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**HYPOTHERMIA AFTER PERINATAL
ASPHYXIA IN RATS**

W. Vreuls, C. Loidl^a, E.H.J. van Dijk^b, Y. Temel^b, A. Blokland^b, A.D. Gavilanes^a, W. Honig^b, J.H. Vles^c, H.W.M. Steinbusch^b and C.E. Blanco^a

^aDepartments of Pediatrics, ^bPsychiatry and Neuropsychology and of ^cNeurology, Maastricht University and Maastricht Academic Hospital, Maastricht, Netherlands

Perinatal asphyxia (PA) is a major cause of death and neurological injury in newborns. Previous studies have demonstrated that hypothermia increases the survival rate after PA in male rat pups and prevents morphological changes in the cortex and neostriatum. It is known that these areas are involved in motor and cognitive functions. The aim of this study was to evaluate the effects of gender and hypothermia during PA on the consequences of PA. Uteri were obtained by hysterectomy of pregnant rats in their last day of gestation. Perinatal asphyxia was induced by transient immersion of the fetuses while they were still *in utero*; the period of immersion varied from 5 to 20 minutes in water of 37°C or for a period of 20 or 100 minutes in water of 15°C. Cognitive functions were studied by the Morris water maze test at 1.5 months; motor and exploratory activity were studied by the open field test at the age of 5 months. Motor hypoactivity was observed only in male rats that had suffered severe PA (20 minutes of PA at 37°C). Hypoactivity was not present in female rats in the severe PA group. The

survival fraction after severe PA was 22% in males and 46% in females. The application of hypothermia during asphyxia was able to prevent motor hypoactivity in the male severe PA group. No alterations were found in the learning and memory capabilities of the different groups. These findings support the hypothesis that hypothermia can prevent the long-term effects of PA. Future research directed toward explaining the mechanisms for the resistance to PA in female rats could be useful for developing strategies to prevent brain damage.

**STUDYING GENE EXPRESSION
IN *ESCHERICHIA COLI* AFTER ANTIBIOTIC
EXPOSURE: A NEW APPROACH TO
IDENTIFYING NOVEL ANTIBIOTIC TARGETS**

M. O'Shea, L. Piddock and C. Spreadbury

The University of Birmingham, Birmingham, UK

The increasing emergence of bacterial resistance to existing antimicrobial agents reduces the therapeutic efficacy of those agents in the treatment of infectious disease, and represents a threat to public health. One approach of responding to this problem is to identify new molecular targets against which novel antibacterial agents may be developed. Analysis of differentially expressed mRNAs synthesized in response to stress-stimuli can be used to aid identification of new genes. The detection of differential gene expression in *Escherichia coli* induced by exposure to a quinolone antibiotic was undertaken using the novel technique of RNA fingerprinting by arbitrarily primed polymerase chain reaction (RAP-PCR). Total RNA was isolated

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from nalidixic acid-treated and untreated cultures of *E. coli* at regular intervals over 30 minutes. Fingerprinting of RNA populations was performed by RAP-PCR using two random, arbitrary primers: a 10-bp primer for first-strand cDNA synthesis followed by a 20-bp primer for second-strand synthesis. Unique products were isolated, cloned and sequenced. A differentially amplified RAP-PCR product 590-bp in size originating only from bacterial cultures exposed to nalidixic acid for 30 minutes was identified. This product was cloned and sequenced. Comparative sequence analysis demonstrated that approximately 465-bp of the cloned cDNA possessed significant sequence homology with an internal region of the gene *clpB* (GenBank accession number M28364). This gene encodes ClpB, a heat shock protein. Heat shock responses are one of a number of regulatory mechanisms used by bacteria to facilitate their survival when challenged by stressful environments, including exposure to antibiotics. These results illustrate the enormous potential of RAP-PCR as a tool for use in the molecular dissection of gene expression and the isolation of differentially expressed mRNAs encoding proteins which may have important roles in bacterial infection and resistance. Design and synthesis of inhibitors of such proteins would afford an alternative strategy for antimicrobial chemotherapy in response to the growing problem of antimicrobial resistance.

EFFECTS OF CIGARETTE SMOKING ON PULMONARY FUNCTION IN STUDENTS

Z. Brzozaa, J. Szpunar, B. Labuz,
and W. Saucha

Silesian Medical School, Katowice, Poland

There is much data concerning the influence of cigarette smoking on the function of the respiratory system. Most studies have focused on people who have smoked cigarettes for many years, but few have evaluated changes of lung function in young people associated with a short history of smoking. The purpose of the current study was to assess measurable effects of smoking in young adults who have smoked for three to six years, as well as to determine the prevalence of cigarette smoking in this group. For our research, 126 students at Silesian Medical School (Atowice, Poland) were studied. Standardized questionnaires were used to obtain demographic data and smoking history. Students were divided into categories dependent upon the number of cigarettes smoked per day. Subjects who had

