ACTA MEDICA (HRADEC KRÁLOVÉ)

2000, Vol. **43**, No. **2**

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ABSTRACTS FROM "THE FOURTH SCIENTIFIC CONFERENCE OF THE CHARLES UNIVERSITY FACULTY OF MEDICINE AND UNIVERSITY HOSPITAL", 8 DECEMBER 1999, HRADEC KRÁLOVÉ

Abstracts of papers of "The Fourth Scientific Conference of the Charles University Faculty of Medicine and University Hospital" containing summaries of research projects completed through different grant agencies.

Isolation of troponin T in the myocardium of rats and rabbits

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Myocardia from male Wistar rats (n=10) and from medium size Chinchilla male rabbits (n=10) were used for analysis of intracellular compartmentation of regulatory protein troponin T. 50 mg of heart tissue was homogenized in Tris buffer solution (0.05 mol/L Tris, 2 mmol/L EDTA, and 0.05 mmol/L dithiotreitol, pH 7.0) and stirred at 4 °C for 1 h. Thereafter the insoluble molecules were sedimented by ultracentrifugation (1 h, 100,000 g, 4°C). The pellet was washed and the centrifugation was repeated. The supernatants were combined for cytosolic fraction (fraction A). The structurally bound troponin T was extracted from the resulting pellet in ions buffer solution (0.4 mol/L potassium chloride, 0.1 mol/L potassium dihydrogen phosphate, 0.05 mol/L dipotassium hydrogen phosphate, 0.04 mol/L sodium pyrophosphate, and 0.01 mol/L magnesium chloride, pH 7.0) and stirred for 1 h at 4°C. After centrifugation (1 h, 100,000 g, 4°C) this procedure was repeated once. The two supernatants were pooled to measure myofibrillar fraction (fraction B). The remaining pellet was resuspended in ions buffer and after an overnight incubation it was centrifuged (30 min, 20,000 g, 4°C). The supernatant was saved (fraction C). The concentration of proteins was measured using the Coomasie Plus Protein Reagent Kit (Pierce). In the rat myocardium the concentration of proteins in fraction A was 11.83±2.17 mg/g, in B was 6.33±1.55 mg/g and in C 14.48±2.98 mg/g, i.e. the total extracted proteins were 32.44±3.99 mg/g of the wet tissue. In the rabbit myocardium the concentration of proteins in fraction A was 20.50 ±1.84 mg/g, in B 9.04 ±1.74 mg/g and in C 15.92 ±2.73 mg/g, i.e. the total extracted proteins were 45.47±3.14 mg/g of the wet tissue. This isolation will be followed by the measurement of cardiac troponin T using a commercial kit (Roche) and by electrophoresis.

Supported by Grant: GA ČR No. 305/98/P261.

The lack of changes of cardiac troponin T following repeated i.v. administration of Oracin and NO-1-B

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NO-1-B (3,9 – dimetoxybenfluoren hydrochloride) and Oracin ((6-[2-(2-hydroxyethyl)aminoethyl]-5,11-dioxo-5,6-dihydro-11H-indeno[1,2-c]isoquinoline hydrochloride) are considered to be new potent antineoplastic drugs. The cardiac side effects of repeated i.v. administration of these cytostatics (once a week, 10 administrations) were followed in three groups of rabbits: 1) Oracin (10 mg/kg; n=7), 2) NO-1-B (12 mg/kg; n=7), 3) NO-1-B (24 mg/kg; n=6) and were compared with the control group (saline 1 ml/kg; n=6) and the group with experimentally induced cardiomyopathy (daunorubicin 3 mg/kg; n=13). The venipunctures for biochemical examination were performed in the following time intervals of the experiment: before and 24 h after the 1st (weekly) administration of the drug; before and 24 h after the 5th administration; before and 24 h after the 8th administration, and before and 24 h after the 10th administration. The concentration of cardiac troponin T (cTnT) was mea-

sured using a commercial kit (Roche, Basel, Switzerland). In the daunorubicin group cTnT levels after the 8th administration were significantly higher (0.31± 0.11 μg/l) in all animals with premature deaths compared with the rest of surviving animals (0.04±0.03 μg/l). Cardiac TnT after administration of Oracin and NO-1-B in both doses were always within the physiological range (lower than 0.1 μg/l) during the whole experiment. These data were supported by further followed-up parameters. It is possible to conclude, that no significant deterioration of cTnT as a predictive marker of cardiomyopathy and heart failure was found during repeated i.v. administration of Oracin and NO-1-B which is very important for their further use.

Supported by Grants: GA UK No. 272/1998C, IGA MZ No. 4 212-3 and Research Project CEZ: J13/98:111500002.

Study of molecular mechanisms of atherogenesis in familiar hyperlipidaemia

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Abnormal metabolism of fatty acids has been associated with hyperlipidaemia and atherosclerosis. Saturated fatty acids (SUFA) increase the risk of the development of coronary artery disease (CAD). Cholesterol-raising activity appears to be limited to lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0). Long-chain (n3) fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), as well as (n6) fatty acids (mainly linoleic acid and its derivative gamma-linolenic acid [C18:3n6]), have been reported to possess lipid-lowering properties. Although atherogenic effects of the individual dietary fatty acids and dietary cholesterol on fasting serum lipids and lipoproteins have been studied extensively, possible interactions among fatty acids or with lipoproteins are only poorly understood. Therefore, the aim of this study was to apply a combination of bioanalytical techniques to study the composition of fatty acids in plasma, erythrocyte membranes and serum lipoproteins in hyperlipidaemic subjects under aggressive hypolipidemic therapy. Blood samples from 5 hyperlipidaemic patients undergoing chronic treatment with low-density lipoprotein (LDL) - apheresis were analysed for lipids and fatty acids in serum, lipoprotein fractions and erythrocyte membrane by capillary gas chromatography (GC), reversed-phase high-performance liquid chromatography (LC), spectrofluorometry and spectrophotometry. LDL-apheresis has been associated with significant changes in the metabolism of fatty acids in relation to the triglyceride-rich lipoproteins. Monounsaturated oleic acid may exert its hypotriglyceridemic effect via VLDL, IDL, LDL and HDL fractions. Polyunsaturated fatty acids, associated with triglyceride metabolism via IDL or VLDL, are the linoleic, gama-linolenic and docosahexaenoic fatty acids.

Supported by Grant: IGA MZ No. NB/4548-3.

The role of phenotype and genotype of familial hyperlipoproteinaemia in atherogenesis of coronary artery disease

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Twenty eight men (aged 34-77 years) who underwent an elective coronary angiography for coronary artery disease (CAD) were studied. They were divided into Group A (luminal narrowing <50%; n=11) and Group B (luminal narrowing >50%; n=17). Capillary gas chromatography was used for determination of fatty acids. Retinol and alpha-tocopherol were analyzed by reversed-phase high-performance liquid chromatography; other parameters were determined spectrofluorometrically and spectrophotometrically. A severe coronary atherosclerosis in Group B was associated with higher serum LDL/HDL cholesterol ratio, triacylglycerols, and phospholipids ($p<0.05$). Erythrocyte membrane fatty acids C14:0, C16:1 and C22:6n3 were significantly higher in Group B ($p<0.05$). We found significantly higher plasma polyunsaturated fatty acids (PUFA) C18:3n6 in Group B, whereas plasma linoleic acid was not changed significantly. There was a significant increase of IDL-C18:0, LDL-C14:0 and HDL-C22:6n3 PUFA in Group B. We conclude that disturbances in SUFA and PUFA metabolism are associated with coronary atherogenesis. Such abnormalities may include an enhanced extrahepatic transport of C14:0 SUFA via LDL and its incorporation into cell membranes, and an enhanced clearance of anti atherosclerotic C22:6n3 PUFA via serum HDL.

Supported by Grant: IGA MZ No. NB/5205-3.

Diagnostics of Helicobacter pylori using the 13-carbon urea breath test

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Title of presentation: Prevalence of Helicobacter pylori in the Czech Republic – preliminary data based on the 13C-urea breath test in 389 symptom-free persons. Gut 1999; 45, Suppl 5: A106. Authors: Jan Bureš, Petr Dítě, Marie Kopáčová, Václav Voříšek and the Czech Helicobacter Pylori Study. Aims and conclusions: The Czech Helicobacter Pylori Study Group has started an epidemiological study to determine Helicobacter pylori (HP) prevalence using the 13C-urea breath test (13C-UBT) in the Czech Republic. 13C-UBT was positive in 49 % (192/389), which is significantly lower than previously assumed. The Study continues. Title of the presentation: Comparison of different protocols for 13C-urea breath testing of Helicobacter pylori infection in healthy subjects. Gut 1999;45(Suppl 5); A191. Authors: Kopáčová M, Bureš J, Voříšek V, Konštacký M, Rejchrt S, Palička V. Conclusions: 13-UBT is an accurate test to diagnose HP. Citric acid solution as a test drink and 20 or 25-minute breath sampling intervals are optimal for the 13C-UBT in healthy volunteers. Title of the presentation: Detection of Helicobacter pylori by means of 13-C urea breath test: methodology (in Czech). Diabetol Metabol Endokrinol Výž 1999;2, in press. Authors: Bureš J, Palička V, Kopáčová M, Voříšek V, Rejchrt S, Živný P. Conclusions: The 13C-UBT is a simple, non-invasive and global test for HP detection. The test reflects the hydrolysis of 13C-labelled urea by HP urease. This paper gives the methodology of performing the 13C-UBT based on our own study (2,808 examinations). Title of the presentation: Detection of Helicobacter pylori by means of 13C-urea breath test: clinical reproducibility of the test (in Czech). Klin Biochem Metab 1999;7(28): in press. Authors: Kopáčová M, Bureš J, Voříšek V, Konštacký M, Rejchrt S, Živný P, Palička V. Conclusions: Reproducibility of positive or negative result was 100 % (testing 27 healthy volunteers). Mean inter-test range was 14.4 %. Cut-off 3.5 was used.

Supported by Grant: MPO No. PZ-Z2/30.

Causes of positive pressure in the cavity of the middle ear in tympanometric examination

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Authors evaluate a group of 62 patients where they diagnosed 66 times positive pressure in the cavity of the middle ear during tympanometric examinations. Based on their own experience and data in the literature they found that positive pressure in the middle ear may be caused by an air current during politzeration or catheterization of the auditory tube (a similar effect can be produced also by sneezing, blowing of the nose, crying), acute tubotympanic catarrh, acute otitis media in the initial or regressive stage, or as a result of pressure changes in the middle ear during general inhalation anaesthesia using nitrous oxide. Based on statistical processing of the results by the non-paired t-test the authors reach the conclusion that in the majority of patients the positive pressure in the middle ear is not associated with conduction deafness and that there is no relationship between the pressure and hearing loss. There is however a relationship between the compliance of the conduction system and the presence of a conduction disorder. The stapedial reflex can be evoked in cca one half of ears with a positive pressure in the middle ear. However, there is no relationship between the pressure and evoking the stapedial reflex. The stapedial reflex is however more readily evoked in ears with a greater compliance of the conduction system. References: 1. Ostergard CA, Carter DR. Positive middle ear pressure shown by tympanometry. Arch. Otolaryngol 1981;107:353-6. 2. Smith CG, Paradise JL, Zang TI. Modified schema for classifying positive-pressure tympanograms. Pediatrics 1982;69: 351-4.

Supported by Grant: IGA MZ No. 4100-3.

Cytology of living cells cultivated in vitro during practical classes

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This project is a part of our long lasting endeavour to improve the quality of practical classes in medical biology and genetics. In the past we focused on the development of laboratory facilities for molecular biology. This year we aimed our effort at modern cytology which uses mammalian cells cultivated in vitro. The majority of financial support (1,815,000 Kč) was used for the modernisation of a student microscopic laboratory. It includes 35 students' microscopes with phase contrast optics, one microscope for teachers, a data projector, a digital TV camera, a digital camera, a computer, and a biological thermostat. The rest of the funding (144,000 Kč) was used for essential reconstruction of electricity in the student laboratory and for necessary consumables and chemicals for practical classes. There was no funding for travel expenses or for personal cost. We were able to completely reconstruct the existing student microscopic laboratory and to build a new modern laboratory for microscopy of living cells cultivated in vitro. These technical improvements enable us to introduce the new practical classes based on the observation of living human cells cultivated in vitro. The main advantage is that we bring the principle of evidence based medicine to the practice. During practical classes each student can perform experiments with cells cultivated in vitro, analyse results and prepare presentations. The entire learning method is thus oriented to multimedia applications. Based on our first experiences we have prepared new handouts for practical classes and new syllabi for the next semester. This new chapter will be included in our new student textbook for practical classes. Students' opinions about this innovation will be evaluated by means of a questionnaire.

Supported by Grant: FRVŠ No. 1287/1999.

Flow cytometry analysis of the dynamic changes in mitochondrial membranes during cell death

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Cell death continues to be one of the most intensively studied biological phenomena. Nevertheless, a lot of controversy remains at the cytological level. One unsolved question is the role of biological membranes in this process. In our study we compare the data obtained with time lapse videomicroscopy with those of flow cytometry. As a model drug for the induction of apoptosis we used etoposide (Vepesid inj., Bristol Mayers, 10 microg/ml). As cell models, stabilised cell line Hep-2 grown in the DMEM and a cell line HL-60 maintained in RPMI with 10% foetal calf serum were used. The transmembrane mitochondrial potential was monitored by a specific fluorochrome DiOC6 (Molecular Probes) in flow cytometry assessment. To properly interpret the transmembrane potential changes, we have also analysed the same potential after the treatment with several inhibitors of mitochondrial functions. Based on these results we selected carbonyl cyanide m-chlorphenylhydrazone as a standard inducer of the mitochondrial membrane damage. Dynamics of membrane changes was measured during the first 24 hours after treatment with etoposide. Flow cytometry results indicate the changes in cell morphology, which stay in agreement with the time lapse observation. Although the changes in mitochondrial potential can be observed as early as after 6 hours after the beginning of treatment, some cells retained their positive staining even after 24 hours. This observation only confirms our previous findings that suggest heterogeneous response. Heterogeneity along with the presence of several subpopulations after the etoposide treatment further obscure and complicate the analysis and interpretation of biochemical data.

