic clearing of the active lesions immediately following the procedure (Fig 2). Postoperatively, the patient applied petroleum jelly to the surgical site until the wounds healed. Complete re-epithelization occurred within two weeks, and there has been no recurrence of the lesions noted in either axillar area during the 12-month follow-up period, which is still ongoing.

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DISCUSSION

Hailey-Hailey disease (Benign familial pemphigus) was first described 1939 by the Hailey brothers (2). This rare blistering disease is an autosomal dominant-inherited genodermatosis with incomplete penetrance. A positive family history is present in approximately two thirds of patients (3), while the rest of cases are believed to be new mutations, involving a defect in a calcium ATPase.

The pathophysiology of Hailey-Hailey disease is still not fully understood. Reports in the literature note that the underlying pathologic process is acantholysis and that the fragility of epidermis is secondary to a defect in the adhesion complex between desmosomal proteins and tonofilaments (4). This defect appears to involve interfollicular epidermal cells, whereas the adnexal keratinocytes are usually spared of the acantholytic process (5,6). Histologically, a widespread incomplete suprabasal acantholysis is the trademark of Hailey-Hailey disease, causing the well-known "dilapidated brick wall" appearance of the lower epidermis (5).

The clinical findings of Hailey-Hailey disease include vesicles and bullae arising on apparently normal skin. After the bullae rupture, erosions are seen, which may impetiginize. The condition manifests predominantly in the axillae, groin and intertriginous areas. The marked predilection for these sites is believed to occur because of the dense population by adnexal structures such as hair follicles, apocrine and eccrine glands. Hailey-Hailey usually appears only after puberty, as the adnexae mature (7). Clinically, the differential diagnosis includes intertrigo, candidiasis, and frictional or contact dermatitis (4).

The onset is mostly between the second and third decade of life and is triggered by friction and excessive sweating (6). It has been postulated that benign familial pemphigus may be expressed in any part of the body when various external stimuli insult the skin with an underlying primary defect (3). These triggers include trauma, friction, warm and humid environment, UV radiation, contact allergens (including ingredients in topical therapy), and infectious agents (bacteria, yeasts, HSV). A case of total-body generalized Hailey-Hailey disease has been reported in the literature (8). Super-infection of the lesions, particularly by *Staphylococcus aureus* or *Candida* species, is common, and is a trigger for further acantholysis and maintenance of pathologic process.

Hailey-Hailey has a variable, usually chronic course, with periods of remissions and exacerbations. Recurrences are more often and more severe in the hot summer months. The condition is often debilitating, both physically and psychologically. The patients suffer from pruritus, burning, intense pain and restricted mobility, which can be depressing. Malodorous discharge greatly affects social activity and patient's lifestyle. In severe cases, the condition may give rise to temporary or permanent disability.

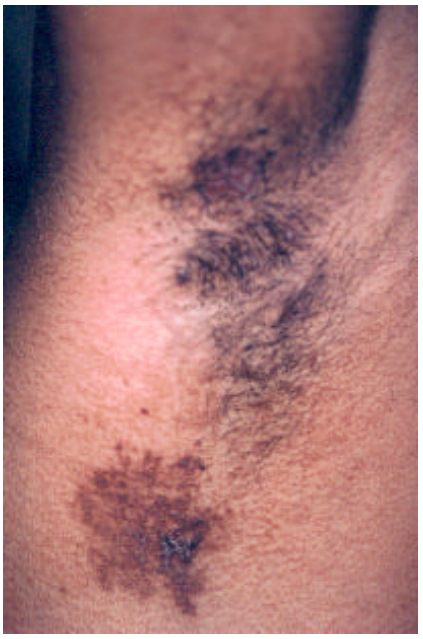

Figure 1: Pre-operative photograph of dry crusted plaques in the patient's left axillar area.

The treatment of Hailey-Hailey poses a challenge. Numerous conservative modalities have been used, ranging from topical and systemic therapy with antibiotics, antifungals, and coticosteroids to dapsone, methotrexate, thalidomide, etretinate and even cyclosporine (3,5,9). Whereas these treatment approaches may improve or even control the disease in a short term, they have not been shown to be effective on a long-term basis or for severe chronic forms of relapsing disease.

Surgical intervention has been introduced to control difficult cases refractive to medical therapy. The first successful report came in 1966 from Biro and Madday, who performed full-thickness excision of lesional skin followed by split-thickness grafting from the thigh (10). Kumar described the case of a patient with Hailey-Hailey disease who was treated by excision of lesional skin and subsequent primary closure of the wound (3). Since then, several authors presented favorable results obtained by excision of involved skin with subsequent grafting onto lesional sites. However, because of the morbidity and potential complications associated with wide excision of intertriginous folds (scar contracture with subsequent restricted mobility, graft failure, infection, thromboembolic disease and poor cosmetic results (9), alternative modalities had to be pursued.

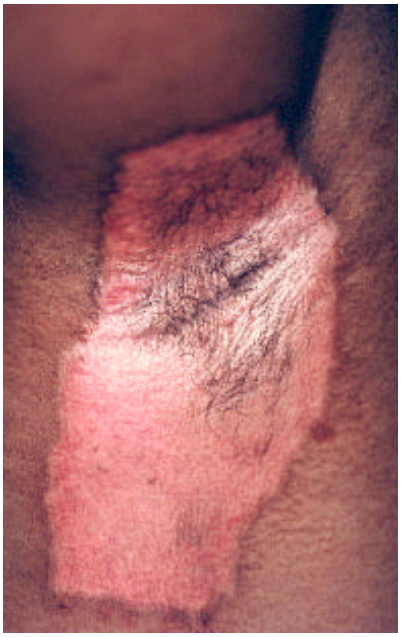

Fig 2: Two-week post-operative photograph of the patient's axillar area. Remarkable clearing of lesions is seen.

Belhaouari et al. (11) first suggested dermabrasion therapy in 1983, and in 1989 Hamm described successful dermabrasion in four cases of the Hailey-Hailey disease resistant to conventional management (12). Kirtschig et al. (12) subsequently reported two cases of patients in whom dermabrasion led to a long-standing absence of active skin lesions. Metze et al. (13) performed histological, ultrastractural and histochemical study of lesional and non-lesional skin of 18 patients with Hailey-Hailey disease. They note that none of the adnexal epithelia expressed the intrinsic defect of cell adhesion. Their finding offered an explanation for the success of dermabrasion - after complete removal of the involved epidermis, re-epithelization would occur from the skin appendages.

Recently, the use of carbon dioxide laser emerged as an effective option for treatment of numerous epidermal and dermal dermatological lesions, including benign and malignant growths, keloids, vascular deformities, warts, tattoos, etc. (14). The first report of successful carbon dioxide laser abrasion of Hailey-Hailey disease came in 1987 from Don et al. (3), who treated the inner aspect of the left thigh of a 50-year old man. The area remained disease-free during an 8-month follow-up period, as contrasted to the untreated opposite thigh. Kartamaa and Reitamo (15) described the use of continuous CO2 laser in six patients with Hailey-Hailey disease, demonstrating substantial improvement of disease-affected areas in most patients post CO_2 laser. The therapeutic success of the laser is attributed to its ability to selectively destroy the diseased epidermis while leaving the dermal structures intact (14). The adnexae, which presumably do not express the adhesion defect are responsible for re-epithelization following the ablation of the affected keratinocytes (6). Compared to dermabrasion, CO2 laser ablation is a careful, low bleeding method with less postoperative pain and rapid healing of the erosions. It also offers a safer work environment relative to dermabrasion.

More recently, Christian and Moy (9) suggested the use short-pulsed short-dwell carbon dioxide laser for the treatment of Hailey-Hailey disease. Lasers with short pulse durations generally cause less residual thermal damage than the ones with relatively long pulse duration, and are therefore associated with less erythema and faster healing. The authors presented a case of a 26-year-old woman with a 10-year history of refractory axillary Hailey-Hailey disease. They treated the right axilla with two passes, and the left one with three passes, repeating the procedure, with two additional passes, three years later. At a 3.5-year follow-up, recurrence was noted in the right axilla.

We went on to use UltraPulse 5000 short pulse laser therapy at four passes on our patient. The benefit of our intervention is demonstrated by the absence of recurrence of active lesions in either axilla over a 12-month follow-up period, which is still ongoing. We suggest that short pulse CO_2 laser therapy is a valuable treatment modality of chronic, symptomatic, localized plaques of benign familial pemphigus, particularly when conservative therapy has been unsuccessful. Further controlled studies, with more patients and longer follow-up periods will be needed to determine optimal laser settings, as well as to establish the long-term effects of the therapy and duration of remission.

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REVIEW ARTICLE

Aging of the Cerebral Cortex

Tak Pan Wong*, Ph.D.

ABSTRACT Significant structural trimming of neuronal structures in the cerebral cortex has long been considered as a primary cause of various age-related cortical dysfunctions. While recent findings provided additional data to support this notion, current understanding of cortical neuronal functions in aging also revealed the relationship of neuronal plasticity and imbalances between different neurotransmitter systems with the formation of age-related cortical dysfunctions. Manipulating these age-related alterations in neuronal function may be a novel therapeutic approach in the treatment of cortical dysfunctions in aging. This review will focus our current understanding of age-related changes in neuronal structures and functions in the cerebral cortex. Implication of these age-related alterations will be discussed.

The importance of the cerebral cortex in various motor and cognitive functions have drawn scientists' attention to the study of its age-related modifications in the last few decades. Although substantial structural and neurochemical changes in the aged cerebral cortex have been frequently reported, these changes displayed both temporal and regional specificity with age. In the following sections, critical findings regarding modifications in the aged cerebral cortex will be reviewed.

STRUCTURAL CHANGES

Shrinkage of the aged cerebral cortex

The most striking feature of aging brains is their shrinkage (1-3). The advent of magnetic resonance imaging (MRI) has provided an accurate non-invasive proof of cortical shrinkage with age (4). This age-related shrinkage also coincides with the weight loss (5,6) and expansion of the ventricular volume in the aged brain (7). For instance, human brains from individuals over 60 years old have been shown to be 17% lighter than brains of young adults (8).

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It is important to note that this age-related shrinkage is region specific. For instance, Haug and coworkers (9) have shown that areas 7 and 17 (parietal and occipital cortex) exhibited no shrinkage in aged brains, while >15% atrophy was found in areas 6 and 11 (extrapyramidal and orbital cortex).

Neuronal loss

Extensive neuronal loss in the aged brain has long been suggested to be the primary factor explaining age related neuronal shrinkage. Cortical neuronal loss in the aged brain was first reported by Brody's group (1). Further study of cell loss in the neocortex showed that primarily large neurons are lost during aging (1,10,11), although loss of small neurons have also been reported (8,12). Indeed, Meier-Ruge and coworkers (13) have hypothesized that 100,000 neurons in the human brain disappear daily resulting in a 19.7% reduction in cell number at the age of 80.

The occurrence of extensive neuronal loss in aged brains was questioned by a finding from Haug and coworkers (14). Haug's group found that using the common method for tissue preparation, young cortical tissue actually shrinks more than old tissue in histological preparations. Since most studies of cell count had been based on cell density measurement, Haug's group raised the possibility that the number of

neurons in young brains was overestimated. After correcting for shrinkage, Haug's group observed no neuronal loss but an increase in neuronal density after a decrease in brain volume in the aged neostriatum and cerebral cortex (15). An independent study from Terry and coworkers also supported no age-related cell loss in the cerebral cortex (11). Recent findings of no age-related loss of cortical neurons in both monkeys (16) and humans (17) further weaken the significance of neuronal loss with age (for review, see 18).

One of the major factors in causing the brain shrinkage with age is the loss of white matter. MRI studies have revealed significant loss of the white matter in aged brains (19,20). In particular, Guttmann and coworkers reported that the loss of white matter is more serious than the loss of gray matter in the cerebral cortex from aged human subjects. Similar findings were obtained from Peters and coworkers using monkeys as their animal models (21). They also showed that the loss of white matter in the aged monkey neocortex correlated closely with their age-related cognitive decline. Damage of myelinated fibers with advancing age has been shown to be the prime factor causing the loss of white matter volume (22,23). Interestingly, oligodendrocytes, which are responsible for the formation of myelin in the brain, displayed various age-related modifications, including swollen processes, inclusion of aging pigments in their cell bodies, and aggregation with other oligodendrocytes (24). These changes in oligodendrocyte may be related to the loss of myelinated fibers in aged brains. In summary, there is no conclusive evidence supporting a significant loss of neurons with age. Instead, loss of white matter could be an important factor in contributing to the overt brain shrinkage.

Dendritic loss in aging

Dendrites in the brain are important neuronal structures for synaptic contacts. They account for 90% of the total surface area of a neuron's receptive surface area (25,26). Synapses make contacts on dendritic shafts and specialized dendritic structures called dendritic spines. Most synapses containing excitatory neurotransmitters like glutamate establish contacts on dendritic spines (27,28). Significant age-related loss of dendrites in the cerebral cortex has been first reported by Scheibel and coworkers (29). These age-related dendritic losses include both shortening (30,31)and fewer dendritic branches (32,33). Losses in basal dendrites might have some regional specificity. For instance, more accentuated loss of basal dendrites was reported in the deeper cortical layer V when compared to superficial layers II/III (34,35). It is also important to note that dendritic losses in aged brains are not an inevitable process. For instance, no loss of dendrites in layer II pyramidal neurons has been reported in the entorhinal cortex of aged rats (36).

Loss of dendritic spines is another consistent change in aged pyramidal neurons. Spine loss on basal dendrites has been frequently reported (see 30,31). This loss is so prominent that up to 50% decrease in dendritic spines could be found, representing a much higher rate of loss than the mean of 10-20% loss of dendrites in the age brain (37,38). Taken together, these studies provide evidence for a substantive loss of dendrites and dendritic surface of pyramidal neurons in aged brains. This significant loss of dendritic structures may limit the availability of postsynaptic substrate in aged brains for synaptic connections.

Synaptic changes

Synapses are the most important structures for neuronal communication. These structures link neurons inside the brain by directionally conveying neuronal information with different neurotransmitters. The importance of these structures in cognitive function has been recently addressed in the studies of synaptic loss in Alzheimer's disease (AD, see 39,40). Studies of synaptic loss during normal aging have been massively explored in the last two decades.

Quantitative studies using electron microscopy revealed significant loss of synapses with age in laboratory animals (41,42) and humans (43,44). This age-related structural change also displays regional specificity. For instance, in Wistar rats aged from 3 to 17 months, there was a 22% decrease in the synaptic density of the associative cortex, but only a 9% decrease in the motor-sensory cortex (45). In fact, some cortical regions, such as the piriform cortex, have been shown to be devoid of aged-induced decline (46). In addition, not every kind of synapse is altered equally with age. Adams's group has reported age-related loss of asymmetrical synapses, but not symmetrical synapses, in the layer I region of the somatosensory cortex in aged humans (43).

Apart from synaptic loss, age-related modification of synaptic structure has been reported. Adams and Jones (41) showed that terminals in the parietal cortex of aged rats contain fewer mitochondria, synaptic vesicles, reduced vacuolar and tubular cisternae, and displayed smaller presynaptic area. Fewer mitochondria were also observed in postsynaptic dendritic spines in the same study. The loss of these intracellular structures may compromise metabolism and function of synapses in the aged brain. However, loss of synapses and changes in presynaptic structure

has been shown to be accompanied by an increase in the mean length of postsynaptic active zone (47). These structural modifications in the remaining synapses of the aged brain may represent a compensatory phenomenon to maintain normal cortical synaptic function.

In the cerebral cortex, the available evidence so far points to significant structural loss with age. They include disappearance of dendrites, dendritic spines, and synapses in the aged cerebral cortex. Since the number of neurons probably remains rather stable in the aged cerebral cortex, the loss of these pre- and postsynaptic structures would result in a substantial loss of interneuronal connections in the aged cerebral cortex. In the following section, I will summarize age-related modification of two different neurotransmitters, which play important roles in neuronal communication.

NEUROCHEMICAL CHANGES

Age-related modification of different neurotransmitter systems in the cerebral cortex has been extensively documented. The present evidence would indicate that neurotransmitter systems are affected differentially by aging. For instance, studying the concentration of serotonin, norepinephrine, and dopamine in the cerebral cortex of rat brains at different developmental stages has shown that while serotonin concentration remains unchanged until very old aged (3 years), levels of norepinephrine and dopamine progressively decrease starting at 1 year of age (48). Thus, instead of resulting from a general decline in neurotransmission, the reduction of neuronal function in the aged brain is more likely caused by an imbalance between different neurotransmitter systems. The following passages will discuss the major excitatory and inhibitory neurotransmitters, which are glutamate and g-aminobutyric acid (GABA), respectively.

Glutamate

Glutamate is the most important amino acid in mediating excitatory synaptic transmission of the cerebral cortex (49,50). Efferent fibers from the cerebral cortex to either extracortical or cortical regions utilize glutamate as a neurotransmitter (51,52). Most glutamate immunoreactive neurons are pyramidal neurons. Since glutamate also plays important roles in cellular metabolism, its role as a neurotransmitter has been historically debated vigorously (53,54); however, its role as a major neurotransmitter is widely accepted today. There are four major types of glutamate receptors (55,56). N-methyl-D-aspartate (NMDA) receptors, a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, and kainate receptors belong to the family of ligand-gated ion channels. The last group of glutamate receptors is the G protein-coupled metabotropic glutamate (mGluR) receptor.

The multiplicity of glutamate functions in the nervous system makes the presence of glutamate a poor indicator of glutamatergic synaptic function. Indeed, both decrease (57,58) and no change (59,60) in the basal glutamate level have been observed in aged rat brains. Studying the evoked release of glutamate also revealed conflicting results. For instance, using the same strength of electrical stimulation that elicited an increase in glutamate release in the prefrontal cortex of young rats failed to induce any change in glutamate release in the same cortical region from aged rats (61). However, glutamate release caused by high extracellular potassium, which induced depolarization of neurons, is actually higher in brain tissue from older rats (62).

By far the most consistent age-related change in the glutamatergic system is the loss of glutamate receptors. Significant decreases in the mRNA level of glutamate receptors were found in the aged cerebral cortex (63). Among different glutamate receptors, NMDA receptors are preferentially altered in the aged cerebral cortex. Decreases in NMDA binding was shown in the monkey's parietal and occipital cortex (64,65). In rodents, decreases in the number of NMDA receptors with age has also been shown (66,67).