a history of asthma or other cardiopulmonary symptoms were not included in the study. Using the LUNGTEST apparatus, we measured the following parameters of lung function: forced expiratory volume in one second (FEV₁), forced vital capacity (FVC), ratio FEV₁/VC (Tiffeneau Index) and forced expiratory flow (FEF₇₅, FEF₅₀, FEF₂₅). The results suggest that students who smoke have lower values of lung function parameters than nonsmokers, and also indicate that the effect of cigarette smoking on pulmonary function may be more detrimental in women than in men. Finally, the prevalence of cigarette smoking in this group of medical students was compared with data for the general population of Poland. It was determined that there are no differences in prevalence of cigarette smoking between women in our School and women in the general population; however, the data suggest that men studying medicine are less likely to smoke cigarettes than men in the general population of Poland.

ACTIVITY OF MATRIX METALLOPROTEINASES-2 AND -9 IN CERVICAL CANCER

M. Bogusiewicz

Department of Physiological Chemistry, University School of
Medicine, Lublin, Poland

Matrix metalloproteinases-2 and -9 (MMP-2 and -9) are enzymes that degrade collagen type IV, which is the main structural protein of the basement membrane (BM). Breakdown of the BM is a crucial step in neoplastic cell invasion. Thus, MMP-2 and -9 are strongly implicated in the spread of malignant tumors. The aim of this study was to purify MMP-2 and -9 as well as to evaluate their activity in cervical cancer. Materials for the study were eight specimens of squamous cell cervical cancer (CC) (stage Ib and IIa according to FIGO classification) and eight specimens of normal cervix (NC), all taken during operations. The extraction of MMPs comprised two steps, namely Triton and heat extraction. After overnight dialysis of obtained extracts, protein content estimation was carried out using Bradford's method. Zymography (substrate electrophoresis) in 10% SDS-polyacrylamide gels containing 0.8mg/ml of Type A Porcine Skin Gelatine (Sigma) was applied for detection of MMPs. After electrophoresis under Laemmli conditions, gel slabs were incubated in zinc containing 50mM Tris buffer with *p*-aminophenylmercuric acetate at 37°C for 18 hours. Gels were then stained with Coomassie Brilliant Blue R-250. Gelatinolytic activity of MMPs

was detected as unstained bands. Zymography revealed activity of latent (72 kDa) and active (67 kDa) forms of MMP-2 as well as latent (92 kDa) and active (83 kDa) forms of MMP-9. Activity of MMP-2 differed significantly among the particular cases but not between CC and NC groups. However, higher activity of the 67 kDa MMP-2 form was observed in CC cases. Activity of MMP-9 was noticeably higher in CC cases in comparison to NC cases. Results of the current study suggest that MMP-9 plays an important role the invasion of cervical cancer. Nevertheless, the exact role of MMP-2 is not completely clear. Enhanced activation or insufficient inhibition of MMP-2 are suggested to be involved in the spread of cervical cancer.

PHOSPHORYLATION OF PURINE RIBOSIDE BY RECOMBINANT ADENOSINE KINASE

K. Tkacz, A.C. Skladanowski and W. Makarewicz

Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland

Previous experiments have shown that during incubation of cultured rat thymocytes with an adenosine analogue, purine riboside (PuR), there is a strong cytotoxic effect as well as formation of purine riboside triphosphate (PuTP). These processes were inhibited by specific adenosine kinase inhibitors, suggesting that adenosine kinase is responsible for PuR metabolism *in vivo*. The structure of the adenosine kinase gene was discovered and the first recombinant adenosine kinase was obtained as a result of human cDNA expression in the bacterial system. The aim of this study was to examine the basic properties of the recombinant adenosine kinase with purine riboside as a substrate. PuR phosphorylation activity was measured by the formation of PuTP. An anion exchange HPLC procedure was used to measure PuTP formation (column Sepharon SGX, NH₂, 7 μ m, 30000 TP). Enzyme activity was assayed after the sequence of reactions PuR+ATP→PuMP+ADP, PuMP+ATP→PuDP+ADP, PuDP+ATP→PuTP+ADP were carried out in one incubation mixture by adenosine kinase, myokinase and creatine kinase, respectively. Adenosine kinase was a rate-controlling step in this sequence. As a result we found that PuR is a substrate for the recombinant adenosine kinase. A complex relationship was shown to exist between enzymatic activity and the concentration of the substrate. The measured PuR concentrations were in the range of 5-200 μ M. The concentration of PuR at which the reaction first occurred was 20 μ M. The reaction rate increased with

increasing PuR concentrations up to [PuR] = 50 μ M, at which concentration the reaction rate reached a maximum. Concentrations of PuR above 100 μ M inhibited the enzymatic activity, reducing the reaction rate below its maximal level. These data confirm the well-known inhibition of the native enzyme by an excess of adenosine. Enzymatic activity was dependent on the enzyme concentration. We conclude that recombinant adenosine kinase phosphorylates PuR *in vitro*. Purine riboside might be a convenient substrate for measuring adenosine kinase activity.