Supported by Grant: GA UK No. 274/98.

Possible effects of restenosis implantation of radioactive stents with a very low activity, brought about by a cyclotron

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In 1999 we wanted to implant radioactive stents in patients, but we had to perform a new measurement for the Agency of Nuclear Security of the Czech Republic. We had to perform irradiation tests and a detailed dosimetric assessment of the staff's radiation exposure. A new measurement was used for the detailed measurement of stent irradiation homogenity. The radiation calibration was performed with respect to stent weight. Dosimetric measurements as well as the exposure calculation were performed both with and without the use of safety equipment (guards, gloves, etc...). The mechanical properties of irradiated material are being tested by ing. Kotek at VUT Brno at the same time.

Supported by Grant: IGA MZ No. 4786-3/98.

Intestinal barrier damage induces liver injury and an increase of liver DNA synthesis in rats

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In addition to its role in absorbing nutrients, the intestinal mucosa provides an important barrier to potentially harmful luminal toxins, bacteria, and antigens. The damaged intestinal barrier plays a possible role in the pathogenesis of some diseases (e.g. celiac disease, drug-induced enteropathy, inflammatory bowel disease, etc). The massive flux of antigens through the small bowel mucosa can lead to a systemic reaction and multiorgan systemic failure. Because relatively little attention has been paid to the impact of intestinal ischemia on the liver, we have decided to study the effect of ischemia-reperfusion injury of the small intestine on liver damage and liver DNA synthesis. The experiments were performed in two series on male albino Wistar rats with an initial body mass of 220-230 g. Intestinal ischemic-reperfusion injury was induced by an occlusion of the superior mesenteric artery (SMA) for a period of 15 min. The lactulose-mannitol (LAMA) test was used to measure the intestinal permeability 24, 48, 72 hrs and 7 days after SMA occlusion. The extent of liver damage and liver regeneration after SMA occlusion was determined by an assessment of serum activity of AST and ALT, liver DNA synthesis, and mitotic activity of hepatocytes in above mentioned intervals after SMA occlusion. The results were compared with those obtained from laparotomized (LAP) rats. Increased intestinal DNA synthesis found 24 hrs after SMA occlusion documents an induction of a regenerative response of the small intestine after its ischemia-reperfusion injury. An enhanced serum concentration of malondialdehyde evidences that lipoperoxidation participates in intestine injury. Significant elevation of the serum activity of AST and ALT (24 hrs after SMA occlusion) followed by a stimulation of DNA synthesis (72 hrs after occlusion) is probably caused by an enhanced load in the liver of toxic agents due to the increased permeability of intestine.

Supported by Grant: GA UK No. 1/1999/C.

Population pharmacokinetic/pharmacodynamic modelling in clinical pharmacology

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Population PK/PD modelling offers the possibility of gaining information about pharmacokinetics and response from relatively sparse observational data from the therapeutic drug monitoring database or from large scale clinical trials, where the inter-individual variability is at a maximum and the data obtained from each patient are sparse and hence it is not possible to characterise the individual's pharmacokinetic-pharmacodynamic profile. The aim of the project is to explore: 1) the rationale and concepts of the population approach and statistical issues involved in the approach; 2) population methods in clinical pharmacokinetics/pharmacodynamics with special attention to therapeutic drug monitoring and post-marketing dose adjustments. Results: The population pharmacokinetics of carbamazepine (CB) in patients with epilepsy were investigated with the help of NONMEM. The factors evaluated for their possible effects on CB clearance (CL) were: total body weight (TBW), age, sex, and valproic acid (VA), primidone (PM) and phenytoin (PH) comedication. A total of 758 steady-state serum concentrations obtained in 472 (240 female and 232 male) patients were analyzed. The model found best to describe data was CL=0.61*TBWexp(-0.54). The results show that CL (l*h-1*kg-1) exponentially decreases with TBW. Concomitant therapy increased CB CL, suggesting induction of CB metabolism by 13% (VA), 79% (PM) and by 52% (PH), respectively. TBW was a better predictor of CL than age. There was no inter-sexual difference in CB CL. With this population model, CB doses that would be suitable for individualization of therapy are proposed.

Supported by Grant: FRVŠ No. OCB15.10.

Interactive atlas of histopathology

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The interactive atlas of histopathology is composed of a list of histopathologic slides together with digitized microscopic pictures of each pathologic process in tissues and organs. Individual histologic pictures are shown in various magnifications and for better understanding of morphology freehand schemes are also used. Together with schematic and histologic pictures we show a text portion of the atlas explaining the theory of an individual pathologic entity. There are also included text portions explaining basic histology (links) of tissues and organs. The atlas is designed as a system of linked web pages accessible for users via the faculty server (Intranet).

Supported by Grant: FRVŠ No. 49050.

Exposure of membranes used in GTR

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Guided tissue regeneration (GTR) is mostly accomplished by physical barriers (membranes) – frequently grafts are also used. The purposes of the membranes are: 1. to assure space for regeneration, 2. to prevent invasion of unwanted cells into the area of regeneration, 3. to protect the wound during healing. The exposure of membranes making possible bacterial infection is an important clinical problem. There are many factors playing a role in the exposure of membranes. Results: Exposures of membranes.

	augmentation	periodontitis
Vicryl membranes	13 (20) 65 %	9 (9) 100 %
Gore-Tex	1 (5) 20 %	4 (11) 36 %
Bio-Gide	3 (11) 27 %	0 (1) ?

Conclusion: The exposure of membranes is rather frequent. The highest number of exposure was in group 1 (Vicryl membranes), followed by group 2 (Gore-Tex) and group 3 (Bio-Gide). All exposed Vicryl membranes were eventually removed; exposed membranes of groups 2 and 3 remain in place or were re-epithelized.

Supported by Grant: IGA MZ No. 4802-3.

Preliminary experience with contrast enhanced ultrasound cystography in the diagnosis of vesicoureteral reflux

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Purpose: To evaluate the diagnostic accuracy of contrast enhanced ultrasound cystography with ascending administration of Levovist (CUSC) for the detection of vesicoureteric reflux (VUR) in children. Materials and methods: Between September 1998 and June 1999 we performed 45 CUSC examinations in 43 children with either intermittent

urinary tract infections or unexplained dilatation of pyeloureteric systems. Based on results of micturation x-ray cystouretrography (MCUG), which served as a "gold standard" in this prospective unbiased study, we evaluated 89 pyeloureteric units. Results: MCUG revealed 46 refluxing pyeloureteric units. CUSC detected VUR in 36 of them; among 10 false negatives we found only VUR of grade II or III. The parameters of the diagnostic accuracy of CUSC for depicting VUR in comparison to MCUG were as follows: sensitivity 82 %, specificity 100 %, positive predictive value 100 %, negative predictive value 81 %. Conclusion: Contrast enhanced ultrasound cystography is a clinically useful method for the detection of VUR in children. The possibility of establishing a grading system of VUR by means of CUSC should be further investigated within a larger cohort of examinations. References: Eliáš P et al. Čes Radiol 1999; 53(Suppl. 1):4-8.

Supported by Grant: IGA MZ No. 4688-3/98.

Photo-patch tests - practical experience in the 2nd year

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The epidemiology of contact photoallergy in our population is not well known at present. Therefore the basic aim of our project was to establish the scale of diagnostic methods in the assessement of skin photosensitivity state using photo-patch tests. We tested some substances (TROLAB® set provided by HERMAL comp.). These substances can cause photoallergy, phototoxicity, and/or contact sensitivity. We used an assessement of the minimal erythema dose and its individual seasonal variation (1.2) to determine the time required for delivering the UV-A dose of 5 J/cm^2; this dose was evaluated as the suberythemal dose. There were two groups of probands according to the presence or absence of photosensitivity objections. Photosensitivity troubles were present in 17 persons; 10 persons (controls) had no symptoms of photosensitivity. Contact sensitization was detected in 9 cases (53%) in the photosensitive group, although in 5 controls (50%), a lower incidence of contact sensitivity was also detected. Photoallergic reaction appeared on the healthy skin of 2 controls. A positive photoallergic response was found in 5 persons within the photosensitive group. The results of studies conducted with sunscreens showed that propanedione, cinnamates and oxybenzone were the most frequent photoallergenic substances. These data suggest that some photosensitive troubles could be caused by a skin response to the photoactivated sunscreen's ingredients. Thus the physical (non-photoallergenic) forms of sunscreens are recommended for essentialy sensitive people, especially when being used from childhood. References: 1. Ettler K. J Eur Acad Dermatol Venereol, 1999;12(Suppl. 2):S313-S4. 2. Ettler K, Nožičková M. Čs Derm 1999;74(6):239-42.

Supported by Grant: IGA MZ No. 4689-3.

The effects of combined therapy on persons with increased body weight

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The aim of the study was to assess the effects of physical activity on the therapy of persons with increased body weight. We compared the usual dietetical/pharmacological therapy without physical activity (a group of 18 females; average age of 48) with the usual dietetical/pharmacological therapy supported by long-term physical activity (combined therapy; a group of 18 females; average age of 43). Next we compared a combined therapy involving directed and controlled physical activity with a combined therapy involving individual physical activity. Actual health state of the examined persons was estimated before the initiation of physical exercises (February 1998), after 4 months of directed and controlled physical activity (June 1998), after 2 months of individual physical activity (September 1998) and after 9 months of directed and controlled physical activity (June 1999). The anthropometrical, physiological and biochemical parameters and markers of motion ability were found. The group of persons with combined therapy involving long-term directed and controlled physical activity revealed a significant decrease of heart rate and blood pressure (both systolic and diastolic), a significant decrease of the LDL-cholesterol levels and a significant improvement of motion ability. The group of persons without physical activity revealed only a significant decrease of LDL-cholesterol levels. The most considerable reductions of individual body weight (as much as 14 kg) were found in the group with combined therapy involving directed and controlled physical activity, but the average (group) changes between actual health state examinations were not statistically significant. On the basis of our results, the combined therapy involving long-term directed and controlled physical activity was designated a more efficient form of therapy for persons with increased body weight.

Supported by Grant: GA UK No. 275/98C.

Environmental exposure of children living in Hradec Králové to PAH

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The aim of this study was to assess the intake of polycyclic aromatic hydrocarbons (PAH) by children living in a city. Four groups of children (3-6 years old) living in Hradec Králové (Czech Republic) were chosen: two groups from a kindergarten situated in the center with a higher traffic density ("polluted" area) and two groups from a kindergarten situated in the border part of the town with a low traffic density ("non-polluted" area). Food consumption was recorded in all children and PAH intake from foodstuffs was estimated.The ambient air samples were collected at the playgrounds and inside the kindergartens during 3 days in summer 1997 and 3 days in winter 1998. Urine samples were collected in the morning and in the evening. Mean outdoor total PAH concentration (sum of 12 individual PAH) in the "polluted" area was approximately 3 times higher than that in the "non-polluted" area. Indoor concentration in the "polluted" area was more than 6 times higher than that in the "non-polluted" area in summer and 3 times higher in winter. In both areas, the contribution to the total pyrene absorbed dose from food consumption was much more important (85-99.7 %) than that from inhalation. The estimated daily-absorbed dose of pyrene from the soil represented approximately 0.02 % of the total absorbed dose. Significantly higher urinary concentrations of 1-hydroxypyrene (1-OHP) were found in the evening, as compared to the morning, samples of children from the polluted kindergarten in summer and winter. No. differences in urinary concentrations of 1-OHP were found between the summer and winter season at each kindergarten. In conclusion, the food seems to be the main source of the total pyrene and total PAH intake in observed children, even under a relatively high PAH air exposure in the city.

Supported by Grant: SEAD of NATO, Brussels No. 970418.

Helicobacter pylori. Some selected clinical problems

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Two main problems will be discussed: 1) The relationship between gastric autoimmunity, Helicobacter pylori (Hp), and the state of gastric mucosa. Aims: to evaluate the significance of parietal cell antibodies (PCAb) directed to the cytoplasm of parietal cells, and the anticanalicular and antiluminal antibodies; to assess the relationship of gastric autoantibodies to Hp and to gastritis. It was shown that PCAb unlike the cytoplasm does not correlate in all cases with gastric autoimmunity; the false positivity is caused by contemporarily present antithyroid antibodies. There are cases of chronic gastritis where the etiology is unknown (no Hp, no gastric antibodies). The absence of PCAb (cytoplasmic) does not exclude gastric autoimmunity (anticanalicular and antiluminal antibodies present). The autoimmune type of gastritis could exist in rare cases without gastric antibodies, as well as cases with HpAb in the serum but without Hp in the gastric mucosa. Gastric autoimmunity may participate in the etiology of chronic gastritis simultaneously or in cooperation with Hp. 2) Optimal therapy for Hp infection under our conditions: we could prove that a combination of omeprazole 2x20 mg or pantoprazole 2x40 mg with clarithromycin 2x250 mg and metronidazole 2x500 mg give analogical results as were presented in other studies from western Europe (MACH 1, etc.). The good results (290 patients included in the study) in our country could be explained by the low incidence of Hp strains resistant to metronidazole.

Supported by Grant: IGA MZ No. IZ/4210-3.

Mycobacterium avium subsp. paratuberculosis in Crohn's disease

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In recent years, attention has been devoted to the possible etiologic role of Mycobacterium paratuberculosis (MP) in Crohn's disease (CD). MP is an etiologic agent of paratuberculosis (Johne's disease) in ruminants. Both diseases are characterized by chronic granulomatous lesions, and with clinical signs of enterocolitis. The aim of the study was to search for MP in CD, to identify isolated strains by RFL analysis, together with a look at the situation of paratuberculosis in ruminants in the Czech Republic. Surgical tissue samples were obtained from both diseased (49 pat.) and normal (34 pat.) segments of intestine. MP was isolated in 3 cases from diseased tissue (isolated RFL types: A-C10, D-C12, B-C1), in no case from normal tissue. The same RFLP types of MP identified from CD were also identified in cattle. A substantial increase of paratuberculosis seems to be parallel with the increase of CD. The isolation of MP is a rare finding in patients with CD. A possible explanation: the culture of MP takes a long time (up to several years) to be cultivated; spheroplast as a possible form is not cultivable, but a transformation to bacterial form is

possible after a long incubation; MP possibly initiates an immunological process followed by disappearance of MP from the tissue. In spite of our negative results of antibody to MP with the crude antigen, the positive results with the specific antigen 36K (p36) support the view that MP may play a role in the etiology of CD (el-Zaatari, 1999).