Apart from a decrease in NMDA receptors in the aged cerebral cortex, studies of the change in different NMDA receptor subunits with age also displayed significant age-related modifications. For instance, mRNA level of both NR1 and NR2B subunits of the NMDA receptor have been shown to decrease preferentially in the aged cerebral cortex, whereas no age-related change was observed in the NR2A subunit (68). The modification of subunit expression may alter the receptor composition of NMDA receptor in the aged brain and lead to age-related changes in the binding properties of this receptor (69,70) and/or physiological properties such as desensitization (71).

Various findings suggest that kainate and AMPA receptors may exhibit greater resistance to age-related change than NMDA receptors. Binding studies performed with homogenized cerebral cortex revealed significant decrease in NMDA but not AMPA and kainate receptors (72). This lack of change in the AMPA and kainate subtype may be due to several factors. First, the age-related change of AMPA and kainate receptors may be restricted to fewer cortical regions than the NMDA subtype (73). In addition, the loss of AMPA and kainate receptors may occur at a later time point of

aging than NMDA receptors (74). Finally, the smaller decrease in AMPA receptor with age may be due to its plasticity towards age-related insults. For instance, separating the age cohort by their cognitive performance revealed an increased in the binding of AMPA receptors in the aged-impaired group (75). Although little has been done on the age-related change in metabotropic receptors binding, a decrease in the density of metabotropic receptors has also been reported in the frontal cortex (74). Taken together, these findings support a significant loss of postsynaptic glutamatergic receptors, especially the NMDA subtype, in the aged brain.

It is however important to note that a decrease in receptor density does not always give rise to lower excitatory synaptic function in the brain. For instance, while the density of NMDA receptor decreased in an accelerated senescence mice model, the level of glutamate and the amount of glutamate release in both the hippocampus and cerebral cortex was increased (76). In addition, decrease in NMDA receptor density has been shown to parallel by an increase in the affinity of these receptors in aged brain (66,77). Whether these potential compensatory changes in receptor function could maintain a normal excitatory synaptic function in the aged cerebral cortex remains to be established.

GABA

GABA is the major inhibitory neurotransmitter in the cerebral cortex (78). This neurotransmitter is present mainly in intrinsic neurons (79,80). Indeed, 10-15 percent of cortical neurons have been shown to be GABAergic (81). GABA receptors in the cerebral cortex can be separated into the GABAA and GABAB subtypes.

Decrease in GABAergic parameters with age has been frequently reported. For instance, the level of GABA in the cerebrospinal fluid from aged human is lower than in younger controls (82,83). In the cerebral cortex, decrease in the GABA content with age has been reported in preparations using either synaptosomes (84) or microdissected tissues (58). GABA transport also decreases significantly with age (85). Finally, a decrease in GABAB receptor mediated postsynaptic current has been observed in the aged brain (86).

While there is evidence to support a decline in the level of GABA with age, no evidence supported a decrease in GABAergic neurons in the aged cerebral cortex (87,88). Unlike glutamate receptors, binding studies revealed inconsistent alterations in the level of GABA receptors in the aged cerebral cortex. For instance, binding of GABAA receptors in aged brains is either lower than (89,90) or similar to the level of that in young brains (65,91). Interestingly, although significant decreases in the level of mRNA of different GABAA receptor subunits with age have been reported (92,93), there was not an age-related change in protein expression of different GABAA receptor subunits (94). Finally, no change in the binding of allosteric ligands at GABAA receptors with age was observed. Studying the binding of benzodiazepine site at the GABAA receptors also revealed no change with age (95,96). Binding of GABAB receptors also revealed little modification with age (97). These observations suggest that GABAergic receptors could be less vulnerable than glutamate receptors in aging.

Despite the lack of any modification in the binding of GABAA agonists and modulators, binding of the GABAA receptor-coupled ionophore in the cerebral cortex is decreased significantly in the aged brain (91,92). A decrease in picrotoxin binding, which requires an open receptor/channel, was also observed in the aged cerebral cortex (98). These findings suggest that the kinetic/structural properties the GABAA receptors, instead of its density, are affected in aging. However, it is not clear whether these changes would result in a substantial decrease in the inhibitory neurotransmission in the aged cerebral cortex.

FUNCTIONAL CHANGES

Decreases in the functional capacity of the central nervous system with age occur universally in all living organisms. For instance, significant alteration in the gait control, sleeping cycle, and learning and memory with age are the three commonest neural impairments in aged humans (for review, see 99). While these functional alterations in aged brains may be related to the structural and neurochemical modifications I have summarized in the last few sections, mechanisms underlying these age-related deficits are still largely unknown. The formation of complex behavioral responses relies on an even more complicate activation and inactivation of different group of neurons, whose activities are in turn determined by countless synaptic inputs. Age-related modification of cortical activities from systemic to synaptic levels will be discussed.

Systemic level

Knowing that performing certain cognitive or sensorimotor task can evoke reproducible brain activities in particular cortical area, alteration in the response pattern with age can be an indication of the

age-related functional modification of the studied cortical area. Using electroencephalography (EEG), which reveals electrical activities from a group of cortical neurons or a cortical area, cortical brain potentials evoked by performing memory tasks have been shown to be diminished and delayed in the elderly (100,101). Brain potentials in response to sensory inputs also have a longer delay in aged subjects (102,103). Measurements of EEG in rodents also revealed significant decreases in amplitude (104,105) and a delay in the appearance of evoked brain potential (106). Apart from a decline in evoked cortical activities, alteration of the pattern of brain potentials can also be found in the aged cerebral cortex (107).

Using functional magnetic resonance imaging (fMRI) also revealed a positive correlation between the reduction in cortical activation and cognitive performance. For instance, decrease in cortical activities in aged people has been matched with the decline in working memory formation (108). Comparing the activation of cortical tissue upon auditory stimulation also revealed significant age-related decreases (109). Thus, results from these noninvasive recording techniques support a decline in evoked cortical activity with age.

Cellular level

Studies of spontaneous activities of cortical neurons found significant decrease in the firing rate (110,111). In addition to single unit recordings, measurement of activity from multiple neurons simultaneously in the parietal cortex also revealed significant decrease in discharge rate in aged rats (112). However, a decrease in neuronal firing rate is not necessarily a universal phenomenon of aging. Lack of age-related change in spontaneous neuronal firing rate has also been reported (113,114). The inconsistency in age-related changes in neuronal firing rates may be due to the methodology used in these studies. Alternatively, loss of spontaneous neuronal activities may be restricted to specific brain areas only. In addition, factors which determine the firing of a neuron, including the threshold for the action potential, strength of excitatory and inhibitory synaptic inputs, can be differentially affected in aging (for a review, see (115)). Thus, understanding the change in these cellular and synaptic parameters with age may provide important information to the modification of neuronal firing in the aged cerebral cortex.

Apart from decreases in firing rate, modification of neuronal firing pattern with age may play important roles in age-related deficits. For instance, neurons in the suprachiasmatic nuclei from aged rats displayed aberrant firing patterns, which may be the basis for the decline in circadian rhythms with age (116). However, no evidence so far shows a similar modification of cortical neuronal firing pattern with age.

Synaptic level

While the loss of synaptic structures with age is a widely accepted modification in the aging cerebral cortex, little is known about the functional significance of this structural loss in the cerebral cortex. Most of our current understanding of age-related changes in synaptic function stems from studies of the hippocampus.

In the CA1 region, significant loss of synapses in the CA1 area have been shown to match with a decrease in evoked synaptic potential (117). Loss of evoked monosynaptic GABAB-mediated synaptic potential IPSPs has also been observed in the CA1 area (86,118). However, compensatory changes to maintain the magnitude of synaptic potential have also been reported. For instance, studying the modification of perforant path - dentate granule cell synapses in aged rats showed a significant reduction in the maximal field excitatory synaptic potential in aged rats, which is matched with the loss of synaptic terminals in this area. However, for a given magnitude of stimulation, a larger synaptic potential was obtained in aged rats, suggesting that the strength of remaining synapses in aged rats are in fact higher (119,120). Indeed, compensatory changes in the CA1 area of aged rats have also been reported. For instance, the NMDA receptors mediated EPSP has been shown to increase in the aged CA1 region (121). These compensatory alterations in synaptic function may explain a relatively slight or minimal age-related functional change concomitant with a substantial structural loss in aged brains (122).

Another well-known modification of synaptic function in the aged hippocampus is the reduced capability in the formation of long term potentiation (LTP). Repeatedly stimulated afferent fibers have been shown to induce an enduring increase in synaptic transmission, which has been regarded as a cellular mechanism of learning and memory (123). Both activation of NMDA receptors and nitric oxide have been shown to be important in the LTP induction (for review, see (124)). In aged rats, the threshold for the induction of LTP is increased (125,126), and the decay of LTP is accelerated (127,128).

Plasticity

Significant plastic changes in aged brain have been widely reported. In the presence of substantial synaptic loss, both an increase in the number of dendrites (129) or enlargement of remaining synaptic boutons (130) have been shown in aged tissues. Interestingly, the topographic organization of sensory inputs in the

somatosensory cortex is modified with age (131). In addition, performing the same cognitive function can activate different cortical structures in young and aged brains (132)). Since topographic rearrangement of sensory inputs in the cerebral cortex can be induced after damages of sensory afferents (for review, see 133,134), the topographic changes in aged brain may be the consequence of age-related structural loss in the aged cerebral cortex.

Although aged cerebral cortex still displayed a certain degree of neuronal plasticity, various evidences point to a reduced tolerance of the aged cerebral cortex towards lesion or detrimental influence. For instance, stress induced increase in glutamate release is five times higher than the level found in younger animals (135). This increase in extracellular levels of excitatory neurotransmitters could result in damage of neuronal tissues in the aged brain (for review, see (136). Lesion studies also revealed similar decline in plasticity of the aged cerebral cortex. Lesions of the cerebral cortex after hypoxic insults are more severe in old rats than in younger controls (137). While lesion of the nucleus basalis magnocellularis caused an upregulation of GABA receptors in the frontal cortex of young rats, no modification of GABA receptors was observed in aged rats (138). Indeed, the structural and functional changes in the aged cerebral cortex discussed above may limit the available resource for coping with insults.

CONCLUSION

While the majority of studies in the literature regard age-related reduction in cortical synaptic structures as the primary substrates of age-related decline in learning and memory, the characteristics of these age-related structural losses also shed light for possible manipulation of the loss of cortical functions in aging. Firstly, reduction of synaptic structures in aged cerebral cortex displays regional and temporal specificity. For instance, glutamatergic and GABAergic have been shown to exhibit different extent of modification in aging. The imbalance between these neurotransmitter systems may have a more direct impact to cortical functions than solely a morphological trimming of synaptic structures in aged brains. Interestingly, we have shown that the ratios of spontaneous glutamatergic and GABAergic synaptic event in normally aged and aged impaired rats are different (139). Restoring the imbalance between different neurotransmitters in aging may be a novel therapeutic approach in treating age-related cortical dysfunction. In addition, while aged brains exhibited compromised neuronal plasticity, aged brains still possess remarkable compensatory capability. For example, we have showed that while pyramidal neurons in aged brain receive fewer synaptic inputs than young rats, frequency of spontaneous synaptic inputs between young and aged rats are similar (140). These compensatory potential in aged brain may be another therapeutic targets for correcting aged-related functional deficits, which in turn not only will benefit the life quality of the aged population, but also will reduce the financial burden of treating aged related dysfunctions in our society.

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REVIEW ARTICLE

Isoniazid, The Frontline of Resistance in Mycobacterium tuberculosis

James B. Whitney B.Sc.† and Mark A. Wainberg Ph.D.*†

ABSTRACT Tuberculosis is an ancient disease that has held close association with humans for millennia. Through persistence, this remarkably successful organism has managed to infect an estimated third of the world's population. Declining rates of tuberculosis in developed nations have masked an emerging epidemic of drug resistant cases that have been reported in almost every country under scrutiny. The recent completion of the genome sequence of *Mycobacterium tuberculosis* has mandated more efficient control and management of this disease. The momentum for this public health imperative will come from information gleaned from advances in genomics and related technologies towards deciphering molecular mechanisms of mycobacterial drug resistance.

INTRODUCTION

Tuberculosis, a disease of great antiquity holds lineage to saprophytic soil organisms whose later introduction as a human pathogen likely coincided with the domestication of cattle approximately 10,000 years ago. Throughout history, tuberculosis has been classified by many names, from Phthisis ("to waste") by the ancient Greeks, to consumption in the 1800's. In 1882 Robert Koch isolated the causative agent, *Mycobacterium tuberculosis* from crushed lung tubercles. Currently, M. tuberculosis is known as the world's leading cause of death from a single infectious agent, with a global prevalence of greater than 1.6 billion persons. (1,2)

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Despite overall declines in TB incidence in industrialized countries during the past three decades, mostly due to conscientious public health measures, there has recently been an important rise in the incidence of TB. (3,4) However, the contention that this is a "new" epidemic may not be entirely accurate when one considers the natural history of this disease follows secular trends that epicycle over the course of a human lifespan. What is "new" about this increasing disease burden is the widespread emergence of multi-drug resistant strains (MDR), which by definition, are strains resistant to at least the major frontline drugs: isoniazid and rifampin. This increase is due, at least in part, to the discontinuation of long course multi-drug treatments combined with patient non-compliance (5). In addition, the predilection of M. tuberculosis for the impoverished has further compounded this problem (6). As well, *M. tuberculosis* exhibits an important synergy with HIV and the role of the latter as a cofactor in TB disease has proven to be a major impediment to the control of both AIDS and Tuberculosis. (4,7,8)

Nonetheless, it is apparent there has been an increased incidence of MDR TB in both developing and

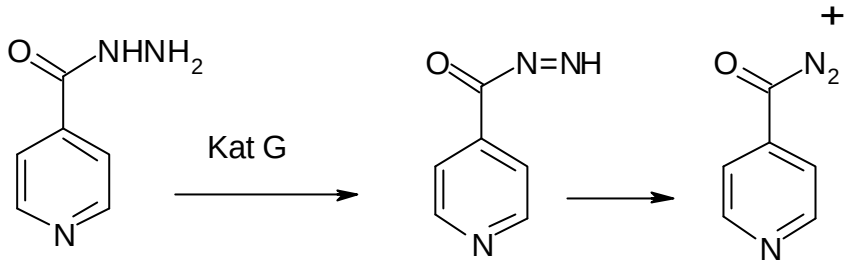

Figure 1. Potential metabolic activation mechanism for Isoniazid. Kat G mediates 2 electron transfers to produce an activated Isoniazid intermediate(s). It is this reactive intermediate that is capable of intracellular acylation of nucleophiles in *M.tuberculosis*, thereby facilitating toxic effects.

industrialized countries, despite the availability of directly observed therapy (DOTS) and BCG vaccine. These recent developments underscore the urgent need not only for new drugs and more efficacious vaccines, but more importantly, a concerted effort into devising methods for timely vaccine and chemotherapeutic development. In light of this urgent need, it may be serendipitous that we are seeing enormous leaps in proteomic and genomic technologies as well as the complete published sequences of the *M. tuberculosis* (H37RV) genome (9,10).

Therefore, this review is intended to consider previous work in light of recent advances using the major frontline antitubercular drug, isoniazid, as a paradigm for mycobacterial drug resistance. Following an abridged historical development of isoniazid, the current understanding of its mechanism of action and mechanism(s) of resistance and the rationale for the exquisite sensitivity of *M. tuberculosis* to isoniazid will be discussed. From there, the current directions of TB research in conjunction with present genomic based approaches will be considered.

ISONIAZID AND THE ROLE OF KatG

Isoniazid (INH), or isonicotinic acid hydrazide (Figure 1), is a synthetic bactericidal agent that was first produced in the early 1900's but was not utilized as an antitubercular agent until 1952. Presently, it is the prophylaxis of choice due to its low cost per dose, relatively low frequency of hepatotoxicity, (11,12) and reasonable bioavailability (13). In conjunction with Rifampin and Pyrazinamide it forms the major front line therapy worldwide (14).

INH enters mycobacterial cells via passive diffusion across the bacterial envelope (15). The minimal inhibitory concentration (MIC) for susceptible strains ranges from 0.02-0.05 mg/ml and is equally effective in *M. tuberculosis* and *M. tuberculosis* complex (*M. bovis, M. microti, M. africanum*) members. Surprisingly, INH exhibits little or no inhibitory activity against other mycobacteria and most prokaryotic pathogens. The reasons for this, and the primary mechanism of action of INH have been the subject of considerable investigation. Much of the current understanding of the in vivo mechanism of isoniazid has been extrapolated from in vitro work; almost exclusively focusing on relatively few bacterial enzymes associated with decreased susceptibility profiles. The first mechanistic insight of INH was revealed in 1954 when Middlebrook and others noted an inverse relationship to catalase-peroxidase activity and INH resistance (16,17).

This implicated the catalase-peroxidase enzyme, or KatG of *M. tuberculosis*. KatG is a hemeB containing dimer with only one functional domain. The other is apparently inactive (18). Its physiological role is protective, combating the low pH found during the "oxidative burst" in human phagocytes, where liberated O_2 radicals are converted to H_2O_2 within the phagosome. KatG activity eliminates this via a "deceptively simple reaction" (18);

$$2\,H_2O_2 \rightarrow 2\,H_2O + O_2$$

Which is in fact a 2-step prosthetic group mediated process:

$$\text{Heme-Fe[III]} + H_2O_2 \rightarrow \text{Heme-Fe[IV]} + H_2O$$
$$\text{Heme-Fe[IV]} + H_2O_2 \rightarrow \text{Heme-Fe[III]} + H_2O + O_2$$

It is clear that KatG plays a pivotal role in virulence and has been found to be essential for persistence in mouse and guinea pig models (18,19).

Curiously, it is this same protective enzyme that is implicated in susceptibility to INH. Specifically, INH is a prodrug that requires cellular activation by KatG producing a reactive species with antimicrobial action. The postulated reaction is shown in Figure 1. There is also evidence that INH effect is potentiated by the presence of peroxide, typically found in activated macrophages (20,21).