VITAMIN K: POSSIBLE EFFECTS ON BONE DENSITY IN EXPERIMENTAL RENAL FAILURE

B. Gyorffy, J. Toth, A. El-Shakmak and A. Szabo

Semmelweis University of Medicine, Budapest, Hungary

Vitamin K is a coenzyme of the gamma-carboxy-glutamase, which carboxylates the GLA-proteins. Among these proteins are clotting factors and the osteocalcin, which is responsible for the binding of Ca⁺⁺ and hydroxyapatite, thus facilitating bone mineralization. In renal failure vitamin D insufficiency develops, leading to renal hyperparathyroidism and a disorder in bone turnover. Our study was planned to investigate whether vitamin K therapy could influence bone metabolism in renal bone disease. Three groups of rats were studied: (i) sham-operated normal animals on normal diet (Ca: 0.95 %, P: 0.75 %), *n* = 10 ; (ii) subtotaly nephrectomised (SNx) animals on a high phosphorus diet (P: 1.2 %), *n* = 10; and (iii) SNx animals on a high phosphorus diet (P: 1.2 %) with vitamin K administration (1mg/kg/d s.c. Konaktion MM for two weeks), *n* = 10. Animals were sacrificed eight weeks after nephrectomy, and plasma, urine and bone samples were taken for analysis. Serum Ca⁺⁺, pH, iPTH, urine Pyridinolin and bone mineral density (BMD) were measured with IRMA, HPLC and radiodensitometry. Hyperparathyroidism was documented with elevated iPTH level in SNx animals 200 ± 182pmol/l and in vitamin K treated SNx animals 151 ± 154pmol/l vs. intact animals 20 ± 12pmol/l (mean ± SD, *p* < 0.001). The pyridinoline/creatinine excretion was strongly elevated in SNx animals 216 ± 96 but less elevated after vitamin K treatment 167 ± 48 compared to intact animals 98 ± 33 (*p* < 0.01). Mean of BMD tended to decrease in SNx compared to intact control (0.65 ± 0.11 vs. 0.73 ± 0.15 OD, *p* = NS), and tended to increase after vitamin K administration in SNx animals 0.73 ± 0.11 OD, *p* = NS). Our results suggest a possible

protective role of vitamin K treatment on bone mineral metabolism in renal hyperparathyroidism, but the difference in SNx animals after vitamin K treatment was not significant.

CLINICAL RELIABILITY OF LACTATE ANALYSIS IN THE DELIVERY ROOM: A COMPARISON OF THREE TECHNOLOGIES EVALUATING THE LACTATE METABOLISM UNDER ANAEROBIC GLYCOLYSIS IN HUMAN PLACENTAL VESSELS

J. Heilmann, A.K. Luttkus and J.W. Dudenhausen

Department of Obstetrics, Faculty of Medicine (Charité), Humboldt University of Berlin, Germany

Since the direct measurement of lactate in umbilical vessels may have a better predictive value for fetal outcome, obstetricians must know the intrinsic change in lactate levels under the anaerobic conditions in post partum metabolism following cord clamping. In this study, the increase of lactate concentration over time was studied in a placental model. Lactate metabolism needs to be quantified before this new technology and evaluation of this metabolite can be implemented into clinical practice in the delivery room. For forensic reasons, any delay of blood gas analysis may result in erroneously high lactate concentrations. In this prospective observational study, a total of 540 blood gas

analyses, including lactate concentration, were performed at three time intervals: 0, 30, and 60 minutes after cord clamping. In the delivery room, under standardized conditions, chorionic plate vessels were punctured three times in the same spot while the placenta was stored at room temperature. Each time 200 μ l of whole blood was obtained from heparinized syringes to measure lactate using two different blood gas analyzers (Chiron 865, Massachusetts, USA and Radiometer ABL 625, Copenhagen) and a photometer (Vitros) for comparison. Compared to the baseline concentrations at time 0, the median increase of lactate in the arterial chorionic plate vessels after 30 minutes was 3.16mM; 3.32mM; 2.01mM for the Chiron, Radiometer and Vitros analyzers, respectively; vein measurements were 2.11mM; 1.72mM and 1.76mM. After 60 minutes there was an increase in the arteries of 6.71mM, 5.5mM, and 3.44mM, and in the veins of 4.13mM, 3.88mM, and 3.68mM. Hence, the new technology of lactate analysis is practical in clinical use. The results are plausible and reproducible for each instrument. The anaerobic metabolism in the placental vessels post partum leads to a significant increase in lactate levels within one hour. Because of the relevant variation of the values, a retrospective calculation should be avoided. Therefore, we suggest performing lactate measurements immediately post partum. As there is no consensus to date concerning gold standard reference values for lactate levels, the clinician should be aware of a potential difference in lactate levels that are obtained utilizing different techniques.