Supported by Grant: IGA MZ No. IZ/4211-3.

Long term influence of hemodialysis and peritoneal dialysis on the immunity of patients with chronic renal failure

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The study has two closely connnected objectives. The first objective is to describe the immune reactions of patients with chronic renal insufficiency (CRF) treated by regular hemodialysis (HD), continuous ambulatory peritoneal dialysis (CAPD), and also patients treated conservatively before starting renal replacement therapy. The second objective is to establish the factors influencing immune responses of these patients focusing on the type of regularly used dialysis membrane, and on parameters of dialysis adequacy and metabolic compensation. Methodology: The study is open to all patients with CRF starting HD or CAPD during next 1.5 years. HD patients are divided into 2 groups (A and B) not differing in age, gender, diagnosis and basic parameters of HD, especially Kt/V; group A is treated only with cuprophane membranes, group B is treated only with polysulphone membranes, and by CAPD. All patients are investigated by a set of immunological tests before starting, in 4 and 12 weeks, 6 and 12 months of the treatment and the results will be statistically evaluated.

Supported by Grant: IGA MZ No. NK4565-3.

Effects of the repeated administration of new antineoplastic agents on the cardiovascular system of rabbits in vivo

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New potential anticancer agents - dimethoxybenfluron (3,9 – dimethoxybenfluron hydro- chloride) and Oracin (6-[2-(2-hydroxyethyl) aminoethyl]-5,11-dioxo-5,6-dihydro-11H-inde-no[1,2-c]isoquinoline hydrochloride) - were studied in respect of their cardiac effects and compared with daunorubicin. The cardiovascular effects of repeated i.v. administration of these cytostatics (once a week, 10 administrations) were studied in three groups of rabbits: 1) Oracin (10 mg/kg; n=7), 2) dimethoxybenfluron /DMB/ (12 mg/kg; n=7), 3) DMB (24 mg/kg; n=6) and were compared with the control group (saline 1 ml/kg; n=15) and the group with experimentally induced cardiomyopathy (daunorubicin 3 mg/kg; n=15). In addition, biochemical, haematological and histological parameters were followed up. Toxic effects of daunorubicin were demonstrated (significant increase in the PEP:LVET ratio, 20% mortality, signs of haemato- and nephrotoxicity, histological picture). Administration of new anticancer drugs mostly did not induce significant changes in PEP:LVET (Oracin - max. increase to 115.2%, DMB-12 - 101.4%, DMB-24 - 117.5%), cardiac TnT was always within the physiological range, no premature deaths occurred. Histological examination did not reveal pathological changes of the myocardium. It can be concluded that the administration of new antineoplastic agents - dimethoxybenfluron and Oracin - did not induce signs of cardiotoxicity in rabbits in vivo. References: 1. Macháčková J et al. Acta Med (Hradec Králové) 1999;42(3):89-92, 2. Adamcová M et al. J Cancer Res Clin Oncol 1999;125(5): 268-74.

Supported by Grant: IGA MZ No. 4212-3 and Research Project CEZ: J13/98:111500002.

Pharmacokinetic model of low dose methotrexate

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Low dose methotrexate (i.e. 5 - 30 mg MTX per week), administered to patients suffering from severe forms of psoriasis, became the drug of choice, following topical treatment (i.e. tar, corticosteroids) and PUVA. Long term drug administration (i.e. months, years) is necessary. The effect of MTX results from an intracellular enzyme inhibiting activity (i.e. AICAR system and DHFR) after drug intake and polyglutamation, resulting in an inner cell storage and sustained action. Immunological effect seems to be mediated by adenosine. A too high amount of a too rapidly released adenosine can result in lastitude and nausea. On the other hand, gradual drug intercellular accumulation can result in late toxic effects - i.e. depletion of folic acid, liver fibrosis or cirrhosis, trombocytopenia and megaloglastic anemia. Previous studies with low dose methotrexate showed a great interindividual variability in drug pharmacokinetic parameters (on the contrary, intraindividual variability was very low). The individualisation of drug administration and dose prediction could be very useful for obtaining optimal plasma drug concentrations and avoiding adverse effects of the treatment, mainly a depletion of folic acid. Our first task is to quantify pharmacokinetic parameters in each patient after the first administered dose (i.e. 5 mg MTX in one group or 2.5 mg in the other one). The following parameters (measured or calculated) are useful: plasma MTX concentrations and AUC, Tmax, Cmax and MTX urine excretion. It is very important to monitor the rate of MTX concentrations in erythrocytes during the first three months of treatment (erythrocyte - a model of drug cellular storage). The second problem is to provide individualization in the dosing schedule (the prediction of dose and, if necessary, the most appropriate route of administration). The goal is to prevent folate depletion and adverse effects as results of inappropriate treatment.

Supported by Grant: GA UK No. 310 56.

Computer room for multidisciplinary educational projects

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The Internet is a rich source of medical information and it can be widely used as early as in undergraduate medical courses. We prepared a preclinical multidisciplinary educational project covering medical biophysics and biostatistics, anatomy, biology, physiology, and pharmacology. Every course enhanced its syllabus by seminars or individual student projects that require the use of multimedia workstations for data processing, modeling and simulation, tutorial programs, or programs for browsing medical resources and publication databases on the Internet. All these projects should be connected with respect to both contents and timing. We provided the students with a technical background of new multimedia workstations and theoretical support. We expect that the students will be able to use modern information resources individually and reasonably. In most courses the students use computers and information resources for these purposes: biophysics and biostatistics - study materials for self study questions on the Internet, data processing and typing the protocols from lab lessons, tutorial programs (EKG, Ultrasound, Statistics), seminars in Internet, Word, and Excel Anatomy - 3D reconstruction of anatomic structures and Virtual Human Anatomy (tutorial programs). Biology - simulation of genetic development of different populations, seminar called "Internet as a Useful Tool for Working with Scientific Information". Physiology - computer simulation of action potential (AxonLab program), individual project "Publication Resources on the Internet". Pharmacology - database of drugs AISLP, computer modeling of drug kinetics in an organism, clinical and pharmacological studies (self study).

Supported by Grant: FRVŠ No. F1442/1999.

Bicarbonate CVVHD application

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1. Research data have been collected during the second year of the grant. 19 patients have been investigated. The data describe metabolic and some hormonal changes during the period of transformation from parenteral to enteral nutrition. The data are still being collected and we have no statistical analysis yet. 2. The aim of the present study is to inform our opinion about the bicarbonate system in continual venovenose hemodialysis (CVVHD) during the treatment of acute renal failure patients with multiorgan dysfunction and hemodynamic instability. A retrospective analysis of patients, treated by CVVHD at the unit of intensive metabolic care in 1998 has been performed. 20 adult patients were treated by CVVHD. The whole length of continual replacement therapy application was 148 days during one year, and the mean duration of one patient treatment was 7.5 days (from 1 to 32 days). The anuric period was from 2 to 40 days; 5 patients were not anuric. 12 patients died. The elevation of blood lactate level above than 7 mmol/l was documented in 7 cases after the starting of CVVHD. The usual lactate solution for continual replacement therapy (40 mmol/l of lactate) was changed to bicarbonate solution, which we prepared immediately before using the same technique as the all-in-one system for parenteral nutrition (saline solution 0.9% 2400ml, dextrose 40% 15ml, natrium bicarbonate 8.4% solution 120ml, Aqua steril. 1000ml, potassium chloride 7.5% 15ml, magnesium sulfate 10% 10ml, Calcium 10% sol. 10ml). One patient recovered, four patients lived more than two days and a transient improvement of hemodynamic stability was achived. We conclude that the application of the bicarbonate system in CVVHD, which is not largely procurable, can improve the treatment of multiorgan failure shock patients.

Supported by Grant: IGA MZ No. 4788-3.

Intraoperative radioimmunodetection of colorectal carcinoma

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Intraoperative radioimmunodetection (IRID) is an intraoperative diagnostic method used during the surgical treatment of colorectal carcinoma for the detection of occult metastases. The method identifies patterns of disease dissemination and could help to make an effective amount of resection. 48 patients were operated on for colorectal car-

cinoma (26 men, 22 women) with the median age of 64 years. The primary tumour was in 41 cases, recurrence of disease in 7 cases. The relations between the IRID positive results and histological examinations were statistically assessed after the 38th operation. The analyses used the NCSS programme. The logistic regressive function was used for synoptic statistic appraisal of the usual descriptive statistics because it is the most suitable method for ascertaining the essence of clinical characteristics. It seems to be important to "calibrate" each patient by taking a series of readings to determine what is normal activity for healthy tissue and normal activity for tumour tissue in comparable tissue. This fact was put in the following ratio: IL/HM divided by TT/HT (IL = radioactivity of infiltrated lymphonodes, HM = radioactivity of healthy mesocolon tissues, TT = radioactivity of tumour tissue, HT = radioactivity of healthy colon tissue). This ratio, index, was a statistically significant factor; it correlated with histological results with r = 0.40 and its significance was estabilished by $\chi 2$ test approximately on 5 % level of significance. The other IRID results up to the number of 48 patients were evaluated by the new method. Using our first experience we tried to find more exact rules for IRID results assessment. The positive IRID results of tumour infiltrated lymphonodes were confirmed by histology in 73.3% of cases.

Supported by Grant: IGA MZ No. 4532-3.

Cytochrome CYP3A4 and amiodarone

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The objective of this study was to determine the steady-state serum concentrations of amiodarone (AM) and desethylamiodarone (DEA) in relation to CYP3A4 activity in humans. We also investigated the in vivo and in vitro influences of amiodarone and its major metabolite DEA on CYP3A4 activity. Methods: The urinary ratios of 6beta-hydroxycortisol (6beta-OHC) to urinary free cortisol (UFC) have been used as the criterion of hepatic CYP3A4 catalytic activity. Twelve cardiac patients were enrolled. Urine samples were collected before amiodarone treatment and during the third day of amiodarone therapy. Blood samples for the measurement of plasma AM and DEA were taken after 182 + 26 days. In an in vitro study the effect of both compounds (AM and DEA) on cortisol 6beta-hydroxylation was studied with human liver microsomes. Results: There was a large interindividual variation in the urinary 6beta-OHC/UFC ratio (range 2.48-13.16) before amiodarone treatment. Nevertheless, we were unable to find a relationship between CYP3A4 activity and AM or DEA plasma concentrations (r=-0.47, p<0.17 and r=-0.21, p<0.56, respectively). On the other hand, patients had a statistically significant decrease (p<0.05) in the urinary 6beta-OHC/UFC ratio during the third day of amiodarone therapy, suggesting in vivo inhibition of CYP3A4. At the same time, DEA inhibited 6beta-hydroxycortisol formation during in vitro experiments, whereas an increase in the rate of 6beta-OHC production was observed in the presence of amiodarone. Conclusion: We did not find any relationship between CYP3A4 activity and steady-state serum concentrations of AM or DEA. Nevertheless, amiodarone therapy decreases the 6beta-hydroxylation of cortisol. The possible mechanism is the competitive inhibition of hepatic CYP3A4. Moreover, DEA greatly contributes to the overall inhibitory effect of its parent compound.

Supported by Grant: GA UK No. 57/97/C.

Leucine and protein metabolism after bilateral nephrectomy in rats. The role of hepatic tissue

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The aim of this study was to evaluate the effect of acute uremia on changes in leucine and protein metabolism in the whole body and in hepatic tissue. Acute renal insufficiency was induced by bilateral nephrectomy (BNX). Twenty four hours later the parameters of protein and amino acid metabolism were evaluated in the whole body using primed constant intravenous infusion of L-[1-14C] leucine and in isolated perfused liver (IPL) using keto [1-14C]isocaproate. The control group consisted of sham operated rats. BNX induced a marked decrease in proteolysis, protein synthesis, leucine oxidized fraction and leucine clearance. The decrease in protein synthesis was higher than in proteolysis. A significant drop in protein synthesis was observed in muscle, gut, heart and spleen. The study with IPL in BNX animals showed decreased oxidation of ketoisocaproic acid and higher concentrations of branched-chain amino acids (BCAA) leucine, isoleucine and valine in the perfusion solution. We conclude that the rapid depletion of body proteins after BNX is caused by a greater decrease in protein synthesis than in the proteolysis associated with an increase of the leucine oxidised fraction. The data obtained in the IPL model indicate that BNX causes metabolic changes which enable resynthesis of BCAA from corresponding branched-chain keto acids in the liver.

Supported by Grant: GA ČR No. 306/98/004.

Effect of hyperammonemia on leucine and protein metabolism in rats

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The cause of muscle wasting and decreased plasma levels of branched chain amino acids (BCAA), valine, leucine, and isoleucine in liver cirrhosis is obscure. In this study we have evaluated the effect of hyperammonemia. Rats were infused with either an ammonium acetate/bicarbonate mixture or a sodium acetate/bicarbonate mixture or saline for 320 minutes. The parameters of leucine and protein metabolism were evaluated in the whole body and in several tissues using a primed constant intravenous infusion of L-[1-14C] leucine. Ammonium infusion caused an increase in ammonia and glutamine levels in plasma, a decrease in BCAA and alanine in plasma and skeletal muscle, a more significant decrease in whole-body protein synthesis than in whole-body proteolysis, and an increase in leucine oxidized fraction. A significant decrease in protein synthesis after ammonium infusion was observed in skeletal muscle while an insignificant effect was observed in liver, gut, heart, spleen and kidneys. We conclude that the decrease in plasma BCAA after ammonia infusion is associated with decreased proteolysis and increased leucine oxidized fraction. The more significant decrease in protein synthesis than in proteolysis, together with the increased leucine oxidized fraction, imply protein wasting in the hyperammonemic rats.

Supported by Grant: GA UK No. 276/98C.