An elegant series of experiments by Zhang *et al*. demonstrated a key role for KatG in the action and resistance to INH. This group sought to identify the gene(s) responsible for INH resistance. A molecular

genetic approach was taken using a lab adapted *M. smegmatis* strain normally susceptible to moderate concentrations of INH (32 mg/ml). From this, a mutant strain was isolated that was viable in concentrations up to 500 mg/ml INH, this particular mutant was transformed with a cosmid library representing the entire genome of the INHs lab strain H37RV. Selection with INH allowed isolation of a hypersensitive clone, that displayed a marked catalase activity, thereby co-locating these two functions to a single genomic fragment (22).

Restriction mapping and Southern blotting localized the INH susceptibility to a smaller 2.9 Kb fragment, which in comparison with *E. coli* was shown to contain sufficient coding capacity for KatG. In addition, Zhang *et al.* provided evidence that over expression of this product could confer a susceptible phenotype in a dose dependent manner to a naturally INH^R *E. coli* strain.

To determine the clinical relevance of this result, a series of eight INH^R clinical isolates were assayed by southern analysis confirming that high resistance of two strains was due to deletion of the KatG gene. It is worth noting at this point that gene deletion is a relatively unusual mechanism of resistance in contrast to what is typically observed in bacterial systems where active efflux, (23) altered affinities of enzymatic targets, (24) and antibiotic inactivation, (25) represent norms in resistance acquisition.

Therefore, although convincing, it is likely that the studies conducted by Zhang *et al*. suffered from errors in sampling since this mechanism of resistance appears in a distinct minority of cases.

A more representative sample was analysed by Heym *et al*. (26). This group used 39 clinical isolates from diverse locales to determine whether mutations in KatG were associated with INH^R. A PCR-SSCP (PCR-single stranded conformational polymorphism) strategy was used, in which the target is first amplified by PCR. Products are then denatured to single -stranded form and run on high-resolution polyacrylamide gels. Sequence alterations can be inferred by altered mobilities in comparison to reference standards. Mutations are then typically confirmed by sequencing (For detailed reviews see 27, 28).

This methodology allowed rapid screening of resistant and control isolates in 12 arbitrary overlapping intervals. Aberrant mobilities were sequenced and indicated that 21 of the 36 resistant isolates contained mutations within KatG with an Arginine to Leucine mutation at position 463 predominating in seven isolates. Five others carried a Serine to Threonine mutation at position 315. An additional three isolates contained a deletion mutation at residues 120-123. The final six mutations in KatG were represented at a singular frequency.

Similar, but less convincing work was reported by Pretorius *et al.*, using a lower resolution PCR-SSCP methodology (29, 28). However, the strength of this work was in the relatively large and geographically diverse samples from Africa, the US and Switzerland. Results of this study and a later one show a high proportion (52%) of African isolates with Serine 315 Threonine (G-C) mutations at codon 315, and additional mutations at codons Thr 275 Ala, Arg 409 Ala, Arg 463 Leu and Asp 695 Ala. Overall, 64% of the observed INH resistance was attributable to mutations within KatG, again suggesting a complete KatG deletion was a rare event. An interesting additional finding of this report was that of a fully INH susceptible control isolate mutated at codon 463, which will be discussed later.

Table 1. Relative activities and isoniazid minimal inhibitory concentrations (MICs) of Bacillus Calmette Guerrin BCG transformants expressing KatG mutants versus reference lab strains.

Strain	Peroxidase (A405)	Catalase (A240)	Isoniazid MIC (mg ml -1)
VC	0.0	0.0	>500
TB-KatG	1.0	1.0	0.5
MAC-KatG	1.9	1.2	1.0
R104L	0.1	0.2	>500
H108Q	0.1	0.0	>500
N138S	0.1	0.0	>500
L148R	0.1	0.2	>500
H270Q	0.1	0.0	>500
T275P	0.3	0.0	>500
W321G	0.3	0.3	>500
D381G	0.0	0.0	>500
S315T	0.6	0.4	90
S140N	1.6	1.5	0.5
A350T	1.3	1.0	0.5
R463L	1.9	1.4	1.0
R463G	0.9	1.3	0.5
L587M	1.3	1.4	0.5

Adapted from Rouse *et al.* (1996). Of note is the S315T mutation that, while conferring only moderate resistance to isoniazid (˜ 90mg ml -1), allows retention of significant levels of associated peroxidase and catalase activities. **MIC**, minimal inhibitory concentration.

Numerous recent investigations have led to similar conclusions. Martila *et al.* found 22 of 24 (91.7%) INH resistant isolates carried a Ser 315 Thr mutation, of which 12 also carried a 463 mutation, all originating from the St. Petersburg area in Russia (30). Clonal spread in this case was not markedly noted by the authors in this study. However, due to the genetic similarity of isolates, it would have been interesting if some evidence had been presented regarding the transmission of drug resistant strains. Unfortunately, studies of clonal transmission of resistant bacteria in this area were not investigated.

The mutational spectrum of KatG may also be a factor of geography as the aforementioned author several years previous found a low prevalence, (three of fifty-four isolates containing mutations at codon 315) in Finnish patients (31). A similar result was also obtained by Rouse *et al*. in a clinical study of 26 INH^R isolates, nineteen of which were from Korea with only a single isolate mutated at codon 315 (33). However, it appears the study may have been limited by the use of isolates exhibiting rather low inhibitory concentrations of ~1 mg/ml. In contrast, an opposite

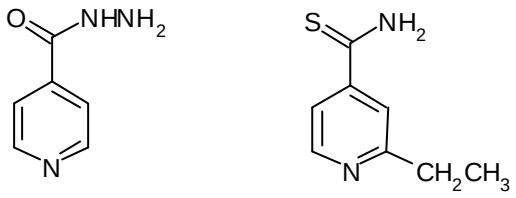

Figure 2. Structures of Isoniazid (INH) and Ethionamide (ETH/ETA)

finding was noted in studies in the Netherlands that showed distinctly higher levels of clinical isolates carrying 315 mutations (32).

Overwhelmingly, the predominance of the 315 mutations in clinical INH r is supported in several other large and well-conducted studies. Haas *et al*. (34) which concluded 64% of KatG mediated resistance was due to mutation of codon 315, as well as an older but extremely convincing study by Musser *et al*. (35) indicating 75% of INH^R isolates contained the 315 or 463 codon mutations.

In considering previous studies, several investigators noted that the majority of mutations seen clinically were of the missense type. This indicated the importance of maintaining some, albeit reduced, KatG function in vivo. The rationale behind this is that even low KatG activity still confers a selective advantage versus nonsense mutations that result in truncated products.

Rouse *et al.* validated this hypothesis in a well-designed study that investigated the impact of specific missense mutations on KatG function. This was accomplished by introducing fourteen genetically defined mutations at thirteen different codons (36). The relative effects of both in vitro enzyme assays and *ex vivo* INH resistance were compared as shown in Table 1. This data reveals the benefit of the Ser 315Thr mutation in contrast to other mutations, which abrogate catalase activity or result in relatively insignificant increases in MIC's. However, this last point may be confounded by the use of the BCG as a transformation target. BCG in general is comprised of a phylogenetically distinct group of attenuated species, harboring a series of mostly uncharacterized deletions (37, 38). This may have some effect on the validity of studies. However, relative concordance between data using this and other laboratory adapted species is reassuring.

Overall, Rouse's data is in agreement with recently published data by Wengenach *et al*. (39). Her report included a thorough biochemical analysis of the properties of the Ser 315Thr mutation in comparison to wild type. The results show a six folds drop in catalase activity but only a two folds reduction in peroxidase activity. Taken together, these studies add credence to the hypotheses that the Ser315Thr KatG mutant is a competent catalase peroxidase harboring reduced affinity for INH.

One of the most commonly seen mutations associated with INH resistance in a clinical setting is the Arg 463 Leu or Arg 463 Ala. However, the prevailing opinion is that this mutation does not appear to confer any selective advantage (39, 40). In particular, Johnsson *et al.* found no difference between purified wild type and the R463L KatG mutant in terms of activity or ability to discriminate isoniazid substrate (40). Despite being completely in vitro, this study combined with others (26,32,33,34,41), concludes the most plausible explanation is that the 463 mutation is a frequent and possibly geographically isolated polymorphism. However, it should be stated there might be a marginal decrease in INH susceptibility between the KatG 463 leu KatG 463 glu, since the 463 leu naturally occurs in M. Bovis, which does show slightly higher MIC. However, evidence of this sort is weak (36).

In conclusion, considerable evidence has shown that INH acts as a prodrug that requires activation by KatG. Approximately 60 - 70% of all observed INH resistance can be directly linked to defects in KatG. As mentioned, this indicates the likelihood of additional resistance effectors, and raises an important question with the exact target(s) of the activated INH product.

InhA AND FATTY ACID SYNTHESIS

As stated, mutations in KatG account for approximately two thirds of INH resistance. Therefore, several groups have postulated additional downstream targets of activated INH in mediating resistance (33, 35, 44). This was initially based on phenotypic evidence that INH affects cell wall synthesis. Also, at a low frequency of bacterial isolations (10-7), INH resistance was not correlated with loss of catalase activity but rather with co-acquisition of ethionamide (ETH) resistance. ETH is a structural analog to INH, indicating a possible common target (Figure 2).

In an effort to identify this target, Banerjee *et al.* utilized a lab derived spontaneous INH/ETHr mutant to construct a genetic library for complementation studies (42). Two ORFs (open reading frames) were identified, termed orf1 and inhA. Subsequent subcloning studies, using both genomic fragments from *M. bovis* and *M. tuberculosis* H37Rv cloned into the *M. smegmatis* MC2155 strain revealed several interesting results. The first is that the InhA product alone was sufficient to cause an INH resistant phenotype. Second, in comparison to the *M. smegmatis* gene the, *M. bovis* and *M. tuberculosis* InhA genes appear to be located within an operon that includes Orf1. Also, the intergenic region for both *M. bovis* and *M. tuberculosis* H37Rv was considerably shorter than that of *M. smegmatis* and may lack complete promoter sequences.

Sequence alignment of InhA showed marked conservation across all mycobacterial strains and

significant homology (40% identity) to the *E. coli* ENV M protein known to be involved in fatty acid biosynthesis. Sequence comparisons also revealed a single nucleotide change at position 94 from serine to alanine as well as a putative NAD+/NADH binding site. The basis of INH resistance was determined by cell free mycolic acid synthesis assays. In the presence of INH wild type InhA was inhibited in a rough dose dependent manner. In contrast, the S94A mutant strain showed 20 folds greater activity under similar conditions. These data are quite consistent with the supposition that KatG activated INH targets mycolic acid biosynthesis. This group also proposed a hypothetical mechanism based on *E. coli* ENV M resistance to diazaborine, (42) that was discarded, based on the crystallization and functional analysis of InhA by Dessen *et al*. (43).

This group confirmed several unresolved questions. First, was the confirmation of the InhA function in mycolic acid biosynthesis. The role of this enzyme was in the catalysis of the NADH specific reduction of 2-trans-enoyl acyl carrier protein (ACP), an essential step in fatty acid elongation. Second, it was shown by microcalorimetry that neither unmodified isoniazid nor ethionamide bound to InhA supporting a role for activation of these prodrugs by KatG. The salient feature of this paper was that while the K_m and V_{max} values of the enoyl substrate for the wild type and S94A mutant didn't differ significantly, the K_m for NADH was 5 fold higher in the S94A mutant.

This indicated the resistance mechanism was related to specific interplay between the enzyme and cofactor, not INH. Crystallization data from the WT and S94A mutant indicated that perturbations in hydrogen-bonding within the NADH binding site impaired its affinity for NADH. As tantalizing as these results were the complete picture was left to a later report by the same group where an attractive mechanism was put forth (44).

In the WT condition there is a preference for NADH to bind first to InhA, followed by an acyl-ACP substrate, initially leaving the InhA-NADH complex available for attack by activated isoniazid. Due to the higher affinity, the WT InhA-NADH-INH complex would result in permanent inhibition of mycolic acid synthesis. Conversely, the decreased affinity of the S94A mutant would promote acyl-ACP substrates to bind first before NADH thereby protecting the enzyme. Furthermore, in a NADH-INH bound condition the lower affinity may promote release of the inhibitory complex.

Although of enormous academic interest, the clinical relevance of the S94A mutant is questionable since the clinically observed mutations in inhA do not include S94A (44). Rather in this study they are reported at residues 16, 21, 78 and 95. The fact that these are still physically near the NADH binding domain is encouraging (Figure 4). As added proof to the proposed mechanism, a paper by Lei *et al*. on isolation of the InhA inhibitor complex showed complete inhibition of InhA by the presence of a tightly bound (K_D< 0.4nm) INH product that had been activated by KatG (45).

Table 2. Resistance-associated mutations and amino acid substitutions in the Kas A codon. Adapted from Mdluli *et al.*, 1998. Depicted diagrammatically in Figure 3.

KasA Codon	Nucleotide D	Amino Acid D
66	GAT -- AAT	D -- N
269	GGT -- AGT	G -- S
312	GGC -- AGC	G -- S
413	TTC -- TTA	F -- L

THE ROLE OF ß-KETOACYL ACP SYNTHASE (KASA) AND INH

There seems to be considerable dispute within the literature as to additional targets of INH (46). Mudluli *et al*. has investigated accumulations of a saturated hexacosanoic acid (C26:0) found under INH treatment (47). This saturated fatty acid was found linked to acyl carrier protein (AcpM) that normally runs at 12 KD in SDS-page gels, however a second 80 KD complex with the same amino terminus was found to be a covalent complex of ß-ketoacyl ACP (KasA) INH and ACPM. Automated sequence analysis of four INH^R clinical samples revealed four different mutations at the kasA loci (Table 2 and Figure 3). Notably, two samples with the mutations G269S and F413L carried no other mutations, adding strength to the association. The remaining two strains carried additional KatG 315 mutations.

By comparison to the crystal structure of the *E. coli* homolog, three of the amino changes were within the catalytic center, the fourth was found to be located at the carboxy terminus and purported to alter protein-protein interactions (47). The clinical importance of KasA in INH resistance still needs to be fully established. A study by Lee *et al.* may assist in this regard (48). A series of 160 INH resistant isolates were sequenced and 10% carried mutations in KasA involving R121K, G312S, G387D, and previously reported G269S. However, 6 out of 32 susceptible strains also contained the G312S alteration, a seemingly similar situation to the KatG 463 mutation. Nevertheless, the possibility of KasA constituting a resistance mechanism should not be ruled out completely, since the R121K and G387D mutations have yet to be reported in a susceptible strain, and mutations in KatG and InhA do not account for all INH^R seen clinically.

Therefore, it seems possible that geographically distinct polymorphisms between epidemiologically disparate strains may be commonplace. One should also consider that both INH action, as well as resistance is likely pleotrophic in nature. This line of thought is supported by a recent study that utilized specific inhibitors of Inh A and KasA to demonstrate that INH

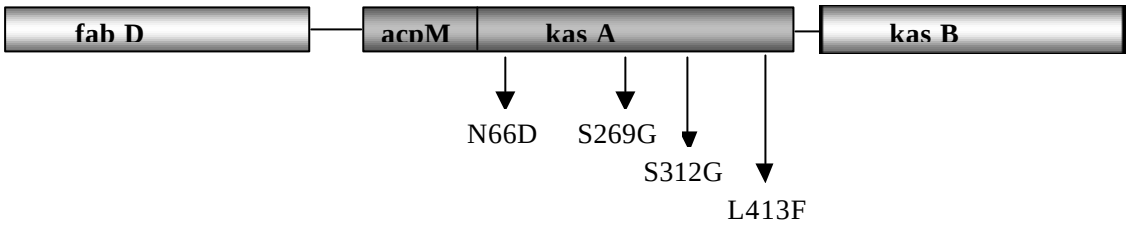

Figure 3. Diagrammatic representation of the kasA codon. Adapted from S. Ramasaswamy et al.,1998.

affects both targets simultaneously albeit by independent mechanisms, thereby arguing convincingly for a role of both targets in INH resistance (49).

THE OXIDATIVE STRESS PARADOX: THE OxyR-AhpC REGULON

The study of both KatG and InhA has provided valuable clues to the action of INH but has also introduced some unresolved questions. The exceptional sensitivity of *M. tuberculosis* to INH lacks rationale in comparison with other bacteria (54). To persist and flourish pathogenic mycobacteria must be able to withstand high oxidative stresses found in macrophages. In gram-negative organisms such as *E. coli* and S. *typhimurium*, the oxidative stress response is controlled by the OxyR protein. OxyR can upregulate several genes including AhpC (encoding acyl hydroperoxidase reductase), Dps (19KD starvation protein), OxyS (a divergent product of oxyR), GorA (glutathione reductase), and most importantly KatG (53). Sherman *et al.* investigated this response in *M. tuberculosis* and several other mycobacterial members (50). Metabolic labeling experiments were conducted under peroxide challenge and 2D protein gels indicated an OxyR like protective response was upregulated by only *M. smegmatis*; all *M. tuberculosis* and *M. tuberculosis* complex members elicited only an upregulated KatG. Deretic *et al.* and others have investigated the oxidative stress regulon in *M. tuberculosis* to determine the basis for these altered expression patterns (51, 52, 54). They specifically sequenced the AhpC gene and its putative regulator OxyR. In the laboratory adapted H37RV strains, both of the OxyR and AhpC genes were divergently transcribed, but the OxyR gene was found to be inactivated by multiple lesions including frameshifts , deletions and stop codons that ablated any gene function.

This result was confirmed in all *M. tuberculosis* strains tested and in all members of the *M. tuberculosis* complex. As added proof, inactivation of the OxyR or AhpC in *E. coli* will also confer INH susceptibility to this otherwise insensitive bacteria (55). When considering the above evidence, it appears that *M. tuberculosis* relies heavily on the defense afforded by their novel cell wall, having essentially eliminated most of the oxidative stress response (except KatG) from their genome.

Naturally, one must consider that the major mutations associated with INH resistance also cause reduced catalase-peroxidase activity in KatG, this, according to Sherman *et al.* is paradoxical since KatG is intimately involved in the survival and pathogenesis of *M. tuberculosis* (53). This group found a strong synergistic effect of H2O2 and INH in KatG positive BCG strains. They conducted a series of expression studies and a pre-induced KatG was capable of increasing survival 35 fold. To determine the compensatory mechanism in KatG (catalase negative) situations, Shermin examined 8 clinical INH^R KatG mutants for altered expression profiles. All 8 isolates expressed a 22 KD protein at considerably higher levels than H37RV controls. This protein was found to be AhpC. Its hyperexpression was a result of point mutations within its promoter region that apparently abrogate the requirement for OxyR regulatory control. The temporal appearance of this mutation was investigated and appears to be the result of a second *in vivo* selection event after KatG mutation. Several groups proposed that the increased AhpC expression could play multiple roles *in vivo* (53, 54). First, it could directly counteract INH effect, or simply compensate for loss of KatG activity thereby increasing overall viability. Sherman reported that while AhpC upregulation provides substantial benefit against peroxide insult it does not detoxify INH. Conversely, Zhang *et al.* supports an independent role for AhpC mutations in the emergence of low INH resistance (54).