Immunoblot configuration as a confirmative serological method in the diagnosis of Lyme borreliosis

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Clinical manifestations of Lyme borreliosis are mostly not pathognomonic. Reliable laboratory tests are therefore necessary to support the clinical diagnosis. A two-step approach for the serological diagnosis of Lyme disease has recently been proposed. All specimens with positive or equivocal results should be confirmed by means of a standardised immunoblot. Analysis of Western blot results is complicated by the lack of established criteria for the interpretation of European Lyme borreliosis blots. The aim of the study: to demonstrate the diagnostic importance of the production of antibodies against specific antigens of three Borrelia burgdorferi genotypes manifested in various clinical forms of Lyme borreliosis in the Czech Republic, after analysis of results to determine whether only one genotype with a high level of reliability can be used or whether it is necessary to use all of them. The samples of serum of 56 patients suffering from Lyme Borreliosis (20 patients with typical clinical signs and 36 individuals with the direct proof of Borrelia-PCR) were collected. The serums will be examined in a short time by immunoblot made from three genotypes of Borrelia. The control group consists of 30 healthy blood donors, whose serums have already been examined. The results are being evaluated presently as well as those of individuals with other diagnoses (Tularemia, EB, Lues). References: Ryffel K et al. Scored antibody reactivity determined by immunoblotting shows an association between clinical manifestations and presence of Borrelia burgdorferi sensu stricto, B. garinii, B. afzelii, and B. Valaisiana in humans. J Clin Microbiol 1999;37(12):4086-92.

Supported by Grant:IGA MZ No. 4549-3.

TIPS created with ePTFE covered stent-grafts - prospective control trial

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Purpose: In prospective, non-randomized control trials the occurence of stenosis, both in general and inside the stent, particularly when using covered and non-covered stents, was compared. Methods: Between October, '97 and March, '99 twenty-seven patients with symptomatic portal hypertension (bleeding from varices or refractory ascites) were treated with TIPS using covered stents. The control group consisted of another 27 patients of the same age. Child-Pugh classification and aetiology of cirrhosis, Wallstent, Spiral Z stent, Jostent and home made stengrafts were used. Results: In the ePTFE group a revision in 5 patients (18.5%) was indicated - instent stenosis in 2 cases, outflow stenosis in 3 cases. In the control group 11 patients (40.7%) underwent angioplasty for stenosis - instent stenosis in 11 cases, outflow stenosis in 2 patients. Conclusion: The use of the ePTFE stentgraft is technically possible, but it is associated with certain technical problems concerning the location of the graft. The use of ePTFE was not associated with a statistically significant reduction of stenosis (5/27 vs. 11/27) p=0.07. We have noted a statistically significant reduction of instent stenosis occurence in the ePTFE group (2/27 vs. 11/27) p=0.05.

Supported by Grant: IGA MZ No. 4558-3.

Mechanical percutaneous thrombolysers for treatment of pulmonary embolism. Experimental study

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Aim: The aim of our study was to test experimentally the safety, efficacy and reliability of three mechanical thrombolysers for the treatment of pulmonary embolism. Methods: The following rotational thrombolysers were tested in twenty-four pigs after iatrogenic embolisation of the pulmonary artery (PA): (1) a high-speed teflon thrombolyser ANGIOCOR was used in four pigs; (2) an ARROW-1 thrombolyser was used in four pigs; (3) an ARROW-2 over a guide-wire thrombolyser was used in twelve pigs. Pulmonary vascular resistance (PVR) was measured before and after the embolisation of PA and after the fragmentation of pulmonary thromboemboli. In six pigs, PVR was measured 1 month after the fragmentation of pulmonary thromboemboli with the ARROW-2 thrombolyser. Results: The ANGIOCOR thrombolyser could safely and efficiently clear pulmonary thromboemboli. However, the deformation of rotors and damage of all thrombolysers occurred after several seconds of their activation. The ARROW-1 thrombolyser was efficient and reliable during the fragmentation of pulmonary thromboemboli. One animal died due to perforation of a branch of the PA with a tip of the thrombolyser. With the ARROW-2 thrombolyser it was possible to easily and reliably break-up thromboemboli in the PA. In one animal the tip of the thrombolyser was trapped in the pulmonary artery and further manipulation with thrombolyser led to rupture of the wall of the pulmonary artery and death of the animal. In all animals, significant increase in PVR occurred after fragmentation of thromboemboli in PA. Conclusion: No. thrombolyser tested in our study was suitable for clinical use.

Supported by Grant: IGA MZ No. 4113-3.

The role of antioxidant balance in ethiopathogenesis of inflammatory diseases of the gastro-intestinal tract with special interest in patients with positive tests for Helicobacter pylori infection

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The aim of this study was to evaluate the activity of highly reactive oxygen species with peroxidation products and antioxidants in patients with antral gastritis and positive Helicobacter pylori infection. The above mentioned parameters were monitored during two time periods - before and after therapy. The results did not show that Helicobacter pylori infection induced any changes in antioxidative balance. The patients with antral gastritis and positive Helicobacter pylori infection had specific disturbances in antioxidative balance, which were not significantly influenced by therapy. As the individuals with disturbancies in antioxidative balance showed modified response during infection, they could be more susceptible to Helicobacter pylori infection as well.

Supported by Grant: IGA MZ No. 4221-3.

Examination of reaction of brain tissue and PC-12 cells to HEMA-EMA copolymer

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HEMA-EMA, a new synthetic hydrogel, was proposed for the encapsulation of dopamine secreting PC-12 cells. The aim of this study was: 1) to assess the biocompatibility of the HEMA-EMA copolymer after implantation into the brain tissue, and 2) to examine behaviour, growth and survival of PC-12 cells with and without the HEMA-EMA polymer. Simultaneously, we examined the immunohistochemical characteristics of the PC-12 cell line. Small diameter (400 μm) polymer capsules were prepared by the submerged jet method and were implanted unilaterally into the brains of adult rats. Animals were sacrificed at 2, 4, 12 and 24 weeks postsurgery. Brains were cut at the implantation site and vibratome and paraffin-embedded sections were processed for a variety of detection methods that allowed identification of cell types participating in host response to implanted material. Acquired results of histological and immunohistochemical examination indicated that reaction of the recipient tissue was mainly caused by injury during implantation (accumulation of siderophages, microglia and GFAP+ reactive astrocytes in the area of the injection tract) while in the surroundings of microcapsules this reaction did not fully develop. The tissue reaction around the implanted capsules consisted of glial and connective tissue components in which the reticular fibres predominated. The polymer was stable – intact microcapsules persisted in the parenchyma six months after implantation. Some foreign body giant cells were observed around the capsules 1 month postimplantation, while later these cells were absent; our results demonstrated that HEMA-EMA polymer did not induce chronic irritation of brain tissue.

Supported by Grant: GA ČR No. 304/98/0267.

Small bowel permeability in patients with inflammatory bowel diseases

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The ground of the small bowel permeability test is the measurement of the absorption of sugars of different sized molecules through the bowel wall. Changes of small bowel permeability values were found in diseases with damaged intestinal barriers, in patients with celiac disease, active forms of Crohns disease and ulcerative colitis. An index of small bowel permeability was counted as the lactulose/mannitol index of sugars absorbed from the gut and excreted into urine during 5 hours. In 1999 we continued with the measurement of small bowel permeability in patients with Crohns disease and ulcerative colitis, their first degree relatives, celiac disease and their first degree relatives. Preliminary results show an increase of small bowel permeability in patients with untreated celiac disease in comparison with patients treated with gluten-free diet and medical checks and an increase of small bowel permeability during clinical relapses of both inflammatory bowel diseases - Crohns disease and ulcerative colitis. The changes were not found in the first degree relatives of the patients with inflammatory bowel disease; the increase of permeability in the relatives of patients with celiac disease suggest the future development of the same disease in these persons. The study will be continued and finished in the year 2000.

Supported by Grant: IGA MZ No. 4529-3.

The nucleic acid differential staining method in vitro and its implementation in biology courses at Charles University Prague, Faculty of Medicine in Hradec Králové

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To visualize DNA and RNA molecules in the cell, one may take advantage of a great number of staining techniques. Unfortunately, not all of them are suitable for a biology course. The Unna-Pappenheim technique of differential staining of nucleic acids permits the use of a light microscope, thereby giving a chance to all students to actively participate in the procedure. This technique uses methyl-green dye for DNA staining and pyronin G solution for RNA staining. After the treatment with a staining solution we can observe the green nuclei, red cytoplasm and nucleoli. The green color of the nucleus disappears after exposing cells to deoxyribonuclease whereas ribonuclease causes the cytoplasm and nucleoli to decolor. Proteinase, which does not interact with either of the nucleic acids, brings no changes to the coloring of cells. The main goal of this project was to test four variants of treatment. The procedure was employed as follows. The continuous cell line Hep-2 was used. Cells were grown according to standard culture conditions in Petri dishes, with two coverslips per dish. Cells on each coverslip were fixed with methanol for 5 minutes and air-dried. After staining with methyl-green-pyronin for 30 minutes, coverslips were rinsed in distilled water, air-dried and mounted as permanent preparations. Rinsing in xylene and alcohol has not come up to our expectations because it has caused strong decolorization. The color differences in structures containing either DNA or RNA molecules were not very distinct. Based on these experiences we prepared protocols for the methyl-green-pyronin method which will be introduced into practical classes in the next summer term.

Supported by Grant: FRVŠ No. 1290.

Carbon dioxide angiography

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During the last 2 years 58 digital subtraction angiographies in 54 patients were performed using either CO_2 alone or in combination with a nonionic iodinated contrast medium. The indication for CO_2 angiography was previous severe adverse reaction to iodinated contrast media (in 8 patients, 10 studies), patients with impaired renal functions or preexisting renal failure (21 patients indicated for peripheral lower leg angiography and 4 patients with hepatorenal syndrome referred for TIPS procedure). There were another 13 patients with retrograde wedged hepatic portography and 6 patients with superior mesenteric angiography indicated for lower gastrointestinal bleeding included in this study. Using CO_2 as a contrast agent the total dose of iodinated contrast media can be lowered by 50 % in patients with preexisting renal function impairment. The whole study can be done by using only CO_2 in patients with a high risk of allergic reaction. Wedged hepatic vein CO_2 - based portal vein opacification was successful in 83 % of the patients. No. case of extravasation was revealed in 6 patients with technically successful superior mesenteric angiography performed for lower gastrointestinal bleeding. The use of CO_2 as an intravascular contrast agent is more technically demanding than iodinated contrast medium imaging. Therefore it was used only in cases where its safety (no nephrotoxicity and hypersensitivity) outweighed its disadvantages. In the wedged hepatic vein CO_2 based portal vein opacification was superior to that with iodinated contrast media.

Supported by Grant: IGA MZ No. 4545-3.

Multiple drug resistance of leukemia cells and its assessment in vitro

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Aims of the project: Detection of multiple drug resistance protein p-170 and other markers (MRP, LRP) on the surface of leukemia cells by flow cytometry. Evaluation of the functional activity of p-170 molecules. Correlation of the presence and activity of p-170 molecules in clinical specimens with immunophenotype and clinical response to therapy. Results: The expression of p-170 molecules on cells of clinical specimens is measured. In selected examples two different monoclonal antibodies directed to different epitopes of p-170 molecules are used. The evaluation of functional activity of p-170 molecules was implemented using the genetically engineered cell line K562-SF1 MIH with expression of p-170 (generous gift of Dr. Jelinek, Institute of Haematology, Prague). The implementation of flow cytometry techniques to assess the process of apoptosis in malignant cells and its link to multiple drug resistance. References: 1. Kodydková K, Krejsek J. Multiple drug resistance of leukemia cells. XIIth Congress of Clinical Haematology, Olomouc, (lecture). 2. Kodydková K, Krejsek J. The resistance of haematopoetic tumour cells to the cytotoxic drugs. (paper, submitted). 3. Krejsek J, Žák P, Toušková M, Vokurková D, Kodydková K, Kopecký O. Gamma/delta T cell lymphoproliferation: a case report. Eur J Haematol 1999;63:1-4.

Supported by Grant: IGA MZ No. M/20-3/98.

Oscillatory model of visual evoked potentials

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On the basis of a spatio-temporal analysis of the motion-onset (M-VEPs) and pattern-reversal (P-VEPs) visual evoked potentials (VEPs) and their decomposition into three independent components (Kremláček and Kuba 1999), a model simulating cortical oscillations was designed. The model consists of three damped oscillators (O1, O2, O3) of an identical construction. The oscillators were serially connected and their processing time was simulated by a variable delay on the oscillator input. Besides this parameter, each oscillator had two additional parameters determining its frequency and a damping property. Particular model parameters for the M-VEPs and P-VEPs in four young volunteers (PZ-A2 lead) were found by the Nelder-Mead optimisation method. The difference between simulated and real data described by normalised root mean that the square error was lower than 13%. The O1 contributed to the N75 and P100 peaks of the P-VEPs and was supposed to simulate the primary visual area (V1). The activity of the O2 corresponded to the P-VEPs (N145) and to the M-VEPs (N160). It likely mimics an activity in the V2, V3A and MT extrastriate visual areas. The O3 with a late contribution (peaking at about 300 ms) is expected to model central cognitive functions, usually connected with sensory perception. Literature: Kremláček J, Kuba M. Global brain dynamics of transient visual evoked potentials. Physiol Res 1999;48:303-8.

Supported by Grant: GA UK No. 56/97/C.

A system of hypertext programs for teaching probability theory and statistics in medical schools

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Statistics is taught within the biophysics course in our medical school. This course is mandatory in the first semester. It uses a textbook that meets the requirements of a one semester statistics course for medical students. This textbook was written before 1994 and the technology available today allows us to create more illustrative and interactive textbooks. The purpose of this project was the design of a hypertext instruction program to cover the topics of statistics and probability theory to suit the needs of a medical school. The programs may be used both in undergraduate and graduate courses. The instruction is enhanced by graphs and solved practical problems. A glossary is also attached. Mandatory parts, which are those the student is supposed to know and understand, are extended by parts allowing deeper understanding of the topic. These parts are supported by mathematical proofs which enable better consistency of instruction in a mathematical sense. The proofs do not use any higher mathematics, so any student with a high school knowledge of mathematics should be able to follow them. The simplicity of user interface is the main point to emphasize. A help function is included to help beginners. To create these programs we used the system Authorware Professional 4.0. A test generator is also available together with a list of examples.