The relevance of this mutation should also be questioned; particularly in light of two recent clinically based studies (56, 57). The first, sequenced the AxyR-AhpC region of 229 *M. tuberculosis* isolates recovered from infected humans and animals, where the KatG and InhA regions had been sequenced and reported previously (56). The most important feature of this study was that most INH^R strains carrying substantially reduced activity of KatG (ie. 315 mutation) lacked any alterations in AhpC or OxyR-AhpC intergenic regions. The second study analysed 57 clinical isolates. Here, 8 compensatory AhpC promoter mutations were identified in 8 catalase negative KatG defective strains whereas the corresponding region of 25 catalase positive INH^R isolates were unaltered (57). Taken together, these results show little evidence for an independent role for

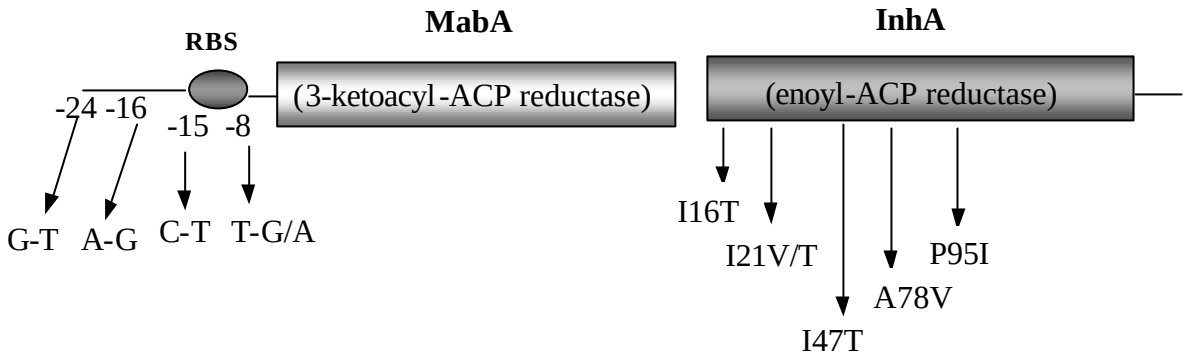

Figure 4. InhA and upstream regulatory element mutations implicated in INH resistance Adapted from S. Ramasaswamy et al 1998.

AhpC in INH resistance which appears at a low frequency similar to what is found in catalase negative strains. These strains may be of clinical significance.

THE "GLOBAL" APPROACH TO ISONIAZID RESISTANCE

Collectively, it is apparent there is controversy over the identification of the molecular correlates of resistance of MTB to isoniazid. There is also some lack of agreement concerning several mutations and/or polymorphisms being causally linked and clinically relevant to INH resistance. The main problem appears to be that previous genotypic studies of *M. tuberculosis* may have incorrectly estimated the importance of specific drug mutations due to errors in sampling, as well as the inclusion of MDR isolates and inappropriate controls. Also, the biochemical characterization of many proposed targets (resistant or susceptible) has inevitably used non-representative lab strains or non-pathogenic species for ease and safety, which may not accurately reflect that seen in the infected individual.

These errors, combined with a reductionist approach to drug resistance, has only revealed a fragmented and convoluted picture of *M. tuberculosis* resistance to isoniazid. To alleviate further discrepancies, a more holistic or "global" approach should be taken that makes full use of genomic databases, tools and methods. A comprehensive review of this subject can be obtained from several sources (58-65).

Several recent studies have undertaken this approach and have yielded a wealth of information. The first study by Wilson *et al.* uses micro-array hybridization to determine changes in expression patterns under INH exposure (66).

The basic methodology is summarized elsewhere, however in brief, at time zero (pre INH) and each successive time point (post INH), mRNA was isolated pelleted mycobacterial culture. These RNA species were differentially labeled with fluorochrome tags in a reverse transcriptase reaction (RT) to produce cDNAs that were used as probes for microarray hybridization. The resulting pattern is analysed via software and intensity changes allow discrimination between relative changes in mRNA expression.

This enabled the documentation of a highly induced gene cluster encoding components of the FAS II fatty acid operon. Several of these genes (acpM and kasA) have been previously reported, corroborating previous studies, and the methods used in this study. The induction of these genes were observed as early as twenty minutes post- treatment. Three additional INH induced proteins were also reported, the first of which is FbpC which is an abundant exported protein involved in mycolic acid maturation in the outer wall. FbpC has also been previously reported (67,68) since it constitutes one of the 3 highly homologous proteins comprising the 85 C Ag complex. As an added note, this complex has been proposed as the site of interaction with human fibronectin and therefore is a primary target for rational drug design and vaccines.

Several novel proteins lacking characterization were also found, 2 acetyl-CoA dehydrogenases (FADE23, FADE24) and an efflux protein EfpA as well as a subunit of AhpC. Further investigation of the relevance of transcriptional responses was analyzed in INH^R strains under conditions of INH and ETH treatment. The first results of this experiment showed a similar upregulation pattern by ETH as observed by INH. The second experiment of INH^R strains revealed no significant alteration of gene expression upon INH insult. Taken together, these results agree with previous literature yet provide a more complete view of all the players involved and the inclusion of several new therapeutic and prophylactic targets.

A similar approach was employed by Allard using a differential expression customized amplification library (Decal) (69). One important feature of this method is the ability to resolve four-fold differences in mRNA expression without confounding from constitutively expressed mRNA (housekeeping genes). Although beyond the scope of this paper, an additional method described by Brenner *et al.* allows the resolution of "a

few tens of mRNA copies per cell" (70). Both methods are quite amenable to bacterial systems as they do not rely on poly adenylation of mRNA transcripts and both require no previous sequence knowledge.

The Alland paper was directed specifically towards the effect of isoniazid on the genomic expression of Mycobacterium tuberculosis, similar to the work of the Wilson group. Their results showed an upregulation of three previously unknown isoniazid-induced genes, IniA, IniB and IniC, all of which have putative functions in cell wall synthesis or a hypothesized protective role in response to cell wall destruction. A later report has characterized the rather unique ini BAC promoter. A molecular genetic analysis described a regulatory region and putative repressor that was specifically induced by a variety of cell wall inhibitors, but only in actively replicating cells (71,74).

The final report by Piatek *et al.* is an excellent example of the use of a molecular epidemiological approach to drug resistance (72). The work utilized a PCR based molecular beacon assay. Briefly, this assay utilizes specifically paired fluorogenic PCR primers which allows real time monitoring of multiple PCR products in a single reaction (i.e. multiplex PCR), The assay itself is relatively quick and results can be analyzed within several hours (72, 73).

The populations used in this study were distinct. The first was from a reference lab in Spain. This population was known to have an overrepresentation of MDR clinical isolates. The second study population was from a community medical center in New York. The use of these two populations was to contrast and thereby preclude many previous mistakes in assessing the clinical relevance of mutations. The study was also used to characterize the assay and it was found to be particularly effective as a predictive screen for INH^R with a sensitivity and specificity of 85% and 100% respectively for detection of mutations in the KatG, InhA and AhpC loci. Results of stratified analysis of the sample populations showed dramatic differences in the ability to discriminate INH^R. Ninety-four percent of isolates from Spain contained mutations associated with INH^R versus only seventy-six percent of New York isolates. Analysis to exclude the confounding of MDR revealed 94% and 96% of isolated from Madrid and New York respectively contained INH resistance mutations. Restriction to New York isolates only showed a strong correlation of INH^R and MDR (94%) compared to only 44% of single drug resistance (i.e. INH^R) mutations.

The authors then investigated the possibility of the KasA mutation accounting for the discrepancies. No mutations were found in codons (66, 312, 413) previously reported to be associated with INH r (48). One previously reported mutation, G269S, was found in 10 isolates but were equally distributed between resistance and susceptible controls, suggesting no association with INH^R. As previously mentioned for KatG, this likely constitutes a polymorphism.

The authors went on to address the possibility of INH^R being the end result of a temporal sequence of mutations that have previously been unassociated with resistance. The hypothesis of a series cumulative mutations (i.e.."a genetic barrier") imparting resistance while interesting, is not a novel one, and in this particular situation it remains yet unfounded.

FUTURE DIRECTIONS

To resolve the previously stated issues, in the interests of public health will require accurate assessment of resistance mutations, polymorphisms, and their relevance (i.e. those being both sufficient and necessary to effect drug resistance) in a clinical setting. Although the primary target(s) of INH are elements of the FASII system, necessary for mycolic acid synthesis, the predominance of mutations seen in patients seem to be localized to KatG.

Further study of these and related problems using burgeoning new technologies, and information focusing on molecular pathogenesis should provide important new insights into mycobacterial metabolism, the genetic basis of mycobacterial drug resistance, and host response. This approach will inevitably lead to new targets, and drug leads. These will hopefully translate to prophylactic and therapeutic interventions in the next several years.

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REVIEW ARTICLE

The Use of Fibrinolytic Therapy in Acute Ischemic Events

Danny Del Duca, B.Sc.*

THE BASIS OF ISCHEMIC EVENTS

The loss of tissue perfusion represents the pathological basis underlying acute cerebrovascular events. The occlusion of the brain's blood supply by intravascular thrombotic and/or atherosclerotic phenomena results in a disruption in tissue oxygen and glucose delivery. This inevitably impairs the cell's capacity to synthesize ATP. Despite initial attempts to maintain consistent ATP stores through anaerobic glycolysis and adenosine release, the starving tissue experiences loss of membrane ionic gradients, secondary to the inactivation of the Na+/K+ ATPase pump. A significant increase in intracellular calcium concentration is triggered by the activation of voltage-gated calcium channels, the activation of ligand-gated calcium channels, and by the inactivation of the Na+/Ca+ exchanger. In addition, massive release of the main excitatory, inhibitory, and monoamine neurotransmitters (many of which become excitotoxins at high concentration) is also observed. The above excitatory transmitters, such as glutamine, for example, play an important role in the over-stimulation of the NMDA receptor, which in turn promotes excessive calcium influx (1). The cytotoxic effects of calcium are associated with the activation of lipases, proteases, and endonucleases, as well as xanthine oxidase and neuronal NO synthase, capable of either direct or indirect destruction of cellular structures (2).

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RECOMBINANT TISSUE PLASMINOGEN ACTIVATOR

The therapeutic basis for the use of recombinant tissue plasminogen activator (t-PA) rests in its capacity to interact with and enhance the function of plasmin, a nonspecific protease which digests fibrin. Plasmin is the central enzyme of the physiologic fibrinolytic system, and is formed from its inactive precursor, plasminogen, through the cleavage of a single peptide bond by various plasminogen activators including endothelial-derived t-PA, urothelium-derived urokinase, streptokinase, and factor XIIa. Endogenous t-PA, a 527 amino acid serine protease, is released in response to stasis produced by vascular occlusion, and is largely specific for activating fibrin?bound plasminogen since it specifically associates with fibrin through the recognition of particular lysine residues (3). The site-sensitive nature of this agent has been viewed as a favorable aspect for therapeutic potential. However, the concentrations of t-PA achieved during pharmacotherapy far exceed those in the physiologic range, thereby reducing the likelihood that systemic activation of plasminogen will not occur. The action of t-PA is antagonized by plasminogen activator inhibitors-1 and -2. The half-life of the active species is 5 to 10 minutes, and clearance occurs chiefly by hepatic metabolism. Moreover, therapy with t-PA is expensive, costing several times more than streptokinase per therapeutic dose.

Despite the important potential for re-establishing blood flow to infarcted regions, one of the main limitations of the use of t-PA has been its association with hemorrhagic events, related to reperfusion injury, in certain patients exposed to this treatment. While it is

clear that cell death occurs within the context of ischemic insult, variable numbers of cells may proceed to die after blood flow resumes, by necrosis as well as by apoptosis. It is hypothesized that cellular damage may be initiated during reoxygenation through the generation of oxygen free radicals by parenchymal and endothelial cells and infiltrating leukocytes. Cellular antioxidant defence mechanisms may also be compromised by ischemia, favoring the accumulation of free redicals. Finally, ischemic injury is associated with the production of cytokines and increased expression of adhesion molecules by hypoxic parenchymal and endothelial cells. These agents recruit circulating polymorphonuclear leukocytes to reperfused tissue; the ensuing inflammation causes additional injury (4). It follows that the above deterioration in the integrity of the reperfused tissue may result in hemorrhagic complications associated with thrombolytic therapy. The most likely mechanism is related to cerebral vascular dysfunction, and this represents the most significant limitation in the clinical use of t-PA in acute ischemic stroke. Other mechanisms which have been proposed to explain the mechanism of t?PA's hemorrhagic toxicity include the lysis of fibrin in physiological thrombi at sites of vascular injury, and the evolution of a systemic lytic state that results from the systemic activation of plasmin, which produces fibrinolysis and destruction of other coagulation factors, especially factors V and VIII (3). For this reason, the combination of neuroprotective pharmacotherapy with thrombolysis has been suggested as a means of reducing the untoward effects of reperfusion. Proposed methods include the concurrent management of t-PA patients with antiexcitotoxic, antiapoptotic, and/or free radical scavenging agents (5).

RECENT FINDINGS AND ONGOING CLINICAL DEBATES

The drug alteplase (tissue plasminogen activator) was recently approved for use in acute stroke in the United States (1996) and Canada (1999). According to national treatment guidelines, administration is recommended within three hours after the onset of ischemia, with the objective being the degradation of the intraarterial occlusion to restore blood perfusion before significant neuronal death ensues. However, the safety and efficacy of this therapy are still under scrutiny. In a recent survey of American neurologists, 30% reported being concerned about its efficacy, while 61% admitted being concerned about the risk of intracranial hemorrhage, the most important adverse effect of t-PA regimens (6). Indeed, cerebral angiography conducted soon after the onset of most ischemic conditions has demonstrated the presence of arterial occlusion in up to 80% of acute infarctions (7). It follows, then, that attempts to promote clot degradation are well justified, given that such intervention can potentially limit brain injury if administered in a timely manner, and before the process of infarction has been completed.

NINDS STUDY

A large, randomized, placebo-controlled study (8) conducted by the National Institute of Neurological Disorders and Stroke (NINDS) in 1995 represented a landmark contribution in favor of this therapeutic approach, and played no small role in encouraging the nation-wide approval of alteplase in the US, shortly thereafter. The study, involving 624 patients treated with intravenous tissue plasminogen activator at 0.9mg/kg body mass, within 3 hours of symptom onset, was divided into two parts. Part 1 measured changes in neurologic deficit 24 hours after the onset of stroke, as an indication of t-PA's immediate clinical effect, while Part 2 assessed different aspects of recovery at three months after treatment. Although no significant clinical improvement was observed within the first 24 hours, a statistically significant improvement in the condition of the t-PA-treated patients, compared to controls, was noted at three months. Patients treated with t-PA were 30% more likely to have minimal or no disability after three months of initial presentation. The beneficial effects occurred in patients with all subtypes of stroke, including suspected lacunar infarction, and were sustained at 1 year. This was accompanied by the finding that exposing patients to this therapy caused no significant differences in mortality (17% total mortality of treated patients versus 21% control). However, symptomatic intracerebral hemorrhage was seen to be associated with t-PA exposure. Within 36 hours after onset of stroke, hemorrhage took place in 6.4% of individuals given t-PA, but only in 0.6% of placebo cases, and at three mouths, 61% of those having hemorrhages died. The final results suggested that this therapy involved a 3% chance of early mortality attributable to intracerebral hemorrhage.

The NINDS study was among the first to indicate that earlier initiation of treatment and the use of lower doses might be important factors in reducing the incidence of t-PA-induced hemorrhagic events. Moreover, the sustained favorable clinical outcome after t-PA treatment was recently shown to be present in patients at 6 and 12 month periods after treatment (9), indicating a sustained benefit of t-PA for cases managed within three hours of initial insult. The importance of the administration of treatment within three hours of stroke onset was suggested by Clark et al. (10), whose work demonstrated that a similar treatment protocol followed at times beyond 3 hours

of initial symptomology failed to show a benefit in individuals treated with t-PA compared to controls. The apparently favorable results of the NINDS work led to the development of a treatment protocol for t-PA which included the use of doses of 0.9 mg/kg, administered no later than 3 hours after the onset of symptoms. In addition, the exclusion of patients deemed ineligible for this therapy was also stipulated. This involved the screening of treatment candidates for the presence of risk factors predisposing to the development of hemorrhagic complications with t?PA therapy, such as evidence of elevated blood pressure, advanced age, high blood glucose levels, or evidence of prior cerebral events on initial CT imaging studies. Indeed, as discussed below, there is evidence suggesting more favorable clinical outcomes in patients treated in concordance with this protocol, while most morbidity associated with t-PA may be caused by deviance from it.

CLEVELAND STUDY

This work (11) underlines the significant morbidity that has since been associated with t-PA, and has played no small role in creating skepticism among practitioners. However, it has also highlighted the importance of adequate patient selection in minimizing the incidence of hemorrhagic events. The study involved 3948 subjects from 29 Cleveland area hospitals, admitted over a one-year period (July 1997 to June 1998). Of these patients, 70 were treated with t-PA within three hours of initial insult, but 15.7% of these patients subsequently suffered symptomatic intracerebral hemorrhage. Despite the fact that the number of deaths associated with this latter complication was only 37% (generally lower than the above work), the overall higher rate of hemorrhagic complications is disturbing. In addition, the authors note that the in-hospital mortality rate for the t-PA-treated patients (15.7%) was significantly higher than that for control patients not receiving the drug (7.2%).

Katzan et al. (2000) state, most importantly, the fact that up to 50% of the t-PA patients in the above trial were reported to have been treated in a manner that deviated, in one or more ways, from the National Treatment Guidelines established following the NINDS (1995) work. This inclusion of high risk patients in the study is, indeed, thought to have played a significant role in the higher incidence of complication-related mortality, and only provides further proof of the importance of carefully screening patients before treatment is commenced. In fact, this finding is congruent with work by Karbalai et al. (12) who report on a series of 69 patients treated with intravenous t-PA. It was found that while the incidence of hemorrhage was as high as 27% in cases deviating from established protocol, the corresponding value was only 5% for patients treated without deviations. Similarly, Tanne et al. (13) report that the incidence of hemorrhage was 11% in patients with protocol deviations, compared to 4% when no deviations were present, thereby suggesting that strict adherence to protocol guidelines is important.

ECASS STUDIES

Further insight unto the controversy surrounding t-PA may be gained be analyzing the results of the European Cooperative Acute Stroke Studies, ECASS I (14) and II (15). The ECASS I trial reported a 20% incidence of hemorrhagic events in the t-PA group, compared to 6.5% controls. Interestingly, this study differed significantly from the NINDS trial in several significant ways. Not only was thrombolytic therapy initiated up to 6 hours after the initial event, but the dose of alteplase was 1.1 mg/kg (compared to 0.9). In addition, 17% of patients included were in violation of the established treatment protocol. Furthermore, while no significant difference in primary end points was initially detected, if the above data are re-evaluated without those patients who should initially have been eliminated from the study, three-month disability scores are found to be significantly lower in the t-PA group (41% vs. 29%). In 1998, ECASS II found no significant difference between patients receiving t-PA compared to controls, in terms of favorable outcome at three months. This may be related to the fact that the t-PA dose used was tapered to 0.9 mg/kg, and that protocol violations were minimized through more effective identification of early infarct signs on pretreatment CT scan analysis, and blood pressure control. The study did, however, draw important conclusions related to additional patient risk factors for the development of hemorrhagic events. These included hypertension, prior congestive heart failure, advanced age, aspirin use prior to stroke, and central nervous system abnormalities (attenuation of density; edema; mass effect) on pretreatment CT scanning. Other studies have added elevated blood glucose levels (16) and evidence of a large infarct in the middle cerebral artery on CT scan (15) to this list.

Taken together, these data point to the unifying theme related to the importance of identifying and excluding high-risk patients during the initial stages of fibrinolytic therapy. Indeed, as efforts to find solutions to this seemingly subjective designation of high-risk patients, many authors have since advocated the development of predictive scoring algorithms that could standardize and perhaps facilitate clinical decision-making procedures. However, it is tempting to argue, as the results of the ECASS II trial demonstrate, that when all efforts to

adhere to treatment protocol are followed, the ultimate statistical benefits of t-PA regimens become questionable. The current debate surrounding the use of t-PA reflects this notion.

THE PROBLEM OF TIME

Among the most important limitations associated with the use of t-PA remains the need to administer the treatment within the initial 3 hours following the acute insult, given the reported reduction in its efficacy as time-to-therapy in prolonged. In 1999, an important randomized controlled trial (17) failed to show any benefits of t-PA administered beyond the established three-hour window. Treatments at times ranging from 3 to 5 hours were associated with increased risk of symptomatic intracerebral hemorrhage, and no remarkable clinical benefits at 90 days. It follows that the advanced age of many of the patients affected often complicates the likelihood of prompt intervention, for reasons related to social isolation, and communication difficulties with care-providers. Indeed, it has been reported that, in one cohort of stroke patients, 29% of those presenting after three hours had recognized their symptoms but had chosen not to seek medical attention (18). Furthermore, efforts to enhance the specific diagnostic efficiency of acute care personnel in the area of acute cerebrovascular insult may also contribute to the more timely initiation of therapy.

CONCLUSION

One may thus infer that ethical implications can arise in the treatment of patients presenting with one or more of the above risk factors which would question their clinical eligibility for fibrinolytic therapy. Proponents of t-PA subscribe to the idea that treatment should not be withheld from patients if it can potentially result in a lower rate of severe disability, despite the fact that very favorable outcomes may be unlikely. Nevertheless, the enhanced probability of hemorrhage in patients presenting with the above risk factors clearly constitutes a challenge in the eyes of most clinical practitioners, and many currently favor a more conservative approach. Indeed, Hacke et al. (19) concluded that the use of widespread intravenous thrombolysis could not be advocated, based on the fact that treating high-risk patients is associated with an unacceptable tenfold increase in the risk of hemorrhagic complications and death.

It is thus safe to conclude that the proven benefits of thrombolytic therapy are reproducible in the clinical setting if guidelines for treatment and management, including the withholding of treatment in higher risk patients, are closely followed. However, while t-PA constitutes an important therapeutic option that is appropriate for some occlusive lesions, it is not indicated for all patients presenting with acute ischemic stroke. The problem of cerebrovascular disease has long posed an insurmountable obstacle for clinical neurologists, and the advent of such a promising therapeutic intervention rightfully deserves to be welcomed with enthusiasm. However, in the face of the documented, potentially harmful consequences of its overzealous administration, it may be wise to argue that it is those physicians who realize the caution and judgment which must be associated with the recent endorsement of t-PA who are most likely to make more effective use of it.

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FEATURE REVIEW

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Patricia N. Tonin, Ph.D.

FEATURE REVIEW

BRCA1 and BRCA2 and Inherited Predisposition to Breast and Ovarian Cancers

Patricia N. Tonin, Ph.D.*

INTRODUCTION

According to the Canadian Cancer Statistics for the year 2001, breast and ovarian cancers will account for 19,500 and 2,500 of new cases of cancer, respectively (1). About 25% to 40% of breast cancer incidence in Canadian women can be attributed to identifiable risk factors (2), which unfortunately are not directly modifiable such as family history of disease. While the majority of breast and ovarian cancers arise 'sporadically' with no known specific etiologic cause, segregation analysis suggests that an estimated 10% of all primary cancers arise because of the inheritance of an autosomal dominant mutant allele (3,4,5,6). This mode of inheritance implies that there is a 50% chance of inheriting the disease-related allele from a carrier parent and that the inheritance of a mutated allele is sufficient to alter susceptibility to cancer (Figure 1). In addition, there is an expectation that cancer cases may 'cluster' in certain branches of the family, where the disease related allele has segregated with the affected individuals, and that the susceptibility allele can be transmitted through the male line (Figure 1) (3,7). Features of hereditary cancer families include multiple affected family members in several consecutive generations, age of cancer diagnosis younger than that in the general population, bilateral cancers in paired organs, and personal history of multiple cancers of specific sites (Table 1). The genetic analysis of families with multiple cases of breast cancer with a mean age of diagnosis before age 50 years and/or ovarian cancer facilitated the discoveries of the breast-ovarian cancer susceptibility genes, BRCA1 (8,9,10) and BRCA2 (11,12,13). A large majority of breast and/or ovarian cancer families (14) and up to 5% of all breast and ovarian cancers have been attributed to germline mutations in either of these genes. Other genes, such as TP53 in Li Fraumeni syndrome (15,16) and PTEN in Cowdens' Syndrome (17,18) confer increased susceptibility to breast cancer, but their contribution to inherited predisposition to breast cancer may be less than 1%. A review is presented on the cancer risk attributed to BRCA1 and BRCA2, options for cancer prevention and detection, the spectrum of mutations that have been described in the Canadian population and the challenges of identifying deleterious mutations in these breast-ovarian cancer susceptibility genes.

CANCER RISK IN MUTATION CARRIERS

In carriers of BRCA1 or BRCA2 mutations, estimates of the cumulative lifetime risk, by age 70 years, of developing breast cancer in females are 28% to 87% and of developing ovarian cancer are 16% to 60% (14,19,20,21,22,23). An important observation is the mutation carriers are at significantly increased risk for developing breast cancer at a young age in comparison to the general population: the risk of developing breast cancer in mutation carriers is15% to 30% by age 50 years in comparison to about 2.3% (1). The lifetime risk for developing ovarian cancer appears to dependent on the gene mutated. Unlike, breast cancer, age-specific penetrance is not significantly skewed toward early onset ovarian cancer in mutation carriers (20,24). Estimates of the life-time risk of developing ovarian cancer is 40% to 66% in BRCA1 mutation carriers and 10% to 27% in BRCA2 mutation carriers (14,19,24,25). Women with BRCA1 mutations are found to have an excess of multiple

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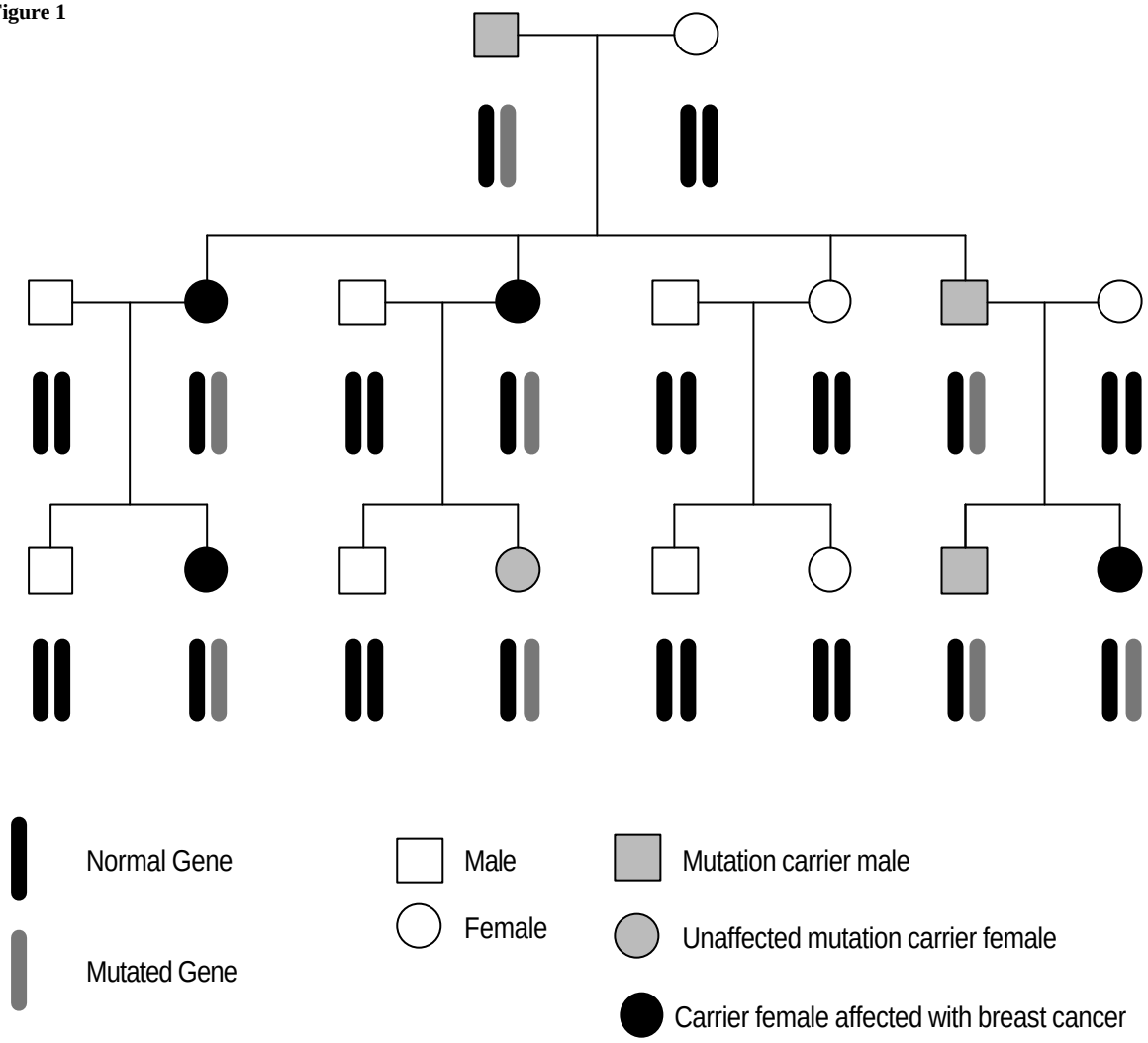
Figure 1



primary cancers of any type (of the breast or ovary) (23,26). Studies by the Breast Cancer Linkage Consortium (BCLC) have reported risk estimates for the development of contralateral breast cancer is up to 52% for BRCA1 carriers and up to 64% for BRCA2 carriers by age 70 years (19,23). Although the estimated risks for cancer in mutation carriers fall within a wide range, they are significantly higher than the lifetime risk for developing breast and ovarian cancers in the general population which are 10.6% and 1.5%, respectively (1).

Male carriers of BRCA1 and BRCA2 mutations are at increased risk of developing breast cancer (27,28,29). Breast cancer in men is a rare disease: in the United States the incidence is less than 1% of the incidence in breast cancer in women (30). Estimates for the lifetime risk (by age 70 years) for men is 0.01% (31). The observation that male breast cancer can occur in the context of family history of female breast cancer prompted researchers to investigate any association with inherited predisposition to breast cancer. Stratton et al reported the linkage of male breast cancer with BRCA2 (32). Germline BRCA2 mutations are to be more commonly reported than BRCA1 mutations (33,34). The frequency of BRCA2 mutations in male

Table 1. Features and types of hereditary breast and/or ovarian cancer families that harbour germline BRCA1/BRCA2 mutations.

Features
- Multiple affected members (first-, second- and third-degree relatives) affected with breast cancer of the same branch of a family;
- Mean age of diagnosis of breast cancers before age 50 years;
- Multiple primary breast cancers;
- Ovarian cancer diagnosed at any age (HBOC families only);
- Male breast cancer diagnosed at any age (more common in HBC families than HBOC families);
- Transmission of trait consistent with autosomal dominant mode of inheritance (Mendelian).
Types of Families
- Hereditary Breast and Ovarian Cancer Syndrome (HBOC)
- Hereditary (site-specific) Breast Cancer Syndrome (HBC)

breast cancer varies with ethnicity and geographic origins of population studied, as well as the method of ascertainment of the affected male. For example, in Iceland, 40% of male breast cancer cases were identified as carriers of a BRCA2 mutation (28). In contrast only 4% of cases were carriers in a study of male breast cancer cases ascertained in Southern California not selected for family history of cancer (33). A higher frequency (14%) was observed in another American study, and 85% of carriers reported a family history of female breast cancer (34). The rarity male breast cancer cases and variable frequency of mutation carriers in cancer cases of populations of different geographic and ethnic origins presents a challenge for establishing risk estimates and thus guidelines for genetic counselling.

Modestly increased risk estimates for cancers at other sites in carriers of BRCA1 and BRCA2 have been reported (19,23). We reported a large multi-site cancer family linked to BRCA2 that harboured cancers of the prostate, pancreas, larynx and colon in addition to 16 breast cancers where a number of cancers were shown to been mutation carriers (29,35). BRCA1 carriers are at increased risk of developing prostate cancer (RR = 4.1) and colon cancer (RR = 3.3) (19,36). BRCA2 carriers are at increased risk for developing cancers at specific sites. In a study by the BCLC, statistically significant increases in risks were observed for cancers of the prostate (RR= 4.65), pancreas (RR = 3.51), gallbladder and bile duct (RR = 4.97), stomach (RR =2.59) and skin [malignant melanoma] (RR=2.58) (23). Germline mutations have also been reported for fallopian tube cancers, a rare form of cancer found in 1% of all gynecologic malignancies (37,38,39,40). In one study of fallopian cancer cases not selected for family history about 16% cancers were shown to harbour germline mutations (41).

Guidelines for assessing cancer risk in mutation carriers is currently based on studies that have assessed penetrance in the context of individuals with a family history of breast and/or ovarian cancer as less is known about the risk of mutation carriers in the absence of a strong family history of cancer (42). The wide range in risk estimates may be due to factors that modify risk in mutation carriers, such as gene-gene and gene-environment interactions. Examples include, recent studies showing that oral-contraceptive pill use may reduce the risk of ovarian cancer in mutation carriers (43); that smoking may reduce the risk of breast cancer in mutation carriers (44); and that carriers harbouring specific variants of AIB1 that contain at least 28 or 29 polyglutamine repeats have a significant increased risk for developing breast cancer than women who carried AIB1 variant with fewer repeats (45). The smoking history of women and the AIBI variant underscores the potential steroid hormone link associated with breast cancer risk. Cigarette smoke has been found to have an anti-estrogenic effect (46). AIB1 is a transcriptional co-activator that interacts with steroid hormone receptors to enhance ligand-dependent transcription and is required for female reproductive function and mammary gland development (47). Current efforts are aimed at identifying and characterizing these (and other) variables that may modify risk in mutation carriers. However, the analysis and interpretation of the results of gene-environment interactions in known mutation carriers remains complex and has not yet been translated into guidelines for risk assessment. Thus, as the penetrance of high-risk women has been investigated based on the extensive analysis of women with a strong family history for breast and or ovarian cancer, mutation analysis and risk assessment is often limited to these women as they are most likely to harbour germline mutations with high penetrance.

OPTIONS FOR THE DETECTION OF BREAST AND OVARIAN CANCERS

The identification of known genetic factors affords the opportunity to identify those individuals at risk under the premise that this knowledge will enhance the accuracy of female breast cancer and ovarian cancer risk prediction and impacts on options for cancer screening and prevention. As the cumulative lifetime risk for developing breast and ovarian cancers is significantly higher in mutation carriers with a family history of disease than the general population, management has been focused on the detection of tumours that arise in this specific context. A number of similar guidelines (research based) have been proposed that are largely based on risk assessment in the context of a strong family history of breast and/or ovarian cancer and then were adapted to include families with proven BRCA1 or BRCA2 mutations when direct mutation detection became possible. The options for cancer detection include monthly self breast exams starting at age 20 years; annual clinical breast examination starting at age 25 years and annual mammograms starting at ages 25 to 35 years for the detection of breast cancer; and pelvic ultrasound and examination and serum CA-125 testing (a marker of primary ovarian cancer or recurrence) for ovarian cancer detection (42,48) .