Supported by Grant: FRVŠ No. 1277/99.

Objective evaluation of brain cortical dysfunctions

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A set of newly developed visual stimuli (1) was used in diagnostic examination of 135 patients with neuro-psychiatric disorders (migraine, dementia, depression, multiple sclerosis, dyslexia). It was proved that a significantly higher sensitivity of electrophysiological testing is achieved when reactions of associate sensory areas are evaluated. (2, 3). In comparison to other groups of patients, those with migraine do not display any delay of the recorded potentials but have significantly increased amplitudes. This might confirm the hypothesis about increased irritability of the migrainous brain and could be used for an objective assessment of therapy. In about 50% of dyslexic subjects, a magnocellular deficit of the visual pathway was verified. However, its normalisation in adolescence has no clear correlation to reading parameters. References: 1. Kremláček J, Kuba M, Kubová Z, Vít F. Simple and powerful visual stimulus generator. Comp Meth Programs Biomed 1998;58:175–80. 2. Kuba M, Kremláček J, Vít F, Kubová Z, Gayer D, Szanyi J. Complex testing of the visual pathway via multiple evoked potentials. Clin Neurophysiol 1999;110 (Suppl.: S115. 3. Kuba M, Kremláček J, Vít F, Kubová Z, Gayer D, Szanyi J. From visual sensation to cognition. Book of Abstracts - 37th Symposium of the International Society for Clinical Electrophysiology of Vision (Eilat, Israel, April 11 – 16, 1999):94. 4. Szanyi J, Kuba M, Kremláček J. Electrophysiological findings in dyslexia (in Czech). Lék Zpr. LF UK Hradec Králové 1999;44:113–20.

Supported by Grant: IGA MZ No. 4800-3.

Modelling of visual processing of motion

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After description of motion-related visual evoked potentials (M-VEPs) characteristics (1) and determination of the most important stimulus parameters (2), we completed the electrophysiological study of visual processing of motion through construction of a model explaining M-VEP formation (3,4). This model reflects well both the topography and dynamics of brain cortical areas and also includes contributions of main motion stimuli parameters. It fits well within the existing anatomical concepts of the visual pathway and it even extends understanding of motion perception because it introduces evidence about centro-parietal activation in some more complex visual stimuli, e.g. in centrifugal motion ("expansion"). This might have a high diagnostic value in case of some selective involvements of these cortical areas (e.g. in cognitive disorders and multiple sclerosis). The introduced model of visual perception of motion can help to predict some disorders on the basis of a simplified time-limited electrophysiological examination. References: 1. Kuba M, Kubová Z. Visual evoked potentials specific for motion-onset. Doc Ophthalmol 1992;80:83-9. 2. Kubová Z, Kuba M, Spekreijse H, Blakemore C. Contrast dependence of motion-onset and pattern-reversal evoked potentials. Vision Res 1995;35:197-205. 3. Kremláček J, Kuba M. Global brain dynamics of transient visual evoked potentials. Physiol Res 1999; 48:303–8. 4. Kremláček J, Holčík J, Kuba M. Visual evoked potentials model of magnocellular system. Biol Cyber 1999–in press.

Supported by Grant: The J. S. McDonnell Found. Cognit.Neurosci., USA No. 93-44.

Results of phonosurgery operations at the ENT Dept. Hradec Králové in 1999

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The goal of the grant "Phonosurgical treatment of voice disorders" is the introduction of the following new phonosurgical procedures to the current practice: 1) Microsurgery of benign vocal cord lesions using the Bouchayer technique, 2) Teflon injections to vocal cords, testing of other reasonable injectable materials, 3) Thyreoplasty type I, 4) Ejnell's operation for bilateral recurrent nerve paralysis Results in the year 1999:1) Mediofixation techniques: 12 operations have been performed by now In 4 cases the vocal cords were medialized using teflon paste and in 8 cases we decided for a thyreoplasty I operation. The indications for the surgery were vocal cord atrophy (4 cases), and vocal cord paralysis (8 cases). 3 patients also suffered preoperatively from aspirations. We have observed improvement of average frequency voice extent in the entire patient collection up to 2 octaves postoperatively. The voice intensity range (whisper – loud speech) improved to 16dB on average. Phonation time became better at 11 seconds. No. significant changes have been observed at spirometry. Aspiration disappeared in 2 cases and decreased in the third one. 2) Laterofaxation techniques: In 1999 we have performed 8 laterofixations using the Ejnell technique; in one case an arytenoidectomy was performed. No. re-operation was necessary in 1999. All patients showed improvement of respiration, all temporary tracheostomies were successfully closed. 3) Microsurgery of benign vocal cord lesions: We have achieved an improvement of skills in the new Bouchayer microsurgical technique for the surgical treatment of vocal cord pathology located at the lamina propria superficialis. The broad spectrum of treated pathologies includes Reinke's edema, vocal cord cysts, noduli etc. We have been observing less postoperative scarring at the lamina propria compared with Kleinsasser endolaryngeal surgery, which has been used so far, as well as quicker postoperative healing.

Supported by Grant: IGA MZ No. 4528-3.

The importance of effective atrial contraction for sequential pacing

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The aim of the planned study is to assess the importance of effective atrial contribution for the selection of an optimal pacing regimen in patients in whom a DDD pacemaker has been implanted. The most important question to be answered is whether quantification of the left atrial contractile function is of importance for the proper selection of the pacing mode. During 1999, the newly developed method (preceding research project) and all other techniques of investigation were applied in only 7 patients due to delayed funding. The data are so scarce that it is impossible to give even preliminary results. It is expected that the rest of the patients (30 in total) planned for the year 1999 will be examined in the coming year.

Supported by Grant: IGA MZ No. NA 5403-3.

Changes of visual functions in myopes twelve months after PRK

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1. 110 myopes undergoying photorefractive keratectomy (PRK) with refraction from -0.25 D to -12.0 D were divided into 4 groups: A: up to -2.75 D, B: -3.0 D to -5.75 D, C: -6.0 D to -8.75 D and D: -9.0 D to -12,0 D. Best corrected visual acuity (BCVA) using a computerized method with Landolt rings and contrast sensitivity (CS) using an adjustment method with ascendent and descendent approach to threshold contrast adaptation on a computerized system of the Contrast sensitivity 8010 type were examined in patients before and 1 year after PRK. 20 emmetropes of the same median age were evaluated as a control group. 2. Preoperative BCVA and CS in myopes of all four groups was significantly lower ($p < 0.05$ up to $p < 0.001$), compared to controls. Decrease of functions was proportional to refraction. 3. With increasing refraction fewer patients were within ±0.5 D and ±1.0 D after surgery. 4. Twelve months after PRK, BCVA in the group A reached the level of controls, CS in the same time interval was equal to control even in groups A and B. 5. According to the results of our study, PRK is a suitable method for myopia up to -6.0 D. References: Langrová H, Hejcmanová D, Peregrin J. Čs Oftal 1999;55(6):333-43. Langrová H. Změny zrakových funkcí po fotorefraktivní keratektomii (Changes of visual functions after PRK. Hradec Králové, 1999. - Thesis)

Supported by Grant: GA UK No. 59/97.

Quality of life in psychiatry - transcultural adaptation and validation of the questionnaire

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Objectives: To validate the Quality of Life Enjoyment and Satisfaction (Q-LES-Q) Questionnaire (1) in the population of depressive patients in the Czech Republic and to actively involve students in the research activities involving direct contacts with the patients. Methods: Q-LES-Q (self-administered quality of life questionnaire) consists of 8 divisions (tests), seven specific and one general. The transcultural adaptation process within the group of depressive patients admitted to the psychiatric ward (F32 according to NKM-10) was followed. In the pilot phase translation and retranslation were performed, then comprehensibility with 18 patients was assessed. Raters' concordance in the evaluations of specific psychometric scales was compared. Reliability was assessed using the test-retest and internal consistency approach. The questionnaire was administered twice, once on admission, the second time within the week after admission. Validity was assessed in comparison to the psychometric scales Clinical Global Impression (CGI), Hamilton Psychiatric Rating Scale for Depression (HAMD) and Beck Depression Inventory (BDI). Results: Data from 56 patients were gathered. Reliability part of testing was performed with the 24 patients, validity assessments were based on 93 measurements. Statistical evaluation is currently in process. Conclusions: Statistical evaluation will ascertain the methodological value of the Q-LES-Q as an instrument for measurement of the quality of life. Students were actively involved in the clinical and research activities. References: 1. Endicott J, Nee J, Harrison W, Blumenthal R. Quality of Life Enjoyment and Satisfaction Questionnaire: a New Measure. Psychopharmacol Bull 1993;29(2):321-6.

Supported by Grant: FRVŠ No. 1289/99.

Serum ceftazidime levels during cardiopulmonary bypass

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Background: The aim of our study was to assess the adequacy of our regimen using ceftazidime (CTZ), ciprofloxacin (CPF) and clindamycin (CLIN), as prophylactic antibiotics and to verify whether cardiopulmonary bypass (CPB) can modify the time course of antibiotic serum concentrations. This is why their serum levels were measured during open-heart procedures. Our results were compared to standard producers' data. Methods: The prospective study involved 75 consequent coronary patients randomized into three groups receiving 1 g of CTZ or 400 mg of CPF or 900 mg of CLIN i.v. with anesthesia induction. Routine coronary surgery with left internal mammary artery harvesting, moderate body hypothermic (30 s C) CPB with crystalloid cardioplegia was performed. Serum antibiotic levels were determined at the following intervals: 1/ before application, 2/ skin incision, 3/ prior CPB, 4/ after cardioplegia infusion, 5/ every 20 minutes of CPB, 6/ prior end of CPB, 7/ chest closure. Conventional cylinder-plate microbiological assay was used for antibiotics levels measurement. Results: All serum antibiotic concentrations showed a sharp decrease immediately after the starting of CPB and lasted until CPB ended. After initiation of CPB after cardioplegia administration serum concentrations of CTZ (105 min after initial dose) decreased by, on average 55%, CPF (97 min) by 42% and CLIN (116 min) by 78%. Conclusion: CPB can modify the time course of antibiotic serum concentrations. The serum levels of CTZ and CLIN at the end of the longest procedures were found to be below the MICs for some of the suspected pathogens. We recommend the use of higher antibiotic doses for prophylaxis and the administration of the second dose with protamine sulphate infusion to obtain maximum concentrations of the antibiotic in newly formed blood clots.

Supported by Grant: IGA MZ No. 4103-3.

The cell cycle analysis of multiple myeloma plasma cell population using flow cytometry

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The prognostic factors with relation to proliferative activity of malignant myelomatous clone rank among the most important in the diagnosis of multiple myeloma. In recent years, the flow cytometry has provided new insights into the biology of neoplastic cells, including cell cycle analysis. The introduction of cell cycle analysis with propidium iodide staining technique has allowed recognition of complete cell cycle distribution (G0/G1, S and G2/M phases) of multiple myeloma plasma cell population. Our preliminary results: We evaluated cell cycle distribution in the bone marrow specimens of our patients with multiple myeloma. We used two double staining techniques (CD38/PI and CD138/PI) for determination of myelomatous plasma cell population. In all cases, measurements were performed on an EPICS XL (Coulter) flow cytometer, and the analysis was based on at least 10,000 events. The proportion of cells in the different cell cycle phases was calculated using the MultiCycle AV software. The mean percentage of plasma cells in the patients' bone marrow at diagnosis was 32±14% (median, 27%). The CD38 and CD138 markers were present in all cases; the use of the CD38/PI staining method is easier for the strong CD38 positivity of MM plasma cell population. The individual cell cycle proportions of MM plasma cell population were analogous when we used both CD38/PI and CD138/PI double staining methods (G0/G1-phases 88.0±7.3% resp. 89.3±5.2%, S-phases 3.8± 1.4% resp. 3.8±1.6% and G2/M-phases 5.1±2.8% resp. 5.6±2.5%). We present our first experience with the use of the new flow cytometric technique which specifically identifies MM plasma cells and can calculate their proliferative rate. This method is useful both for the detection of residual disease in MM patients and for the differential diagnosis between MM and MGUS.

Supported by Grant: IGA MZ No. NC/4535-3.

Critical illness myopathy

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Background: Critical illness is connected with skeletal muscle protein depletion and muscle weakness. Muscle weakness can be attributed also to critical illness myopathy (CIM). The incidence of CIM is controversial. Aim: to assess frequency of creatin kinase (CK) elevation as a marker for skeletal muscle dammage in the critically ill. Method: retrospective chart analysis of 253 ICU patients. CK was assessed on admission and then once weekly. The severity of organ failure was expressed as a maximum daily SOFA score. The ANOVA test was used. The following causes of CK elevation other than myopathy were excluded: surgical trauma, CPR, convulsions, defibrillation less than 7 days before testing, hypothyreosis, myocardial damage. Results: After applying exclusion criteria the maximum CK, the SOFA score and the presence of sepsis were studied in 139 patients (91 men, 48 women), age 59.7 (19 - 90) years. The CK values ranged from 0.15 to 273 μkat/l. CK was elevated in 44% pts., elevation higher than twice upper normal range was found in 27%. No. statistical correlation of elevated CK to the presence of sepsis was found. The degree of CK elevation correlated with the SOFA score ($p=0.0003$). The elevated CK values may be compatible with CIM, the real frequency of which is likely to be higher because the CK values may be elevated for a short period of time and thus remain undetected by weekly testing. Conclusions: The frequency of CK elevation as a skeletal muscle damage mar-

ker is very high in the ICU. CK elevation is related to the severity of MOF. Muscle weakness in these conditions can be due to the CIM and the possibility of influencing it by nutritional means is questionable.

Supported by Grant: IGA MZ No. NB 5197-3.