Although randomized trials and population-based programs have provided evidence that breast cancer screening can be cost effective in women between 50 and 70 years of age (49,50), mammography screening in younger women that could be beneficial for mutation carriers is controversial. For ethical reasons

no randomized trials in BRCA mutations carriers are to be expected, and thus the surveillance for breast cancer in these women is likely to be evaluated by observational studies. Due to the low frequency of mutation carriers, a limited number of studies have been published (51,52,53,54). Brekelmans et al. recently reported on a combined retrospective and follow-up prospective study that analyzed the incidence and characteristics of screen-detected and interval breast cancers among 1,198 women who participated in a high risk breast cancer family clinic in the Netherlands (55). In addition to self-breast exams and clinical breast exams, they used magnetic resonance imaging (MRI) as an option for breast cancer detection. Proven BRCA mutation carriers where amongst the high-risk group of women under surveillance. The rates of both screen-detected and interval cancers were highest among the mutation carriers. The results of the study support the conclusions of earlier studies recognizing that there is a relationship between breast cancer risk and rate of cancer detection at screening, and that screening is beneficial and cost-effective in high-risk women. In addition, this study revealed a substantial risk of interval cancers in mutation carriers, which suggests that current screening protocols may be insufficient in this group of high-risk women. This observation underscores the pressing need to further evaluate why this may have occurred: is it a reflection of the inability to detect cancers by mammography or is it a reflection of the aggressivity of this variant of the disease.

Although, the benefits of mammography detection of breast cancers in women below age 50 is controversial, some studies have shown that screening between 40 and 50 years of age can also significantly reduce breast cancer mortality (56). However, due to the number of false positives and psychological toll, it may be efficacious to restrict screening to high-risk women (those with a strong positive history of breast cancer and/or BRCA mutation carriers) (57). MRI screening may improve detection of breast tumours that are undetectable by mammography either because the surrounding breast tissue is too radiodense or the tumour is insufficiently radiodense (55). A recent report by Warner et al. illustrates promising results with combination of MRI, ultrasound and mammography for the detection of breast tumours in proven BRCA mutation carriers (58). Indeed, in this study MRI was able to detect breast tumours not detectable by either ultrasound or mammography. Documentation of long-term survival in mutation carriers that would also demonstrate the long-term benefit of detection by MRI screening (or by other screening methods) of cancers presumably at their earliest stages of development and thus the most likely to respond to treatment is currently underway in the United States and Europe (59).

Effective screening protocols for ovarian cancer with the purpose of detecting primary tumours at early stage disease in mutation carriers are based on current protocols using transvaginal ultrsasonography and clinical pelvic examination (60,61,62). In the general population, the five-year survival of the disease is less than 30% despite recent improvements in treatment protocols (63) and has essentially remained unchanged for the past thirty years. Since there is a clear indication that early detection correlates with increased survival, methods to detect early stage ovarian cancer is pressing. However, the current methods have limited success for effectiveness for detecting borderline tumours, preinvasive and microscopic invasive tumours, and there has been no prospective, randomized trials to test whether screening will reduce morbidity and increase survival of women who are mutation carriers.

The low incidence of mutation positive male breast cancer cases in the context of a positive family history of female breast cancer has hindered progress in establishing guidelines for breast cancer detection in males. However, in some genetic counselling centres in Canada, male carriers of BRCA2 mutations are recommended to perform monthly self-breast examination and annual clinical breast examination, which may include mammography (64).

OPTIONS FOR THE PREVENTION OF BREAST AND OVARIAN CANCERS

In germline mutation carriers all somatic cells harbour a mutated copy of BRCA, and thus the potential for bilateral or double primary cancers is high in women who are mutation carriers in comparison non-carriers. Hence, the options for cancer prevention have included radical surgeries such as bilateral mastectomy and oophorectomy (65). Although carrier status was not determined, a recent retrospective review of Mayo Clinic experience demonstrated that bilateral prophylactic mastectomy was followed by a 90% reduction from the expected number of breast cancers in women at both moderate and high risk due to family history of disease (66).

The benefit of premenopausal oophorectomy by reducing the risk of breast cancer in the general population has been documented (67.68,69). Rebbeck et al. has shown that BRCA1 mutation carriers who underwent prophylactic oophorectomy have a significant risk reduction for breast cancer compared with mutation-positive and age matched women who

did not have oophorectomy (70). Overall, the age of diagnosis of ovarian cancer in BRCA1 mutation carrier is rare below the age of 40 years (71). While the incidence rates for ovarian cancer have been shown to be at least four-fold lower for BRCA2 mutation carriers than BRCA1 mutation carriers (23), the lowest estimate of risk, at 16% is significantly higher than that estimated for the general population. Although the overall mean age of diagnosis for ovarian cancer in mutation carriers is comparable to the mean age at diagnosis of ovarian cancer in the general population (age 56 years), there is some support from anecdotal evidence and recently from a large scale study on population series of 649 women with ovarian cancer not selected for family history, that ovarian cancer in BRCA2 mutation carriers occur later than in BRCA1 mutation (72,73). These age-specific observations could be considered when contemplating an oophorectomy. However, the long-term benefits remain to be validated.

Another consideration of surgical removal of normal ovaries is the risk for developing primary peritoneal carcinomatosis or papillary serous carcinoma or the peritoneum (PSCP) which has been documented in carriers of BRCA1 mutations (74). Based on a data from Crighton University Hereditary Cancer Institute and a review of the literature (75,76,77), Lynch and Casey estimate that fewer than 5% of women who undergo prophylactic oophorectomy because of familial ovarian cancer will develop PSCP, a risk that is significantly lower than the estimated lifetime risk for developing ovarian cancer in mutation carriers (65).

Chemopreventation is another strategy aimed at reducing risk for breast cancer. At least two recent studies have shown that tamoxifen significantly reduces the risk of primary invasive and premalignant breast cancer in women at high risk for breast cancer and of contralateral breast cancer in unselected women (78,79). Recently, Narod et al. demonstrated that tamoxifen reduces the risk of contralateral breast cancer in proven mutation BRCA1 or BRCA2 carriers (80). In women who used tamoxifen for 2 to 4 years, the risk of contralateral breast cancer was reduced by up to 75%. These results are very encouraging and in combination with the results of earlier studies where benefits were observed in high risk women (although carrier status was unknown) question the efficacy of tamoxifen use in cancer prevention in proven BRCA1 or BRCA2 mutation carriers.

GENETIC TESTING FOR BRCA1 AND BRCA2 MUTATIONS

Although a growing body of evidence implicates BRCA1 as a nuclear transcription factor with a role in response to DNA damage and BRCA2 in recombination-mediated repair of double-strand breaks, maintenance of genome integrity and chromosome segregation (81), the relationship to cancer risk and specificity of cancer sites has not been elucidated. Both genes are large and structurally complex. BRCA1 with 22 exons spans a region of approximately 80 kilobases of genomic DNA and encodes a 1,843 amino acid protein (10). BRCA2 is larger than BRCA1 spanning a region of greater than 100 kilobases of genomic DNA and encodes a 3,418 amino acid protein (12,13). Despite the similarity in nomenclature, the genes share no significant homology based on genomic sequence comparisons or no obvious homology to any known gene. However, BRCA1 encodes a protein with a sequence motif at the amino terminus that shares similarity to proteins with zinc-binding domains, and a conserved acidic carboxyl terminus (10). In contrast, BRCA2 encodes a protein that contains no identifiable functional domains based on amino acid sequence composition (12,13). Structurally they both harbour one very large exon (the 11th exon in both genes), that contains 60% of the coding region and the significance of this gene structure is not known.

The identification of carriers of germline mutations largely relies on the mutation analysis of genomic DNA or transcribed mRNA. Various methods alone or in combination for the detection of mutations have been devised based on the observations that BRCA1 and BRCA2 are large genes with many coding exons, that mutations have been identified in all coding exons, that the majority of mutations are private, and that 'deleterious' or disease causing mutations may be complex. Direct sequencing of genomic DNA or cDNA (derived from mRNA) reveals a significant proportion of sequence variants, estimated at about 85% (82). Methods such as single stranded conformation polymorphism (SSCP) analysis, denaturing gradient gel electrophoresis (DGGE), and protein truncation test (PTT) assays also have been used to identify DNA segments containing putative sequence variants that are verified after DNA sequencing (83,84,85). All of these methods have limitations due to their inability to detect large deletions (86,87,88), lack in the sensitivity of detection (such as SSCP); or are technically challenging (such as DGGE). Recently, denaturing high-performance liquid chromatography (DHPLC) which is a method of comparative sequencing based on heteroduplex detection, has been shown to reliably detect a number of different BRCA1 and BRCA2 sequence variants demonstrating that this method has a high degree of sensitivity and specificity and provides a low cost

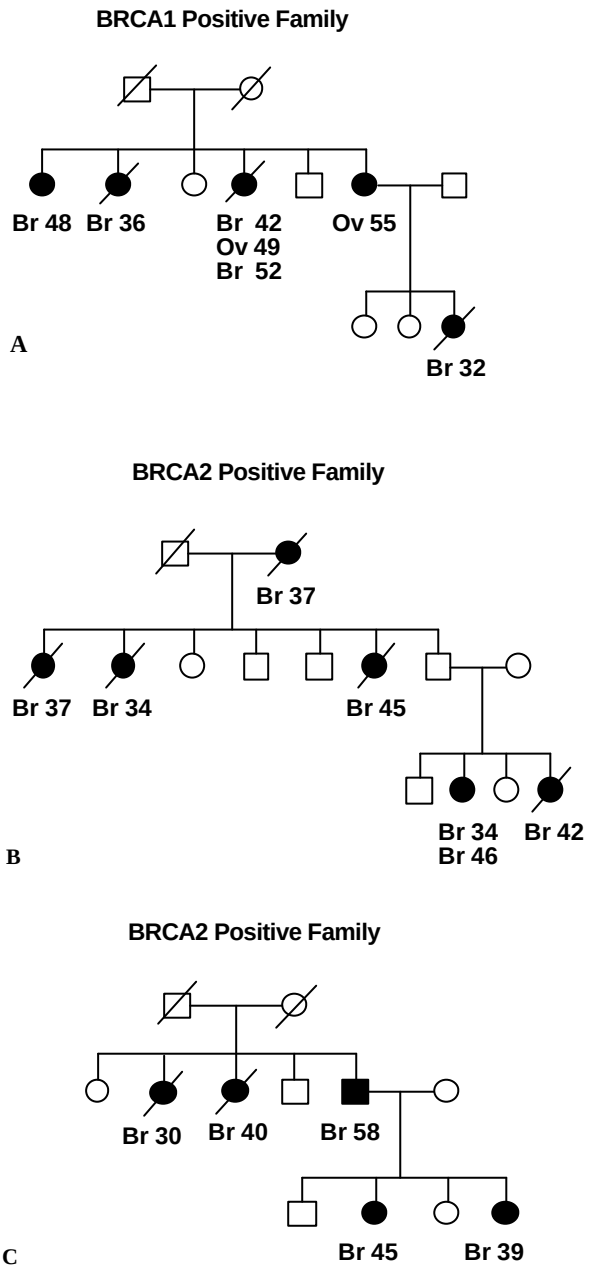


Figure 2.

alternative to direct DNA sequencing (89,90). Because of the wide-spectrum of sequence variants identified it may be necessary to use a combination of different mutation detection protocols. Another consideration that has been explored on a limited basis is that mutations, such as in promoter region(s) of BRCA genes, may alter the transcriptional activity (88, 91) and this alteration may require the use of methods that enable quantifying transcript or protein levels.

The identification of BRCA1 and BRCA2 has afforded the opportunity of identifying carriers of germline mutations by direct mutation analysis where previously the involvement of BRCA was deduced by linkage analysis to markers representative of loci harbouring the putative genes. However, the large size and complex structure of each gene and the spectrum of mutations identified has posed problems for cost-effective mutation screening for high-risk women. The large spectrum of mutations identified worldwide is exemplified by The Breast Information Core (BIC) database which is an open access-online mutation database which lists BRCA1 and BRCA2 mutations (82). A recent review of the database revealed greater than 800 entries for distinct sequence variants for each gene (83). An extraction of entries from Canadian samples is shown in Table 2. This sample is by no means complete or comprehensive as entry of sequence variants in the BIC database is on a voluntary basis and not all entries indicate country of origin of the individual samples for sequencing. The wide spectrum of sequence variations reported for Canadian samples exemplifies the complexity of sequence variants identified in the BRCA genes. Sequence variants include frameshift and nonsense mutations that both result in the introduction of a stop codon leading to chain termination of protein synthesis and thus a truncated protein. Frameshift mutations account for the majority of mutations identified in BRCA1 and BRCA2 worldwide (82). These mutations are deemed 'deleterious' or 'disease-causing' as the resulting truncated protein is presumed to affect the normal function of the gene product. The majority of sequence variants that are missense are termed unclassified variants (82) as the biological consequences of the resulting change leading to specific amino acids substitutions are not known.

The contribution of BRCA1 and BRCA2 varies with spectrum of cancers in breast cancer families (14). Germline mutations in BRCA1 are typically identified families with ovarian cancer (Figure 2A). These cancer families are often referred to as breast-ovarian cancer syndrome families (Table 1). Breast cancer families with no ovarian cancer or contain at least one male breast cancer case are more likely to harbour germline mutations in BRCA2 (Figure 2B and 2C). These families are often referred to as 'site-specific' breast cancer families. However, it is important to emphasize that these phenotypic classifications are not mutually exclusive but reflect the likelihood of identifying a mutation in a particular susceptibility gene and thus could serve as a guide to prioritizing mutation analysis.

Within defined ethnic groups specific, relatively frequent mutations have been identified. Three founder mutations have been identified in the Ashkenazi Jewish families of eastern European ancestry:

Table 2: Spectrum of BRCA1 and BRCA2 Sequence Variants Reported for Canadian Population*

Gene	Designation	Exon or intron (IVS)	Nucleotide	Base change	Codon	Amino acid change	Mutation type or effects†	Reported more than once in BIC	Recurrent mutation mutation in some populations
	185delAG	2	185	delAG	23	stop 39	F	Yes	Ashkenazi Jewish
	C61G	5	300	T>G	61	Cys>Gly	M	Yes	
	Q356R	11	1186	A>G	356	Gln>Arg	M/P	Yes	
	D369N	11	1224	G>A	369	Asp>Asn	M/UV	no	
	R496H	11	1606	G>A	496	Arg>His	M/UV	yes	
	2072insG	11	2072	insG	651	stop672	F	yes	
	S741F	11	2341	C>T	741	Ser>Phe	M/UV	no	
	2800delAA	11	2800	delAA	894	stop901	F	Yes	
	2953del3+C	11	2953	delGTAinsC	945	stop950	F	Yes	French Canadian
BRCA1	P1099L	11	3419	C>T	1099	Pro>Leu	M/UV	yes	
	3450del4	11	3450	delCAAG	1115	stop1115	F	Yes	
	3768insA	11	3768	insA	1217	stop1218	F	Yes	French Canadian
	E1250X	11	3867	G>T	1250	stop1250	N	Yes	
	R1347G	11	4158	A>G	1347	Arg>Gly	M/UV	Yes	
	4184del4	11	4184	delTCAA	1355	stop1364	F	Yes	
	R1443X	13	4446	C>T	1443	stop1443	N	Yes	French Canadian
	5221delTG	18	5221	delTG	1714	stop1714	F	no	
	IVS20+1G>A	IVS20	5396+1	G>A	na	na	S	Yes	Dutch
	5382insC	20	5382	insC	1756	stop1829	F	Yes	Ashkenazi Jewish
	IVS21-8C>T	IVS21		C>T	na	na	S/UV	no	
	983del4	9	934	delACAG	252	stop275	F	yes	
	1119del9/ins10	10	1119	delAACAGTTGT/ insGATACTTCAG	297	stop304	F	no	
	E462G	10	1613	A>G	462	Glu>Gly	M/UV	yes	
	2034insA	10	2034	insA	602	stop 615	F	yes	
	2157delG	11	2157	delG	643	stop 659	F	yes	
	2816insA	11	2816	insA	863	stop 880	F	yes	French Canadian
	4359ins6	11	4359	insTGAGGA	1378	Thr>stop	N	yes	
	D1420Y	11	4486	G>T	1420	Asp>Thr	M/UV	yes	
	G1771D	11	5540	G>A	1771	Gly>Asp	M/UV	yes	
	5699insA	11	5699	insA	1824	stop 1828	F	no	
	S1882X	11	5873	C>A	1882	stop 1882	N	yes	
	6085G>T	11	6085	G>T	1953	stop 1953	N	yes	French Canadian
BRCA2	6174delT	11	6174	delT	1982	stop 2003	F	yes	Askenazi Jewish
	R2034C	11	6328	C>T	2034	Arg>Cys	M/UV	yes	
	6503delTT	11	6503	delTT	2092	stop 2099	F	yes	French Canadian
	7297delCT	14	7297	delCT	2357	stop 2358	F	yes	
	IVS14+6G>A	IVS14	na	G>A	na	na	S/UV	no	
	C2636X	17	8136	T>A	2636	stop 2636	N	no	
	V2728I	18	8410	G>A	2728	Val>Ile	M/UV	yes	
	8474delAG	18	8474	delAG	2749	stop 2762	F	no	
	8765delAG	20	8765	delAG	2846	stop 2867	F	no	French Canadian
	Q2858R	20	8801	A>G	2858	Gln>Arg	M/UV	no	
	8904delA	21	8904	delA	2892	stop 5908	F	no	
	W2989X	23	9198	G>A	2989	Trp>stop	N	no	
	9356insA	23	9326	insA	3033	stop3034	F	yes	
	K3326X	27	10204	A>T	3326	Lys>stop	N/UV	yes	

* Extracted from Breast Cancer Information Core; †Mutation type or effects: F=framesfit, M=missense, N=nonsense, S=splice variant, UV=unknown variant.

BRCA1:185delAG, BRCA1:5382insC, and BRCA2:6173delTT (38,93,94,95,96) (see Table 2). In a study 220 North American Ashkenazi Jewish families (including families ascertained in the Montreal area) we observed that 45% of harboured one of three founder mutations (94). In another study, we have observed six common mutations in our analysis of the French Canadian population of Quebec (72,97,98) (Table 2). Two specific

mutations: BRCA1 4446C>T and BRCA2 8765delAG account for a significant fraction of mutation positive cases where 28 of 41 mutations identified in 97 families harboured one of these specific mutations (97). Haplotype analysis (genotyping of polymorphic markers adjacent or within the genes that would enable deducing parent of origin of each allele) has provided evidence for founder effects in these ethnic groups, suggesting that the mutations arose from common ancestors (99,100,101,102). We also showed that presence of ovarian cancer is a strong predictor of the presence of BRCA1 versus a BRCA2 mutation, a phenotype consistent with observations of mutation spectrum of families not selected for ethnicity (14,97). Specific mutations have also been described in other groups defined by country of origin, such as BRCA2:999del 5 which is the most prevalent mutation identified in breast cancer families in the Icelandic population (103).