Comparison of methods for apoptosis detection in HL-60 cells

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In this study we compared flow cytometric and morphologic methods of apoptosis detection at human promyelocytic leukemia cell line HL-60. HL-60 cells were harvested at 4, 7, 16, 24 and 48 hours after the induction of apoptosis by 3% ethanol. Little change was observed both by flow cytometry (decrease of forward scatter, increase of unprocessed cells staining with APO 2.7 antibody) and viability determination by Trypan-blue staining until after 7 hours. However, after 4 hours we observed morphologic changes in the nuclear and cytoplasmic structures using Diff-Quik stained cytospin preparations and standard light microscopic techniques (50% apoptotic cells). The same results were obtained by flow cytometric measurement of sub-diploid DNA content (sub-G1 cells) and increase of staining with APO 2.7 antibody in cells permeabilised by digitonin prior to staining. After 7 hours almost all cells exhibit apoptotic morphology. After 16 hours the cell size (forward scatter) decreased significantly and 54% of unprocessed cells were APO 2.7 positive. After 24 hours only 6% of cells were alive (high forward scatter) and these cells were APO 2.7 negative. The HL-60 cells did not proliferate during the cultivation in 3% ethanol and after 48 hours were all stained by Trypan blue. HL-60 leukemic cells were CD34-/AC133-, CD33+/CD15+ and only 2% of the cells were CD95+.

	time of incubation (h)			
method	0	4	7	24
morphology	2.8±2.2	55.4±9.8	93.3±3.2	99.0±1.0
subG1 peak	5.4±2.7	59.4±7.4	59.5±9.8	75.4±1.1
APO 2.7 unprocessed cells	2.7±2.2	6.1±2.7	8.1±4.0	78.1±11.5
APO 2.7 permeabilised cells	10.6±6.2	91.1±7.9	96.5±2.1	95.1±1.4

Supported by Grant: GA UK No. 279/98 C.

Innovation of medical curriculum

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Preparatory stage: Analysis of present situation - 5 levels: 1. International level. The main trends in pregraduate education of physicians for the next century, according to the enforcement of international and European association for medical education. In the framework of the project SOCRATES/ERASMUS three British universities were visited for the purpose of study of medical curriculum. Adoption of European concept of pregraduate education of pharmacology has started. 2. National level. Meetings of teachers of radiology from the Czech Republic and Slovakia are organized on a regular basis. Set of objectives of pregraduate education of radiology has been elaborated. 3. Faculty level. Main effort has been concentrated on elaboration of coherent dentistry curriculum (duration 5 years), which is separated from that of general medicine. In accordance with requirements of the EU, practical part is emphasized. Inquiry of the student and teacher opinion on general medicine curriculum has been accomplished. 4. Department level. Surveillance of home faculty curriculum has been elaborated and compared with other Czech faculties. Study included histology and embryology, anatomy, biology and genetics, physiology, pathological anatomy, farmacology, radiology, preventive medicine and hygiene, practical and family medicine. 5. Transdisciplinary level. Close cooperation between departments is extended. Special studies have arisen: a) empiric research (investigation regarding anatomy curriculum), b) theoretical surveillance (new trends of students' review of quality of teaching).

Supported by Grant: CEZ:J13/98111500005.

Children's coping with stress

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Main results of 3 years lasting research: Theoretical surveys: Theories, models and terminology of children's coping with stress, present knowledge about children's coping, humour as a way of coping with stress, crisis and marginal situations, the role of social support, coping with school-induced stress, coping with stress among children as oncological and stomatological patients, the role of parents. Methodological surveys: Main methods of research, main problems associated with diagnostics of children's coping with stress, diagnostics of social support. Translation and testing of new diagnostics methods: R. C. Ziller - drawing of social self-perception of children, I. G. Sarason - Social Support Questionnaire, M. Margolit - Children's Orientation Scale, Children Sense of Coherence Scale (standardization on Czech population), Waldron, Vaeni-WV-PPCI-Scale of Children's Coping with Cronic Pain, J. Monier - SACS-Scale of Multidimensional Approach to Coping with Stress. Empiric research of children's coping: Children's perception of hospital-induced stress, stress factors in nurse's work as potentional source of non-adequate behaviour of nurses in dealing with children patients, role of social support and game therapy during coping with stressful medical procedures, role of social support during coping among oncological children patients, central coordination as a factor of maladaption during coping, statement value of biochemical indicators of stress after injury and medical procedure. Objective interventions into children's coping (trials): Using game therapy for children's preparation for stressful medical procedures. Using a combination of anesthesis and progressive caudal blocking.

Supported by Grant: GA ČR No. 406/97/015.

Metabolites of tryptophan in plasma and urine. Methods and results

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Tryptophan (Trp) in humans is catabolized by several pathways leading to various metabolites of kynurenine and indolic compound formation. A number of diseases are connected with abnormalities in their excretion, but the relation of cause and effect is usually unclear. We introduced a two-step procedure for the detection of defects in metabolism of Trp: 1) TLC is employed when starting the investigation, 2) two HPLC methods were proposed and used at the next step, when pathological findings are to be proved and the individual metabolites quantified. The first HPLC procedure enables the assessment of tryptophan, indolylacry-loylglycine (IAG) and five other indolic compounds. The second method is intended to monitor kynurenine and seven of its catabolites. The same Sep-Pack pre-treated sample of plasma and urine is used for all methods. The reference values and the excretion pattern in some groups of patients (350 in total) were assessed. Hepathopathy, gastrointestinal defects, myopathy and seizures with other neurological symptoms were the conditions connected with changes in the excretion of some metabolites of Trp. A significant decrease of IAG excretion was found in burn patients early after the injury. Urine analyses were carried out on patients with Hartnup disease and benign xanthurenic aciduria, inherited metabolic defects of Trp. The finding of IAG in 5 out of the 56 urine samples of germ-free piglets and rats analysed testifies for its endogenous production in mammals. In other experiments, the Trp effect on the decarboxylation of other aromatic amino-acids in the liver was investigated; only a week inhibition under physiological conditions was recognised. References: 1. Marklová E et al. Chromatogr 1997;45:195-8. 2. Marklová E et al. J Chromatogr 1999;A 860.001-005. 3. Dršata J, Marklová E. Acta Medica, submitted.

Supported by Grant: IGA MZ No. 4097-3.

Experimental model of Huntington's disease: A comparison of kainic acid and ibotenic acid lesion of striatum

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The method of transplantation of foetal neural tissue has been used as a treatment of Parkinson's and Huntington's diseases - mostly with a positive effect. However, many "abilities" of grafted foetal neural tissue have not been completely clarified on an experimental basis yet. In our previous study, we used kainic acid (KA) as a neurotoxic agent for induction of the neurodegenerative process in striatum of the rat brain. On the basis of data from the literature, we would like to carry out another type of excitotoxic lesion – induced by ibotenic acid (IA). 1) The standardisation of ibotenic lesion was made. We compared: unilaterally/bilaterally-lesioned brains, 2 or 4 injection sites in each hemisphere, different stereotactic co-ordinates, different volume of injected solution – 0.25 or 0.5 μl, different concentration of IA – 5, 10 or 20 μg/μl, and different total concentration of IA per hemisphere - 5, 10 or 20 μg/μl. So far brains of 18 rats were examined. Histological evaluation was performed on serial paraffin sections, routinely stained with HE and cresyl violet, and selectively with anti-GFAP antibody. We can conclude that following is optimal for our experiments: bilateral lesion (of caudat nc. head) performed by an injection of IA (concentration 10 μg/μl) into 4 injection sites (of 0.25 μl each), i.e. of the total concentration 10 μg per hemisphere. 2) In comparison with KA lesion, IA lesion seems to be more precise in relation to location and more selective. These findings concerning one week-lasting lesion are only preliminary, and they must be completed with an evaluation of IA lesion in longer time intervals and with correlative behavioural tests of animals.

Supported by Grant: IGA MZ No. 5400-3/99.

The phenotype of peripheral blood leukocytes and systemic immune activation in patients with primary and secondary liver tumors: Implications for adoptive immunotherapy

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In a study aimed at evaluating potential effector cell populations for adoptive immunotherapy we examined peripheral blood leukocyte subsets in 84 patients with primary and secondary liver tumors. The samples were analyzed by flow cytometry. Systemic immune activation was assessed by measuring the urinary neopterin/creatinine ratio. Urinary neopterin was significantly higher in patients with liver tumors compared with controls (182 SD147 vs. 107 SD 37 μmol/mol creatinine, t-test, $P < 0.001$). The patients with liver tumors had significantly lower relative (40 SD 10 vs. 45 SD 9 %, $P < 0.05$) and absolute (659 SD 386 vs. 906 SD 360 cells per μl, $P < 0.025$) numbers of CD3+CD4+, relative (9 SD 5 vs. 12 SD 4 %, $P < 0.05$) and absolute (154 SD 115 vs. 221 SD 83 cells per μl, $P < 0.01$) numbers of CD8+CD28+, absolute numbers of CD3+, relative and absolute numbers of CD19+. Percentages and absolute numbers of CD3+DR+, relative and absolute numbers of CD3+CD69+, relative and absolute numbers of CD14+CD16+, and absolute numbers of CD14+DR+ were significantly elevated in patients compared with controls. The relative and absolute numbers of CD3+CD8+, NK cells, CD3+CD25+, CD8+CD57+ and CD19+CD23+ were not significantly different between the patients and controls. The phenotype was similar in 55 patients exposed to chemotherapy compared with 29 untreated patients. Negative correlations were observed between urinary neopterin, and the absolute numbers of CD3+CD4+ (Spearman rank correlation coefficient, $rs = -0.53$, $P < 0.01$) and CD19+ ($rs = -0.45$, $P < 0.05$) in untreated patients. Marked alterations found in PBL subsets of patients with liver cancer which may limit the availability of potential effectors for adoptive immunotherapy. A marked decrease in CD3+ CD4+ cells is associated with systemic immune activation.

Supported by Grant: IGA MZ No. 4676-3.

Analysis of genetically unmodified neural precursor cells

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The project was aimed at yielding a neural tissue of an optimum composition (via cultivation of neural precursor cells - NPCs) that would meet the requirements of specific neural transplantation strategies. Native NPCs were isolated from brains of fetal rats and mice with the use of EGF and/or FGF-2. Two different approaches were utilized to cultivate NPCs: 1) In vitro neurosphere assay which generates multicellular spheroids consisting of clonally related EGF- or FGF-2-derived cells 2) Monolayer of FGF-2-responsible neuroepithelial cells grown on laminin-PORN surfaces. Cells were grown in primary cultures or in subcultures. The progeny produced by NPCs in each type of culture was subjected to population analysis on the basis of its morphological and immunocytochemical examination. Received data indicated that the progeny of NSCs spontaneously tended to differentiate into neuronal and glial cells. This potential of NPCs was preserved after repeated passages that suggested the cultures contained multipotential neural stem/progenitor cells. Therefore cultures contained a heterogeneous population of cells (immature versus mature cells plus dividing, resting and dying cells). A shift between types of culture (neurosphere assay into monolayer and vice versa) did not alter the potential of cultivated NPCs. Moreover, the procedure enabled us to passage NPCs for prolonged periods of time. When the native NPCs isolated and propagated in vitro were transplanted into brains of adult animals grafted NPCs survived and differentiated into neuronal and glial cells as documented by loss of nestin immunoreactivity and by expression of a variety of neuronal and glial cell specific markers. Our results gave evidence that the cultivation of NPCs provided transplantable cells capable of differentiation after neural grafting.

Supported by Grant: GA ČR No. 304/97/1117.

Innovation of practical classes in human histology

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Histology has never been such an important part of the medical curriculum as it is today. It encompasses much of cellular and molecular biology, many aspects of physiology and biochemistry as well as traditional descriptive microscopic anatomy. Cells are now classified according to their function. The techniques of electron microscopy, cytochemistry, and immunohistochemistry are routinely used to study the histology of specialized cells, tissues, and organs serving as the foundation on which pathology and pathophysiology are built. Using these techniques fine needle biopsy increasingly participates in clinical diagnosis. Modern lectures built up on tri-dimensional ultrastructural reconstructions of cells and tissues accompanied by aspects of cellular and subcellular physiology have been developed at our department since 1984. However, in the practical classes, which we now feel are more important, this interdisciplinary approach has lagged behind. The purpose of this project was: 1) To supply the students and staff with a concise microstructural color atlas of prescribed organs and systems as simultaneously revealed by classical histological techniques, histochemistry, and tri-dimensional electron microscopy. 2) Basic physiologic correlations evoking desired biological thinking have been given. 3) The students should usefully retain an atlas of combined pictures and their legends throughout the pre- and postgraduate study. Such a manual does not exist in this country. Examples of the photo-plates and the style of the text will be presented.

Supported by Grant: FRVŠ No. 1282.

Cataract surgery outcomes at the Department of Ophthalmology in Hradec Králové

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Aims: Describe and exactly evaluate the basic data of all cataract patients operated on at the Department of Ophthalmology in Hradec Králové during a period of 18 months. Methods: The data of all cataract surgeries performed between July 1st, 1997 and the December 31st, 1998 were systematically collected and evaluated in a new computer database system. The mentioned system has been prepared as an autonomous program in collaboration with Erilens Comp. for the data processing for the period between July 1st, 1997 and December 31st, 1999 and for use at other Czech Departments of Ophthalmology since January 1st, 2000. We stored information from cataract operations in detail (method of cataract extraction, type of used viscoelastics, type and dioptric power of implanted intraocular lens (IOL), complications during surgery, type of wound closure etc.) and information about the eye before and after surgery (visual acuity, intraocular pressure, retinal problems etc.). Results: Five surgeons performed 4,259 cataract surgeries (4,146 primary extractions and 113 secondary implantations) during the mentioned period (until June 30th, 1999 it was 6,528 cataract surgeries). 48.9% of them were performed as outpatient surgery. The method of parabulbar anesthesia in 49.5% and subtenonal in 46.7%, scleral incision in 75.5% and clear corneal 24.5% respectively, temporal incision in 17.2% was used by surgeons. A tunnel length of more than 5mm was used in 73.4%, 5mm in 15.3% and less than 5mm in 11.3%. Only 28.7% of wounds were closed with the suture. The anterior capsule was opened using CCC in 94.4%. The phacoemulsification method was used in 95.6%. Posterior capsule ruptures (PCR) were observed in 2.04% during the surgery. The PCR varied from 0.8% to 7.1% in the group of 5 surgeons. Only 10.4% of foldable lenses were implanted because poor economic situation of the insurance office. The most frequently implanted IOL was ORC455F from U.S.A. (840 pcs) and Erica P314A -Erilens Company from Czech Republic (698 pcs).

Supported by Grant: GA UK No. 47053.

Electronic compendium of laboratory medicine

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The compendium of laboratory medicine is prepared in electronic form to use the maximum of scientific news in all branches of laboratory medicine. There is a possibility to use the advantages of electronic form, especially hypertext and linkage of the chapters and term. The structure of the compendium is as follows:

1. Theoretical portion covering the definition of laboratory medicine, total quality management, quality improvement, pre-analytical phase of the investigation, analytical phase (methods and technologies), post-analytical phase of the investigation. The basic statistics, method validation and information are included. The separate chapters are dedicated to molecular biology in clinical applications and pharmacology and pharmacokinetics.
2. A special part is focused on analytes. Everyone of them is described in structural form, starting from the chemical definition and clinical description to the methods of analysis and analytical notes.
3. The clinical part of the compendium is composed of two basic aspects according to the organs and systems: (a) biochemical and pathobiochemical description of the organ (system), including methods from the field of clinical biochemistry for investigation, (b) the common diseases of the organ (system) with the biochemical methods for detection. All descriptions are linked to the appropriate chapters and word using hypertext.

Supported by Grant: IGA MZ No. 4692-3.

Cytokines and antibodies in the serum and supernatants of lymphpocyte cultures in children suffering from primary immunodeficiency

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The aim of this work is to improve the diagnosis and classification of primary immunodeficiency diseases in children according to the phenotype data (both clinical and

laboratory). Children with diagnosed primary immunodeficiency were examined and the laboratory examination was implemented to find out differences among healthy population as well as among the primary immunodeficiency syndromes.Total immunoglobulin level of all classes in serum as well as the concentration of specific antibodies and IgG subclasses were used. Cellular immunity was assessed by flow cytometry examination. ELISA was used for the evaluation of cytokine (IL10, IFNgamma, TNFalpha) levels. According to the literature data, the immunoglobulin levels were low to very low in the antibody and B cell deficiencies as well as in the T cell and combined disorders. Quite interesting results supporting the theory of T cell origin of the disorder in CVID (Common Variable Immunodeficiency) were obtained by measuring the IFNgamma and IL10 levels in the CVI patients. The methods for detection of the heterozygot carriers of CGD (Chronic Granulomatous Disease) by flow cytometry were introduced thanks to the support of the grant. The function of the immune system is to be assessed also by measuring the specific antibodies against defined antigens in the serum.

Supported by Grant: IGA MZ No. 4098-3.

Long term mortality and quality of life after intensive care for critical illness

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Quality of life (QOL) after intensive care and hospital discharge has been considered an important part of assessing the final clinical outcome in critically ill patients. Assessment of QOL should be one of the important factors affecting all decisions concerning medical treatment in the ICU setting. The aim of the ongoing study is monitoring quality of life after ICU discharge. Patients' data are collected from all four participating centers, MOs 36-Item Short-Form Health Survey SF-36 questionnaire (made by the Health Institute and International Resource Center, New England Medical Center Hospitals, Boston, Massachusetts, USA) describing basic aspects of patients daily life (functional capacity, physiological function, emotions, vitality, social relationship) has been applying by investigators to asses QOL in this study. At the present time 650 patients after they have been discharged from ICU were sent letters containing information regarding the study and questionnaire. The Response rate has been about 40% until now. Collected data are stored for that study, in a specially created database, together with other clinical and demographic data obtained for every patient (primary reason for admission, severity of illness score Acute Physiology and Chronic Health Evaluation II, severity of organ dysfunction score according to Goris, sepsis-related organ failure assessment SOFA, length of ICU stay, length of ventilatory support, mortality and ICU treatment cost). Collected data will be compared with the healthy Czech population. The relationship between survival, QOL and ICU treatment cost will be also evaluated. Collecting and recording data is ongoing, results will be presented and published after assembling all data.

Supported by Grant: IGA MZ No. 4530-3.

Complex use of human blood in Medical Biology and Genetics practical classes at the Charles University Faculty of Medicine in Hradec Králové

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Human blood is not only the most precious human tissue but also an important diagnostic and therapeutic material. Therefore, handling human blood is considered an integral part of the medical profession and is simultaneously the main reason why we feel that medical students should be taught to work with it from the very begining of their study. The aim of this interdisciplinary project was to prepare and introduce practical tasks using human blood in practical classes in Medical Biology and Genetics at the Charles University, Faculty of Medicine in Hradec Králové. During our project we have necessitated a change in schedule of students' vaccination against hepatitis B (obligatory for our students before their hospital training) which was transferred from the 2nd to the 1st year of study. The core of our innovation lays in the following practical tasks: cultivation of human blood cells in vitro, microscopic observation of osmotic phenomena in human blood erythrocytes, preparation of karyotype from human blood lymphocytes, X-chromatin observation in human blood smear, and isolation and electroforesis of DNA samples from human blood. The above mentioned tasks resulted in new handouts and new syllabi for the academic year 1999/2000. A new student textbook for practical classes will include these task as well. Students' opinion about this innovation will be evaluated by questionnaire. Our first experiencences will be presented in the form of a short lecture at the biological conference CELLS II in České Budějovice - 9/2000.

Supported by Grant: FRVŠ No. 1287/1999.

Testing of intestinal barrier permeability in rat

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The intestinal epithelium together with mucus, IgA and gut associated with lymphatic tissue provides a barrier against the systemic penetration of antigenic compounds from the gut lumen. An increased permeability has been found in various diseases such as celiac sprue, Crohn's disease and nonsteroidal anti-inflamatory drug-induced enteritis. Intestinal barrier damage can be induced also by an ischemic-reperfusion injury of the small bowel in patients after multiple trauma a burn injury, cardiovascular surgery, etc. The main two aims of our study were: a) to introduce a model for ischemic-reperfusion injury of the small intestine in rats; b) to develop a noninvasive method for the evaluation of intestinal permeability. Materials and methods: The experiments were performed on male albino Wistar rats with an initial body mass of 220-250 g. Intestinal ischemic-reperfusion injury was induced by an occlusion of the superior mesenteric artery (SMA) for a period of 15, 20 or 30 min. The lactulose-mannitol (LAMA) test was used to measure the intestinal permeability 24, 48, 72 hrs and 7 days after SMA occlusion. The results were compared with those obtained from laparotomized (LAP) rats and intact rats. In the second series of experiments rats were sacrificed in the above mentioned intervals after SMA occlusion and tissue samples (spleen, liver, and small intestine) were taken for histological evaluation. Results: 15 min SMA occlusion does not lead to a significant change in the intestinal permeability measured by the LAMA test 24 hrs, 72 hrs and 7 days after gut injury. Significant increase in LAMA test ($p<0.05$) was found 48 hrs after SMA occlusion when compared to both LAP and intact rats. These results led us to prolong the time of SMA occlusion. Increased DNA synthesis in the intestine 24 hrs after occlusion documents an induction of gut regeneration after ischemic-reperfusion injury.

Supported by Grant: FRVŠ No. 1524/1999.

Angiogenesis in breast carcinoma

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Angiogenesis gains increasing attention in recent years, as capillary vessels provide a supply of nutritional factors and also represent a gate for the lymphogenous and hematogenous metastatic spread of the tumor. The aim of the project is: (1) to study the number of capillaries in the tumor and its relationship to the metastatic potency and prognosis and (2) to analyze the differences in the quantity of the capillaries and in prognosis between the groups of tumors with and without previously performed aspiration biopsy. Thirty eight cases of breast carcinoma diagnosed at the Department of Pathology, University Hospital, Hradec Králové in the years 1996-98 have been examined up to now. Endothelial cells have been visualised immunohistochemically using antibodies against factor VIII (von Willebrand factor). Capillary vessels were quantified at 200x magnification in the areas of highest angiogenic activity (hot spots), usually at the periphery of the tumor. The highest microvessel counts were correlated with other factors (age, tumor size and grade, nodal status, expression of receptors, proliferative activity, p53 status). The differences between tumors with and without previous aspiration biopsy were analyzed. All patients were women aged 36 - 86 years (average 61, median 62) The size of tumors was 7-70 mm (average 24 mm). Seventeen cases have been previously examined by fine needle aspiration cytology, and twenty cases were node-positive. The maximum counts of microvessels varied from 44 to 156 (average 100) per microscopic field (area 0.59 mm^2). The counts were higher in node-positive tumors and in tumors without previous aspiration, but the difference did not reach statistical significance. The capillary vessel counts did not correlate with other parameters examined. Fine needle aspiration cytology does not seem to increase the number of intratumoral capillary vessels. Angiogenesis in breast carcinoma does not correlate with other prognostic factors.

Supported by Grant: IGA MZ No. 4559-3.

Preparation and storing of cells for extracorporeal liver support

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Acute liver failure (ALF) is a medical emergency, which despite improvements in modern intensive care still carries a substantial mortality rate. Effective temporary liver support would improve the chance of patient's survival under these circumstances by sustaining the patient until a donor organ for liver transplantation is available. Temporary liver support could provide the condition for spontaneous recovery of the damaged liver. Usually, two types of "bio-artificial" devices utilising hepatocytes (hepatoma immortalised cells or primary pig hepatocytes) are used in an effort to replace detoxifying, metabolic, synthetic and regulatory capabilities of the liver. The major issue facing ALF treatment using a bioartificial system is the source of top-quality viable hepatocytes and the ability to maintain their functional

capacity in time. The aim of our study was to introduce the isolation of pig hepatocytes and to evaluate its viability related to cryopreservation. Hepatocytes were isolated from female Landrace pigs (15 kg) by collagenase (SEVAC) perfusion of the liver. Hepatocytes were suspended at 37°C in Krebs-Henseleit buffer (pH 7.4). The cell viability was assessed by trypan blue exclusion and was always greater than 85 % immediately after isolation. One part of hepatocytes was cryopreserved in DMSO medium and the viability of defreezed hepatocytes was assessed 1 day, 1 month or 2 months after isolation. The other part of cells was cultivated. We found a significant decrease in viability of hepatocytes 1 day after isolation (56 %); a similar decrease in viability was observed also in cells cryopreserved for 1 resp. 2 months. Our results are in concordance with literature data, which document that viability of cells in suspension depends on the cryopreservation process rather than the duration of cryopreservation.

Supported by Grant: FRVŠ No. 1525/1999.

Fluorescence analysis of antioxidant vitamins and neopterin in nonagenarians

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The aim of the present report was to study the relationship between some of the biochemical markers of oxidative balance and mortality in the aged population and to evaluate the role of such biochemical parameters in predicting mortality and morbidity. In a prospective follow up of a group of nonagenarians, biochemical parameters as well as antioxidants, indicators of lipid peroxidation, and neopterin an indicator of immune activation, were studied. Thirty-eight nonagenarians, 29 women and 9 men, aged 92±2 (range 90 - 100) years, followed at the Department of Metabolic Care and Gerontology, Charles University Teaching Hospital entered the study. At the start of the study, samples of peripheral blood and urine were obtained for analysis of the biological and biochemical parameters. The samples of urine and blood were then obtained in 6 - 12 months intervals. The significance of difference between surviving subjects and those who died was examined by the Mann-Whitney U test. Correlation between the variables was studied by the Spearman rank correlation coefficient. The decision on significance was based on P = 0.05 level. Serum vitamin E was significantly higher, and serum urinary neopterin were significantly lower in survivors compared to the subjects who died. No. other parameters were significantly different in survivors and in persons who died. Higher vitamin E levels appear to be associated with survival in extremely old individuals while systemic immune activation, evidenced by increased neopterin levels, has an opposite association.

Supported by Grant: IGA MZ No. G/1-3.

Introducing UV spectrophotometry into practicals in biochemistry

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The aim of this project was to introduce simple methods based on UV spectrophotometry into biochemical practicals. Now we are able to offer our students practical experience with methods widely used in biochemical laboratories. For example, activities of many NAD(P)H-dependent enzymes of clinical importance are determined using UV spectrophotometry. We chose lactate dehydrogenase (LD) as a suitable model. This enzyme catalyzes a reversible hydrogenation of pyruvate to lactate and it is possible to demonstrate how our choice of both the substrate and the coenzyme can influence the direction of this reaction. Transminases (AST, ALT) were also introduced as an alternative model. We used commercial Bio-La Tests LD 105 UV, LD 50, AST UV, ALT UV (LACHEMA) for determination. We prepared four practical problems for students:

- to choose the most suitable wavelength (nm) within the corresponding spectral band for measurement,
- to find the time intervals in which the change of absorbance (delta A/min) is constant, e.g. equals approximately the initial rate of the enzyme reaction,
- to compare LD activity determination in the identical serum sample using two methods based on different principles - a direct measurement using UV spectrophotometry and an indirect method based on stoichiometric formation of a coloured product measurable by VIS spectrophotometry,
- to determine the Michaelis constant for lactate dehydrogenase.

The results of the Michaelis constant determination will be assessed and discussed in the following seminar. These practicals should contribute to better understanding of different topics of chemistry and biochemistry that are notoriously difficult for students.

Supported by Grant: FRVŠ No. 1280/99.

The continuation of longitudinal ERG study in diabetics

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In 80 diabetics (35 patients with normal fundus picture, 24 persons with simple diabetic retinopathy and 21 patients after the retinal panfundus photocoagulation) regular repeated ERG examinations were performed. The results were compared with the normative data obtained from 80 healthy persons using the standard ERG examination with DTL electrodes. Special attention was paid to the amplitudes of the oscillatory potentials. Their diminution indicated the shortening of observation periods to three months and the possibility of laser theraphy in the prevention of the appearence of proliferative diabetic changes. We mentioned the existence of Czech parameters of visual acuity estimation and provided the manufacturing of the testing charts. Finaly we analysed the changes of the oscillatory potentials in hypovitaminosis A. In the next year the ERG study will continue and besides the oscillatory potentials the photopic ERG activity in diabetic patients will be studied. References: Peregrin J, Svěrák J. Electroretinography using DTL electrodes: normative data (In Czech). Lék Zpr LF UK Hradec Králové 1999;44:161-6. Svěrák J, Jebavá R, Hejcmanová D, Peregrin J, Sobotka L. Electrophysiology in hypovitaminosis A. (In Czech). Cs Oftal 1999;55:296-8.