The presence of founder effects, leading to a reduced heterogeneity, facilitates carrier detection and genetic counselling, for certain well-defined populations. As a first screen for mutations, the overall cost of mutation detection is significantly reduced when mutation detection is limited to mutations found at a high frequency in specific populations. Genetic counselling is also facilitated when the prevalence of specific mutations is known in the defined population harbouring recurrent mutations. For example, several studies have shown the prevalence of carriers of the common BRCA1 and BRCA2 in the Ashkenazi Jewish population is known to be high, ~2.5% (104,105,106) and the yield of other mutations in either gene is rare. These findings suggest that screening for mutations may be limited to the identification of the three common mutations identified in the Ashkenazim. In contrast to the Ashkenazi Jewish population, the frequency of the six common mutations identified in the French Canadian population is not known, although we have shown that 10% of women diagnosed with breast cancer below the age 41 years (98) and 8% of women diagnosed with ovarian cancer (72), not selected for family history of cancer, harbour one of six common mutations (97).

INTERPRETING TEST RESULTS

Prior to the discovery that germline mutations in BRCA1 and BRCA2 conferred increased risk to breast and ovarian cancers, empiric risk for hereditary cancer was computed based on both the family history of disease (breast cancer) and the age of diagnosis of the breast cancers (4,7). Figure 3 illustrates a pedigree, Family X, displaying a strong family history of breast and ovarian cancer based on the hallmark features of inherited predisposition to breast and ovarian cancers (Table 1). In Family X, the 32 year women having at least two first-degree relatives with breast cancer diagnosed before 50 years of age (in this case her mother and sister diagnosed at ages 36 and 39, respectively) has a 35%-48% cumulative lifetime risk (by age 70) of developing breast cancer. This estimate is based on estimates that derived from mathematical models that used population-based family history data (4). In the absence of a genetic test to determine carrier status she would be informed that she has at high risk for harbouring a mutation based on the 50% chance of inheriting a disease causing allele from her mother who being affected is the predicted carrier (see Figure 1). Mutation analysis of BRCA1 and BRCA2 has the potential to determine if she carriers a disease causing mutation. The results also have the potential to impact on management when considering options for cancer detection and prevention such that management procedures are concentrated on those at highest risk for developing cancer in this family.

Family X is consistent with the clinical phenotype for inherited predisposition to breast and ovarian cancer (Table 1), and thus has a high likelihood of harbouring a germline mutation in BRCA1 or BRCA2. A number of models exist to compute risk of carrier status in high-risk individuals (7,107,108,109). It is important to emphasize confirmation of pathology of cancer sites as risk assessment is based on the cancer site, age at diagnosis and number of cancers regardless of whether genetic testing is considered an option for improving risk assessment. The presence of an ovarian cancer case in a family of young onset breast cancers increases the likelihood that a BRCA1 mutation segregates with disease in this family. Preferably, genetic analysis is performed on an affected individual in the family because it is currently difficult to interpret negative test results. For example, the conclusion of a test result that yields no obvious deleterious mutation ('negative' test result) cannot be distinguished from the possibility that the mutation assay was lacking in sensitivity and thus the mutation was present but not detectable. This genetic test result would be deemed 'not informative' and would not improve risk assessment and thus risk assessment remains as computed based on family history alone. In circumstances where the frequency and spectrum of specific mutations in a defined ethnic group are known, such as the Ashkenazim for example, mutation analysis has proven useful in improving risk assessment when genetic analysis performed on unaffected individual because a clinical specimen was not available from an affected individual (94). In the event of a positive test result in an affected individual such as the women

diagnosed with breast cancer at age 39 in Family X, the genetic analysis could be extended to other family members in order to determine if they are carriers of the same mutation. The absence of a mutation, as the case of 35-year women in Family X, would suggest that she her lifetime risk for developing breast and ovarian cancers is close to population risk. This women may consider options for breast cancer detection that are open to all women in the general population beginning at age 50 years. However, the presence of mutation, as in the case of the 32-year women in Family X, would suggest that her risk for developing breast and ovarian cancers is significantly elevated above population risk. This high-risk woman may consider options for cancer detection and prevention that are available to high-risk women.

Genetic testing has the potential to improve management for women in high-risk families that have already had a breast cancer diagnosis. For example, the 39 year-old women with breast cancer deemed to be a mutation carrier in Family X is at increased risk for a second breast cancer and ovarian cancer in comparison to the women diagnosed with breast cancer at age 55 and found not to be a mutation carrier and thus likely a sporadic case of breast cancer (Figure 3). Breast cancer is the most common cancer reported in the female population (second to skin cancers) and thus it is not surprising that sporadic cases occur in the context of hereditary breast cancer families (94). As mutation carriers are more likely to develop breast cancer at a young age (prior to age 50 years), it is preferable to test the youngest breast cancer case in the family or an ovarian cancer case, as the likelihood of a sporadic ovarian cancer case in breast cancer family is rare.

THE DIFFICULTY OF INTERPRETING SEQUENCE VARIANTS

Mutations in BRCA1 and BRCA2 are deduced based on the anticipated consequence of a DNA sequence variation on the resulting protein, as there is no assay to assess the functional significance of an alteration in the amino acid sequence composition. For example, this is predicted when a sequence variation results in the production of a truncated protein (see Table 2). However, the significance of protein truncating mutations that arise in the extreme 3' end of the gene, as the case of the mutation BRCA2: K3326X, which occurs in exon 27, is not known. The BIC reports one instance where this variant was observed in the context of another frameshift mutation, BRCA2:6503delTT (92). Missense mutations, resulting in amino acid substitutions, are difficult to interpret in comparison to mutations giving rise to truncated proteins. A common missense mutation in BRCA1, 300T>G, results in an amino acid substitution of cysteine to glycine at amino acid position 61 (C61G). Although this substitution does not alter the hydrophobicity or charge it occurs in the zinc-finger-binding domain, and loss of an amino acid with a sufhydryl side chain may affect the function of the resulting Brca1 protein. The observation that this mutation segregates with disease in breast cancer families suggests that the alteration has significant functional consequences. Thus, in the absence of a biological assay for protein function or concentration, the segregation of sequence variants with disease in a family remains the most effective way to deduce the significance of sequence variants of obvious unknown significance. As segregation analysis is less feasible outside of the research facilities the importance of comparative sequence analyses such as with mutation databases, becomes evident. The rare occurrence of sequence variants in healthy women, and thus association only with breast (and ovarian) cancer cases, is also an indicator of the significance of the sequence variation (92).

CONCLUSIONS

The identification of the breast and ovarian cancer susceptibility genes has improved risk assessment of women in high risk families as the most immediate impact is the ability to distinguish women who carry high risk alleles from those that do not and thus avoiding unnecessary management procedures. Assessing risk is a complicated time consuming process involving pedigree inspection, confirmation of cancer sites and age of diagnosis, and interpretation of mutation detection results. Given the complexity of risk assessment, it is strongly recommended that risk assessment based on personal and family history disease and genetic test results is conducted in the context of highly trained personnel such as genetic counselling service specializing in inherited predisposition to adult onset cancers with management conducted in consultation with breast specialists and gynecologists/oncologists.

For the immediate future there is a need to evaluate the long-term benefits of cancer prevention and detection strategies, improve methodologies of cancer detection and risk, and determine environmental and genetic factors that may modify risk in carriers of deleterious BRCA1 and BRCA2 mutations. In addition, recent efforts have been directed towards the identification of novel breast cancer susceptibility genes. Of the estimated 10% of the all breast and ovarian cancer are due to inherited predisposition to cancer consistent with transmission of an autosomal dominant trait, an estimated 5% of these cancers are due to germline mutations in BRCA1 and BRCA2.

Either the sensitivity of detection is beyond our current means, or there are other novel cancer predisposing genes. Ford et al., presented compelling evidence that up to 67% of site-specific breast cancer families with four or five cases of breast cancer was not due to either BRCA1 or BRCA2 (14). One conclusion is that these 'negative' families may be due to mutations in novel breast cancer susceptibility genes, as the clinical phenotype of family history is not consistent with the involvement of other known breast cancer predisposing genes such as TP53 and PTEN. Earlier studies using smaller number of families have provided supportive evidence for this hypothesis (110,111,112,113). However, the identification of novel susceptibility genes has remained elusive (81) despite an intriguing loci identified on chromosomes 8 (114,115,116) and 13 (117). The identification of genetic factors that modify risk in BRCA1 and BRCA2 mutation carriers and novel susceptibility genes may lead to further improvements in risk assessment of hereditary breast and/or ovarian cancer families.

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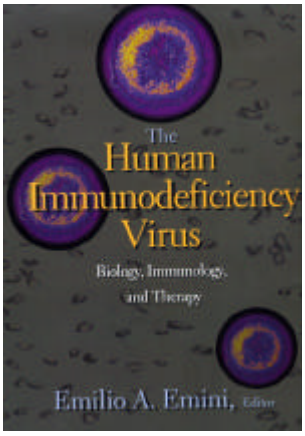
Dr. Patricia Tonin, PhD is a professor of Medicine and Human Genetics at McGill University. She is also a member of the Medical Genetics and Genomics Axis of the Research Institute of the MUHC. Her research team, located in the Montreal General Hospital, has made significant contributions to the study of breast and ovarian cancer, in particular, the characterization of BRCA1 and BRCA2.

BOOK REVIEW



The Human Immunodeficiency Virus: Biology, Immunology, and Therapy
Edited by Emilio A. Emini

Princeton University Press,
2002, 450 pages (Hardcover)
ISBN: 0691004544
$75.00 US

In the past twenty years, "Overwhelming" is probably the best all-encompassing word to describe the HIV/AIDS tragedy. AIDS has been overwhelming in its global spread; overwhelming, in its synergy with other infectious diseases namely, TB and malaria to cause human suffering; overwhelming in the devastating impact it leaves behind as the epidemic tears through the social fabric of developed and developing nations alike. Likewise, our collective effort to curb this pandemic has also been nothing less than overwhelming. In recent years, researchers have acquired a wealth of knowledge with respect to the biology of HIV. Much of this understanding has already been translated into therapeutic treatment that has significantly improved our armamentarium against this disease.

This book "The Human Immunodeficiency Virus" provides a chronicle of the intensity of scientific investigation in an effort to control this disease. Each chapter is written by a different contributor, who is a leader in their respective field. Together they provide a compilation of our current understanding in the diverse area of HIV/AIDS research.

The book itself is highly readable and although each chapter is written so that it may be read independently, the format of the book is constructed to allow a broader appreciation of the expanse of scientific disciplines involved in HIV therapy and prevention, through basic and clinical sciences to social research and preventative education.

The initial chapter although somewhat brief, provides a comprehensive summary of the basics of retrovirology with a particular focus on the causative agent of human disease. The following chapter on HIV genetics although outwardly similar to the first, is not full appreciated until later chapters since its provides a focused perspective on the attributes of HIV that make it such a formidable challenge, particularly in regard to multi-drug resistance and vaccine development.

Chapters three through five focus on antiretroviral therapy. These chapters detail the present viral targets: nucleoside-reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) respectively. The focus of each chapter includes known mechanisms of action, and resistance. These three chapters blend together nicely giving an up to the moment account of current progress, while reviewing of an area of considerable breadth.

The following chapters are naturally the focus of ongoing research into receptor, co-receptor interactions. Later, topics such as integrase inhibitors and accessory functions are particularly well treated.

Following these, as in previous chapters, the book the allows the reader to smoothly transition between the bench top and the clinic into the chapter on translational research.

The importance of the information discussed in the chapter on prevention should not be understated by its relatively late position in the book; perhaps it should have been the first chapter. Prevention is in fact the foremost of available treatments, particularly in resource poor settings where other modalities are only now becoming available, at an unacceptably slow pace. With the intent to both improve the standard of care in these countries and stem the tide of an ever-burgeoning pandemic, drugs and an effacious vaccine must become readily available. In the meantime, prevention certainly constitutes a laudable and readily attainable goal.

The final chapters are suitably reserved for a perspective on vaccines. These are a compilation of HIV/SIV immunobiology and HIV vaccine prospects, unfortunately both are rather brief. In light of the urgent need for a vaccine, prudence would dictate that these topics be allocated a more lengthy discourse. Nonetheless, overall the contributors do manage they do cover theses topics well.

In sum, this book effectively integrates a particularly large and diverse area of research into a single volume that would stand to benefit students, investigators and clinicians.

James Whitney
McGill University AIDS Centre

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Journal articles

1. Bunny B, Coyote WE, Le Pew P, et al. Impact trauma and subdural hematoma. McGill Journal of Medicine 1994; 5: 1-6.

Books

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Internet

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LIPITOR*

ATORVASTATIN CALCIUM

EFFICACY TO REACH TARGET THE FIRST TIME

LIPITOR*
(atorvastatin calcium)
10 mg, 20 mg and 40 mg tablets
<u>THERAPEUTIC CLASSIFICATION</u> Lipid Metabolism Regulator

ACTIONS AND CLINICAL PHARMACOLOGY

LIPITOR (atorvastatin calcium) is a synthetic lipid lowering agent. It is a selective, competitive inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. This enzyme catalyzes the conversion of HMG-CoA to mevalonate, which is an early and rate-limiting step in the biosynthesis of cholesterol.

LIPITOR lowers plasma cholesterol and lipoprotein levels by inhibiting HMG-CoA reductase and cholesterol synthesis in the liver and by increasing the number of hepatic Low Density Lipoprotein (LDL) receptors on the cell-surface for enhanced uptake and catabolism of Low Density Lipoprotein (LDL).

LIPITOR reduces LDL-Cholesterol (LDL-C) and the number of LDL particles. LIPITOR also reduces Very Low Density Lipoprotein-Cholesterol (VLDL-C), serum triglycerides (TG) and Intermediate Density Lipoproteins (IDL), as well as the number of apolipoprotein B (apo B) containing particles, but increases High Density Lipoprotein Cholesterol (HDL-C). [illegible] Elevated plasma TG is also a risk factor for cardiovascular disease, particularly if due to increased IDL, or associated with decreased HDL-C or increased LDL-C.

[illegible] Atorvastatin tablets are 95% to 99% bioavailable compared to solutions.

[illegible] oxidative products. Approximately 70% of circulating inhibitory activity for HMG-CoA reductase is attributed to active metabolites.

Atorvastatin and its metabolites are eliminated by biliary excretion. Less than 2% of a dose of atorvastatin is recovered in urine following oral administration. [illegible] 14 hours, but the half-life of inhibitory activity for HMG-CoA reductase is 20 to 30 hours due to the contribution of longer lived active metabolites.

INDICATIONS AND CLINICAL USE

LIPITOR (atorvastatin calcium) is indicated as an adjunct to diet, at least equivalent to the American Heart Association (AHA) Step 1 diet, for the reduction of elevated total cholesterol (total-C), LDL-C, TG and apolipoprotein B (apo B) in hyperlipidemic and dyslipidemic conditions, when response to diet and other nonpharmacological measures alone has been inadequate, including:

- Primary hypercholesterolemia (Type IIa);
- Combined (mixed) hyperlipidemia (Type IIb), including familial combined hyperlipidemia, regardless of whether cholesterol or triglycerides are the lipid abnormality of concern;
- Dysbetalipoproteinemia (Type III);
- Hypertriglyceridemia (Type IV);
- Familial hypercholesterolemia (homozygous and heterozygous). For homozygous familial hypercholesterolemia, LIPITOR should be used as an adjunct to treatments such as LDL apheresis or as monotherapy if such treatments are not available.

[illegible] hyperlipidemic and dyslipidemic conditions. In 2 dose-response studies in mildly to moderately hyperlipidemic patients (Fredrickson Types IIa and IIb), LIPITOR reduced the levels of total cholesterol (29-45%), LDL-C (39-60%), apo B (32-50%), TG (19-37%) [illegible] were achieved in patients with heterozygous familial hypercholesterolemia, non-familial forms of hypercholesterolemia, [illegible] mellitus. In patients with hypertriglyceridemia (Type IV), LIPITOR (10 to 80 mg daily) reduced TG (25-56%) and LDL-C levels [illegible] with high TG levels (>11 mmol/L).

In an open label study in patients with dysbetalipoproteinemia (type III), LIPITOR (10 to 80 mg daily) reduced total-C [illegible]

In an open label study in patients with homozygous familial hypercholesterolemia (FH) LIPITOR (10 to 80 mg daily) [illegible] patients not on plasmapheresis and of 31% for patients who continued plasmapheresis. A mean LDL-C lowering of 35% was observed in receptor defective patients and of 19% in receptor negative patients (see PHARMACOLOGY, Clinical Studies).

For more details on efficacy results by pre-defined classification and pooled data by Fredrickson types, see PHARMACOLOGY, Clinical Studies.

[illegible] (e.g. poorly controlled diabetes mellitus, hypothyroidism, nephrotic syndrome, dysproteinemias, obstructive liver disease) [illegible] with TG <4.52 mmol/L (<400 mg/dL). LDL-C can be estimated using the following equation:

LDL-C (mmol/L) = total-C − [(0.37 × (TG) + HDL-C)]
LDL-C (mg/dL) = total-C − [(0.2 × (TG) + HDL-C)]

For patients with TG levels >4.52 mmol/L (>400 mg/dL), this equation is less accurate and LDL-C concentrations should be measured directly or by ultracentrifugation.

[illegible] in patients with stable coronary artery disease and LDL-C at least 3.0 mmol/L, in patients referred for percutaneous [illegible] With usual medical care which could include lipid metabolism regulators. The results of the AVERT study should be considered [illegible] LIPITOR there was a trend for a reduced incidence of ischemic events and time to first ischemic event. The results also [illegible] **addition and complementary** [illegible] and would benefit patients referred for this procedure (see SUPPORTIVE BIBLIOGRAPHY).

CONTRAINDICATIONS

Hypersensitivity to any component of this medication.

Active liver disease or unexplained persistent elevations of serum transaminases exceeding 3 times the upper limit of normal (see WARNINGS).

Pregnancy and lactation (see PRECAUTIONS).

WARNINGS

Pharmacokinetic Interactions

The use of HMG-CoA reductase inhibitors has been associated with severe myopathy, including rhabdomyolysis, which [illegible] Atorvastatin is metabolized by cytochrome P-450 isoform 3A4 and as such may interact with agents that inhibit this enzyme. (See WARNINGS, Muscle Effects and PRECAUTIONS, Drug Interactions and Cytochrome P-450 mediated interactions).

Hepatic Effects

In clinical trials, persistent increases in serum transaminases greater than three times the upper limit of normal occurred in <1% of patients who received LIPITOR. When the dosage of LIPITOR was reduced, or when drug treatment was interrupted or discontinued, serum transaminase levels returned to pretreatment levels. The increases [illegible]

Liver function tests should be performed before the initiation of treatment, and periodically thereafter. Special attention should be paid to patients who develop elevated serum transaminase levels, and in these patients, measurements should be repeated promptly and then performed more frequently.

If increases in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) show evidence of progression, particularly if they rise to greater than 3 times the upper limit of normal and are persistent, the dosage should be reduced or the drug discontinued.

[illegible]

LIPITOR should be used with caution in patients who consume substantial quantities of alcohol and/or have a past history of liver disease. Active liver disease or unexplained transaminase elevations are contraindications to the use of LIPITOR; if such a condition should develop during therapy, the drug should be discontinued.

Muscle Effects

Myopathy, defined as muscle aching or muscle weakness in conjunction with increases in creatine phosphokinase (CPK) values to greater than ten times the upper limit of normal, should be considered in any patient with diffuse myalgia, muscle tenderness or weakness, and/or marked elevation of CPK. Patients should be advised to report [illegible]

The risk of myopathy and rhabdomyolysis during treatment with HMG-CoA reductase inhibitors is increased with concurrent administration of cyclosporine, fibric acid derivatives, erythromycin, clarithromycin, niacin (nicotinic acid), azole antifungals or nefazodone. As there is no experience to date with the use of LIPITOR given concurrently with these drugs, with the exception of pharmacokinetic studies conducted in healthy subjects with erythromycin and clarithromycin, the benefits and risks of such combined therapy should be carefully considered (see PRECAUTIONS, Pharmacokinetic Interaction Studies and Potential Drug Interactions). Rhabdomyolysis has been reported in very rare cases with LIPITOR (see PRECAUTIONS, Drug Interactions).

Rhabdomyolysis with renal dysfunction secondary to myoglobinuria has also been reported with HMG-CoA reductase inhibitors. LIPITOR therapy should be temporarily withheld or discontinued in any patient with an acute serious condition suggestive of a myopathy or having a risk factor predisposing to the development of renal failure secondary to rhabdomyolysis [illegible]

PRECAUTIONS

General

The effects of atorvastatin-induced changes in lipoprotein levels, including reduction of serum cholesterol on cardiovascular morbidity or mortality or total mortality have not been established.

Before instituting therapy with LIPITOR (atorvastatin calcium), an attempt should be made to control elevated serum lipoprotein levels with appropriate diet, exercise, and weight reduction in overweight patients, and to treat other underlying medical problems (see INDICATIONS AND CLINICAL USE). Patients should be advised to inform subsequent physicians of the prior use of LIPITOR or any other lipid-lowering agents.

Effect on the Lens

[illegible]

Effect on Ubiquinone (CoQ_{10}) Levels

[illegible] established. It has been reported that a decrease in myocardial ubiquinone levels could lead to impaired cardiac function [illegible]

Effect on Lipoprotein (a)

In some patients, the beneficial effect of lowered total cholesterol and LDL-C levels may be partly blunted by a concomitant increase in Lp(a) levels. Until further experience is obtained, it is suggested, where feasible, that measurements of serum Lp(a) be followed up in patients placed on atorvastatin therapy (see SUPPORTIVE BIBLIOGRAPHY).

<u>**Hypersensitivity**</u>

[illegible] thrombocytopenia, leukopenia, hemolytic anemia, positive ANA, ESR increase, [illegible] epidermal necrolysis, erythema multiforme, including Stevens-Johnson syndrome. Although to date hypersensitivity [illegible]

Use in Pregnancy

LIPITOR is contraindicated during pregnancy (see CONTRAINDICATIONS).

Atherosclerosis is a chronic process and discontinuation of lipid lowering drugs during pregnancy should have little impact on the outcome of long term therapy of primary hypercholesterolemia. Cholesterol and other products of cholesterol biosynthesis are essential components for fetal development (including synthesis of steroids and cell membranes). Since HMG-CoA reductase inhibitors decrease cholesterol synthesis and possibly the synthesis of other [illegible] pregnant women.

[illegible] patient becomes pregnant while taking LIPITOR, the drug should be discontinued and the patient apprised of the potential risk to the fetus.

Nursing Mothers

[illegible] in human milk. Because of the potential for adverse reactions in nursing infants, women taking LIPITOR should not be breast-fed (see CONTRAINDICATIONS).

<u>**Pediatric Use**</u>

[illegible]

Geriatric Use

[illegible] that the safety and effectiveness of atorvastatin in this population was similar to that of patients <70 years of age. [illegible] precautionary measure, the lowest dose should be administered initially (see PHARMACOLOGY, Human Pharmacokinetics; SUPPORTIVE BIBLIOGRAPHY).

<u>**Renal Insufficiency**</u>

[illegible] Similar precautions apply in patients with severe renal insufficiency [creatinine clearance <30 mL/min (<0.5 mL/sec)]; the lowest dosage should be used and implemented cautiously (see WARNINGS, Muscle Effects; PRECAUTIONS, Drug Interactions).

Refer also to DOSAGE AND ADMINISTRATION.

<u>**Endocrine Function**</u>

HMG-CoA reductase inhibitors interfere with cholesterol synthesis and as such might theoretically blunt adrenal and/or gonadal steroid production. [illegible] in adequate numbers of patients. The effects, if any, on the pituitary-gonadal axis in premenopausal women are unknown. Patients treated with atorvastatin who develop clinical evidence of endocrine dysfunction should be evaluated appropriately. [illegible] decrease the levels of endogenous steroid hormones.

Pharmacokinetic Interaction Studies and Potential Drug Interactions

Pharmacokinetic interaction studies conducted with drugs in healthy subjects may not detect the possibility of a potential drug interaction in some patients due to differences in underlying diseases and use of concomitant medications [illegible]

[illegible]

Concomitant Therapy with Other Lipid Metabolism Regulators: Combined drug therapy should be approached with caution as information from controlled studies is limited.

Bile Acid Sequestrants:

Patients with mild to moderate hypercholesterolemia: LDL-C reduction was greater when LIPITOR 10 mg and colestipol 20 g [illegible]

Patients with severe hypercholesterolemia: LDL-C reduction was similar (-53%) when LIPITOR 40 mg and colestipol 20 g [illegible] lower (approximately 26%) when LIPITOR 40 mg plus colestipol 20 g were coadministered compared with LIPITOR 40 mg alone.

[illegible] types of hypercholesterolemic patients (see PHARMACOLOGY, Clinical Studies).

When LIPITOR is used concurrently with colestipol or any other resin, an interval of at least two hours should be maintained between the two drugs, since the absorption of LIPITOR may be impaired by the resin.

Fibric Acid Derivatives (Gemfibrozil, Fenofibrate, Bezafibrate) and Niacin (Nicotinic Acid): Although there is [illegible] of such combined therapy should be carefully considered. The risk of myopathy during treatment with other drugs in this class is increased with concurrent administration (see WARNINGS, Muscle Effects).

Coumarin Anticoagulants: [illegible] patients receiving chronic warfarin therapy (see SELECTED BIBLIOGRAPHY).

Digoxin: [illegible] of digoxin 0.25 mg and LIPITOR 10 mg daily. However, digoxin steady-state concentrations increased approximately 20% [illegible] digoxin should be monitored appropriately.

Antihypertensive agents (amlodipine): In clinical studies, LIPITOR was used concomitantly with antihypertensive agents [illegible] pharmacokinetics were not altered by the coadministration of LIPITOR 80 mg and amlodipine 10 mg at steady state (see Human Pharmacokinetics).

Oral Contraceptives and Hormone Replacement Therapy: [illegible] containing 1 mg norethindrone and 35 µg ethinyl estradiol. Increased plasma concentrations (AUC levels) of [illegible] when selecting an oral contraceptive. In clinical studies, LIPITOR was used concomitantly with estrogen replacement therapy without evidence to date of clinically significant adverse interactions.

Antacids: Administration of aluminum and magnesium based antacids, such as Maalox® TC Suspension, with LIPITOR decreased plasma concentrations of LIPITOR by approximately 35%. LDL-C reduction was not altered but the triglyceride-lowering effect of LIPITOR may be affected.

Cimetidine: Administration of cimetidine with LIPITOR did not alter plasma concentrations or LDL-C lowering efficacy of LIPITOR; however, the triglyceride-lowering effect of LIPITOR was reduced from 34% to 26%.

Cytochrome P-450-mediated Interactions: [illegible] 3A4. Erythromycin, a CYP 3A4 inhibitor, increased atorvastatin plasma levels by 40%. Coadministration of CYP 3A4 inhibitors, such as grapefruit juice, some macrolide antibiotics (i.e. erythromycin, clarithromycin), immunosuppressants (cyclosporine), azole antifungal agents (i.e. itraconazole, ketoconazole), or the antidepressant, nefazodone, may have the potential to increase plasma concentrations of HMG-CoA reductase inhibitors, including LIPITOR (see SELECTED BIBLIOGRAPHY). Caution should thus be exercised with concomitant use of these agents (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Renal Insufficiency and Endocrine Function; DOSAGE AND ADMINISTRATION, SELECTED BIBLIOGRAPHY).

In healthy subjects, coadministration of maximum doses of both atorvastatin (80 mg) and terfenadine (120 mg), a CYP 3A4 substrate, [illegible] unchanged. However, since an interaction between these two drugs cannot be excluded in patients with predisposing factors [illegible] should be exercised when these agents are coadministered (see WARNINGS, Pharmacokinetic Interactions, DOSAGE AND ADMINISTRATION).

Antipyrine: [illegible] system (cytochrome P-450 system). LIPITOR had no effect on the pharmacokinetics of antipyrine, thus interactions with other drugs metabolized via the same cytochrome isozymes are not expected.

Macrolide Antibiotics (azithromycin, clarithromycin, erythromycin): In healthy adults, coadministration of LIPITOR (10 mg QD) and azithromycin (500 mg QD) did not significantly alter the plasma concentrations of atorvastatin. [illegible] which are both CYP 3A4 inhibitors, increased plasma concentrations of atorvastatin approximately 40% and 80%, respectively (see WARNINGS, Muscle Effects; Human Pharmacokinetics).

Patients with Severe Hypercholesterolemia: Higher drug dosages (80 mg/day) required for some patients with [illegible] atorvastatin. **Caution should be exercised in such patients who are also severely renally impaired, elderly, or are concomitantly being administered digoxin or CYP 3A4 inhibitors (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Drug Interactions; DOSAGE AND ADMINISTRATION).**

Drug/Laboratory Test Interactions

LIPITOR may elevate serum transaminase and CPK levels (from skeletal muscle). In the differential diagnosis of chest pain [illegible]

ADVERSE REACTIONS

LIPITOR is generally well-tolerated. Adverse reactions have usually been mild and transient. In controlled clinical studies (placebo-controlled and active-controlled comparative studies with other lipid lowering agents) involving 2502 patients, <2% of patients were discontinued due to adverse experiences attributable to LIPITOR. Of these 2502 patients, 1721 were treated for at least 6 months and 1253 for 1 year or more.

Adverse experiences occurring at an incidence ≥1% in patients participating in placebo-controlled clinical studies of LIPITOR and reported to be possibly, probably or definitely drug related are shown in Table 1 below.

TABLE 1. Associated Adverse Events Reported in ≥1% of Patients in Placebo-Controlled Clinical Trials

	Placebo % (n=270)	LIPITOR % (n=1122)
GASTROINTESTINAL		
Constipation	1	1
Diarrhea	1	1
Dyspepsia	2	1
Flatulence	2	1
Nausea	0	1
NERVOUS SYSTEM		
Headache	2	1
MISCELLANEOUS		
Pain	<1	1
Myalgia	1	1
Asthenia	<1	1

The following additional adverse events were reported in clinical trials; not all events listed below have been associated with a causal relationship to LIPITOR therapy: Muscle cramps, myositis, myopathy, paresthesia, peripheral neuropathy, pancreatitis, hepatitis, cholestatic jaundice, anorexia, vomiting, alopecia, pruritus, rash, impotence, hyperglycemia, and hypoglycemia.

Post-marketing experience: Very rare reports of severe myopathy with or without rhabdomyolysis (see WARNINGS, Muscle Effects; PRECAUTIONS, Renal Insufficiency and Drug Interactions). Isolated reports: thrombocytopenia, arthralgia, and allergic reactions including urticaria, angioneurotic edema, anaphylaxis and bullous rashes (including erythema multiforme, [illegible]

Ophthalmologic observations: see PRECAUTIONS.

[illegible] (see WARNINGS).

SYMPTOMS AND TREATMENT OF OVERDOSAGE

There is no specific treatment for atorvastatin overdosage. Should an overdose occur, the patient should be treated symptomatically and supportive measures instituted as required. Due to extensive drug binding to plasma proteins, hemodialysis is not expected to significantly enhance atorvastatin clearance.

DOSAGE AND ADMINISTRATION

Patients should be placed on a standard cholesterol-lowering diet (at least equivalent to the American Heart Association (AHA) Step 1 diet) before receiving LIPITOR, and should continue on this diet during treatment with LIPITOR. If appropriate, a program of weight control and physical exercise should be implemented. Primary Hypercholesterolemia and Combined (Mixed) Hyperlipidemia, including Familial Combined Hyperlipidemia: the recommended dose of LIPITOR is 10 mg once a day. The majority of patients achieve and maintain target cholesterol levels with LIPITOR 10 mg/day. A significant therapeutic response is evident within two weeks, and the maximum response is usually achieved within two to four weeks. The response is maintained during chronic therapy.

Doses can be given at any time of the day, with or without food, and should preferably be given in the evening. Doses [illegible] Detection and Management of Hypercholesterolemia, Working Group on Hypercholesterolemia and other Dyslipidemias (Canada) [illegible] Adjustments of dosage, if necessary, should be made at intervals of four weeks or more. The recommended dose range [illegible]

Lipid levels should be monitored periodically and, if necessary, the dose of LIPITOR adjusted based on target lipid levels recommended by guidelines.

The following reductions in total cholesterol and LDL-C levels have been observed in two dose-response studies, and may serve as a guide to treatment of patients with mild to moderate hypercholesterolemia:

TABLE 2. Dose-Response in Patients With Mild to Moderate Hypercholesterolemia
(Mean Percent Change from Baseline)[a]

Lipid Parameter	LIPITOR Dose (mg/day)			
	10 (N=22)	20 (N=20)	40 (N=21)	80 (N=23)
Total-C: 7.1 mmol/L[b] (273 mg/dL)[b]	-29	-33	-37	-45
LDL-C: 4.9 mmol/L[b] (190 mg/dL)[b]	-39	-43	-50	-60

[a]Results are pooled from 2 dose-response studies.
[b]Mean baseline values.

Severe Dyslipidemias:

In patients with severe dyslipidemias, including homozygous and heterozygous familial hypercholesterolemia and dysbetalipoproteinemia (Type III), higher dosages (up to 80 mg/day) may be required (see WARNINGS, Pharmacokinetic Interactions, Muscle Effects; PRECAUTIONS, Drug Interactions).

Concomitant Therapy

See PRECAUTIONS, Drug Interactions.

Dosage in Patients With Renal Insufficiency

See PRECAUTIONS.

PHARMACEUTICAL INFORMATION

Drug Substance

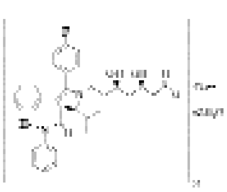
Proper Name: Atorvastatin calcium

Chemical Name: [R-(R*,R*)]-2-(4-fluorophenyl)-β,δ-dihydroxy-5-(1-methylethyl)-3-phenyl-4-[(phenylamino) carbonyl]-1H-pyrrole-1-heptanoic acid, calcium salt (2:1) trihydrate

Empirical Formula: $(C_{33}H_{34}FN_2O_5)_2Ca \cdot 3H_2O$

Molecular Weight: 1209.42

Structural Formula:



Description: Atorvastatin calcium is a white to off-white crystalline powder that is practically insoluble in aqueous solutions of pH 4 and below. Atorvastatin calcium is very slightly soluble in distilled water, pH 7.4 phosphate buffer and acetonitrile, slightly soluble in ethanol, and freely soluble in methanol.

Tablet Composition:

Each tablet contains either 10 mg, 20 mg or 40 mg atorvastatin as the active ingredient. Each tablet also contains the following non-medicinal ingredients: calcium carbonate, candelilla wax, croscarmellose sodium, hydroxypropyl cellulose, lactose monohydrate, magnesium stearate, microcrystalline cellulose, hydroxypropyl methylcellulose, polyethylene glycol, talc, titanium dioxide, polysorbate 80 and simethicone emulsion.

Stability and Storage Recommendations:

Store at controlled room temperature, 15 to 30°C.

AVAILABILITY OF DOSAGE FORMS

LIPITOR (atorvastatin calcium) is available in dosage strengths of 10 mg, 20 mg and 40 mg atorvastatin per tablet.

10 mg: White, elliptical, film-coated tablet, coded "10" on one side and "PD 155" on the other. Available in bottles of 90 tablets.

20 mg: White, elliptical, film-coated tablet, coded "20" on one side and "PD 156" on the other. Available in bottles of 90 tablets.

40 mg: White, elliptical, film-coated tablet, coded "40" on one side and "PD 157" on the other. Available in bottles of 90 tablets.

References:

[illegible] hypercholesterolemic patients. A comparison of atorvastatin, simvastatin, lovastatin and fluvastatin. Pharmacoeconomics 1998; 14(1): [illegible] LIPITOR (atorvastatin calcium) Product Monograph. Parke Davis Div. Warner [illegible] A multicenter double blind 1 year study comparing safety and efficacy of atorvastatin versus simvastatin in patients with hypercholesterolemia. Am J Cardiol [illegible] Estelle Rae C, Campbell LM, Ramirez M, Langan J, Mesia C, Pauciullo P, Sirtori C, Pazos F, Poyoudi R, Newcomb J. The efficacy and safety of atorvastatin compared to pravastatin in patients with hypercholesterolemia. Atherosclerosis 1997; 130:191-197. 5. Data on file. 6. Ontario Drug Benefit Formulary, April 1999.

For a copy of the Product Monograph or full Prescribing Information, please contact:

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