Supported by Grant: IGA MZ No. 4564-3.

Liver perfusion changes after TIPS and their pathophysiological impact

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Transjugular intrahepatic portosystemic shunt (TIPS) is a relatively new type of portosystemic side-to-side shunt created by radiological means. This dramatically broadens the indication range of the shunt and requires new approval of indication criteria. The dramatic change of the liver circulation brings about some typical complications of the shunt - portosystemic encephalopathy and progressive liver failure, the risk of which should be reduced by more accurate indication criteria.Recently studied problem is glucose handling, strongly impaired in cirrhosis. Insulin resistance is usually elevated in a wide range up to overt diabetes, and this condition worsens already not very good prognosis of the patient. From our experience, it worsens also the outcome after TIPS. At our hospital, at the 1st Dept. of Medicine, the study of insulin resistance in cirrhotic patients and its development after TIPS has already started. The preliminary results appear quite heterogeneous and require finding the key influencing factors. At our physiological department we suggest that the dramatic change in liver perfusion after the shunt-creation could be one of them. A slight modification of the method of scintigraphic evaluation of hepatic blood flow using perfusion scan with 99m-Tc, published by University of Muenster in 1997, offers a method to quantify the perfusion changes in the liver. Up to date, 14 patients undergoing TIPS for diverse reasons have been repeatedly investigated using euglycaemic hyperinsulinic clamps to measure insulin resistance and its development. Gained data, as mentioned above and presented at some national meetings, seems to be heterogeneous. Up to now, we have completed repeated scintigraphic measurement in 9 patients. These data are also heterogenous and still unsuitable for statistical analysis. We are prepared to proceed with this promising approach.

Supported by Grant: GA ČR No. 306/99PO14.

Clinical analysis of osseointegrated dental implants with regard to the surface of the fixtures

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One of the key factors on which the successfulness of a dental implant depends is the surface finish of its endosteal part, the fixture. Manufacturers most frequently opt for either a titanium surface, or a surface formed by a hydroxyapatite coating. Within the scope of the working hypothesis, and based on interim clinical results, it may be assumed that a titanium surface of the implant is more advantageous if implanting in a bone of high density is done. If the bone is of poor quality, or if the implant bed is not quite congruent, the alternative with the hydroxyapatite surface should be given preference. We introduced 650 Impladent implants with various fixture surfaces (machined titanium, sanded titanium, hydroxyapatite coated). We monitored particular clinical parameters providing evidence of the overall successfulness of the implants (successfulness of the healing stage, resorbtion of the marginal bone, condition of the marginal mucosa, Periotest values, clinical complications, etc.). Statistical evaluation of the data obtained is a subject of the last study year.

Supported by Grant: IGA MZ No. 4789-3/98.

Observations of the nutrition of infants within the first 6 months of life

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This project has been planned to last two years and prepared in cooperation with co-workers from 6 medical faculties in the Czech Rep. Dr. Schneiderová from the 3rd Medical Faculty of Charles University acts as coordinator of the whole project. The aim of this multicentric study was targeted on studying the nutrition of infants during the first 6 months of their life, as well as the factors that affect the duration of breast feeding, mainly the support by health

workers at various levels. The goal of the first stage was represented by mapping the state of readiness for breast feeding, the approach to breast feeding in maternity hospitals and the nutrition level of infants when discharged from the hospital. Total number of mothers addressed reached 1,104. 1,014 of these respondents entered also the second stage of investigation 6 months later. Results obtained are quite optimistic but still show some reserves, which could be exploited for an increased support of breast feeding in the Czech Republic. From new-borns in our set 93.5% were fully breast fed when discharged from the hospital. By the end of 6th month of age only 25.8% of babies were fully breast fed and 30.4% were breast fed with additional food. So altogether 56.2% of babies were breast fed.These data reflect the effect of activities supporting breast feeding, especially when compared with numbers from early nineties when only 15% babies were breast fed by the end of 6 months of age. When discussing factors that positively influence the length of the breast feeding there should be named especially the social status (married women), positive approach to breast feeding in family, early decission to breast feed (as soon as prior to pregnancy or in its first days). Also the activities of maternity hospitals have an important effect.

Supported by Grant: MZ ČR No. NPZ 203/98.

Influence of passive smoking on child morbidity

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The project "Influence of passive smoking on child morbidity" was planned to last two years. The first year of the study was dedicated to forming a set of respondents from l0 - 14 years of age and assessment of the subjective evaluation of child morbidity. This was done on basis of parents' answers in the questionnaires. Objectivity of child morbidity was done by checking the health documentation of co-operating pediatricians. Inflammations of both the upper and lower respiratory tract, their complications (sinusitis, otitis) and the antibiotic or sulfonamides treatment were studied. The study included 711 children, 365 boys and 346 girls. Due to a higher percentage of brothers and sisters in our set (65% of families had one child only, 35% two and 1% three children) some results were related to the number of families (521). Smoking history showed that 37% of children came from the smoking families. In 15% of them mothers were smokers (8% regular and 6% occasional ones), in 28% of the families fathers were smokers (22% regular and 6% occasional). Nevertheless, only 4% of smoking parents admitted their smoking in rooms where the children were present. This fact may stand for the explanation that no difference in respiratory disease occurence between the smoking and non-smoking families was found. In some cases we have even proven an increased respiratory diseases morbidity in children from non-smoking families. We suppose that smoking history itself can explain only partially the problems of respiratory disease morbidity. It seems to us that other factors, especially ways of heating the flats and the ages of children, will be found to play even a more important role.

Supported by Grant: GA UK No. 281/98 C.

Human brain project

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The Human Brain Project is a US Federal research project joined by many American laboratories. It is sponsored by 16 organizations, e.g. the National Institute of Mental Health, the National Institute on Drug Abuse and the National Aeronautics and Space Admininistration. In its first phase, the project supports research on advanced technologies and novel ways to acquire, store, retrieve, analyze, visualize, synthesize, disseminate and share data about the brain. The goal of the team in this project is to study the structural basis of synapses and their major targets, dendritic spines. Providing three-dimensional reconstructions from a series of electron micrographs are one of the methods that can produce accurate results in this field. The Laboratory of Synapse Structure and Function will make the software and three-dimensional data available for other laboratories via an Internet website. My personal role in the project is to prepare high-quality three-dimensional reconstructions of many different types of presynaptic, synaptic and postsynaptic structures of the brains of animals as well as from pathological and post-mortem human brains. The Laboratory of Synapse Structure and Function provided me with software upgrades and prints from serial electron micrographs. The reconstructions made from these materials are part of a database which will be or already is presented in the Internet website "SYNAPSE WEB". References: http://synapses.bu.edu/

Supported by Grant: NIMH, NIDA, NASA (USA) No. R01- 57351.

The phenotype of tumor infiltrating lymphocytes in malignant ascites

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Ovarian cancer is the leading cause of death among gynecologic cancers. Despite the improvement in survival associated with the introduction of platinum compounds and paclitaxel, many women will ultimately die of the disease prompting the search for new treatment options, including immunotherapy. Advanced ovarian cancer is often associated with the presence of ascites. Malignant ascites offers the unique possibility to obtain, with minimal manipulations, a large number of cells from the tumor microenvironment for analysis or for therapeutic use in adoptive immunotherapy. We have examined by two-color flow cytometry the phenotype of tumor infiltrating lymphocytes (TIL) in malignant ascites of 18 patients with ovarian cancer. Most of the TIL (74 SD 9 %) were CD3+. CD19+ cells represented 7 (SD 5 %) of TIL. The majority of CD3+ cells expressed CD4 antigen (44 SD 14 %), while the number of CD3+CD8+ cells was smaller (28 SD 8 %). Only about half of CD8+ cells expressed CD28 molecule (15 SD 5 %), and 2 (SD 3) % of TIL were CD8+CD57+. Most of the CD3+ cells expressed the RO phenotype (53 SD 11%) while only 13 (SD 9) % had the RA phenotype. The expression of activation markers CD25 and HLA-DR on CD3+ cells was 5 (SD 2) % and 4 (SD 4) %, respectively. CD95 was expressed on 27 (SD 13) % of TIL. NK cells represented 11(SD 7) % of TIL. The expression of CD80 on TIL was below 1% while the expression of CD86 was 3 (SD 2) % and of CD152 2 (SD 1) %. A substantial number (1.7 SD 1.7 %) of all cells in the ascites exhibited dendritic cell phenotype. In conclusion, present data demonstrate that most of TIL are CD3+ and have memory cell phenotype, while only a minority of TIL express activation markers or naive cell phenotype. Most of CD3+ cells are CD4+, and only about half of CD8+ TIL express CD28 receptor. The expression of costimulatory molecules is marginal.

Supported by Grant: IGA MZ No. 5196-3.

Long-term assessment of the quality of care of patients treated for acute myocardial infarction

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Aim. The aim of our study was to evaluate the quality of treatment of 848 patients that were admitted with acute myocardial infarction (AIM) to seven hospitals in the Eastern region of the Czech Republic during the last 16 months. Methods. The following parameters were evaluated: (1) door-needle time; (2) the number of attempts to open a culprit coronary artery; (3) drugs used during treatment of AMI; (4) treatment of complications of AMI. Results. (1) On average, door-needle time was 19 minutes (median was 10 minutes). (2) An attempt to open culprit vessels was performed in 53% of patients with Q-AMI. Fibrinolysis was also performed on 3-31% of patients who were qualified as having a non-Q AMI at discharge. (3) Only 16% of patients (range 1-98%) with AIM were treated with beta-blocker shortly after admission and only 56% of patients (range 47-89%) had beta-blocker at discharge. (4) Treatment of cardiogenic shock was controlled according to values of mean pulmonary arterial wedge pressure in 8% of patients, and only 63% of patients with cardiogenic shock had central venous catheters. Conclusion. Evaluation showed that the treatment of patients with AMI was in most instances in accord with national guide lines. Recognising the therapy, which did not follow the accepted recommendations, is believed to be a valuable result of the study. It shows directly, where can our work be improved. It seems it would be useful to create a national register of patients with AMI and to evaluate it regularly.

Supported by Grant: IGA MZ No. 4544-2.

Ex vivo expansion and maturation of ac133+ selected autologous peripheral blood progenitor cells in breast cancer patients

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Hematopoietic progenitor cells in peripheral blood are used increasingly to restore the formation of blood after high-dose chemotherapy for solid tumors or hematologic cancers. Peripheral blood progenitor cells (PBPC) have been used with increasing frequency for autografting following high-dose chemotherapy. High-dose chemotherapy with hematopoietic rescue appears promising in the treatment of breast cancer. As compared with rescue by autologous bone marrow transplantation, restoration with such cells shortens the period of pancytopenia and reduces the risks of infection and bleeding. To minimalize the contamination of collections of peripheral blood progenitor cells by tumor cells we developed a method of growing progenitor cells ex vivo from a relatively small volume of blood. Over the last few years certain techniques have become available that allow the extensive proliferation of hematopoietic progenitor cells in ex vivo culture systems. The most commonly used method involves a simple liquid suspension culture system supplemented with a range of cytokines. Large increases in total cell numbers and committed progenitor cells can be readily obtained and, with some techniques, significant expansion of primitive hematopoietic cells has been demonstrated. We studied the effect of stem cell factor (SCF), interleukin 3 (IL-3), and interleukin 11 (IL-11) on the proliferation of human CD34+/AC133+ progenitors isolated from leukapheresis products of chemotherapy plus the effects of granulocyte-colony-stimulating factor (G-CSF)- on mobilised patients.

Supported by Grant: IGA MZ No. 4569-3.

Antioxidative defence system in dyslipidemic patients

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Analytical methods used in our work in 1998 were used for examination of the data obtained from patients. We determined the concentration of serum vitamins (A, E, C), serum lipid concentration (cholesterol + triglycerides), serum lipid peroxidations parameters and lipoprotein fractions of vitamins A and E, and lipid concentration in a group of 60 dyslipidemic patients. Our results show good correlation between vitamin E and lipid concentration (cholesterol + triglycerides) in VLDL (r = 0.93) and LDL (r = 0.92) fractions. The positive effect of vitamin E supplementation in dyslipidemic patients is apparent. Vitamin A is present only in HDL lipoproteins fraction. Determination of ascorbic acid (vitamin C) is carried out by capillary electrophoresis (CE). It seems that the ascorbic and dehydroascorbic acid ratio in biological fluids is completely unstable. We recommended a new modification of sample treatment before ascorbic acid CE analysis. Possible effect of apolipoprotein E genotype as an independent risk factor of atherosclerosis, on efficiency of hypolipidemic therapy, was observed in sixty hyperlipidemic patients. Genotypes of apo E were determined by PCR/RFLP method. New method for nitrite and peroxinitrite determination in biological fluids by CE is being validated for the next year. All particular results of studies supported by this grant were given as presentations. (IFCC WorldLab Congress, 4th Czech National Congress of Clinical Chemistry).

Supported by Grant: IGA MZ No. 4540-3.

Diagnostic accuracy of colour coded duplex ultrasonography in patients with peripheral arterial occlusive disease

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Aim. In patients with peripheral arterial occlusive disease (PAOD) severity, localisation and length of arterial affection influence principally the method of treatment. In this study the authors determined the accuracy of information about the peripheral arteries' obliterative character gained by colour duplex ultrasound (CDU). Angiography (AG) was choosen as a comparative method. Materials and methods. Patients with claudications and critical limb ischaemia were included in the study. Arterial system was visualised from subrenal aorta to the level of the ankle. The stenoses were quantified by peak systolic velocity ratio. Absence of the signal in colour as well as spectral Doppler record is the main diagnostic criterion of obliteration. Obliterations in the femoropopliteal segments were divided into short ones (shorter than 10 cm) and long ones (longer than 10 cm). Results. 130 patients (166 lower extremities and 1121 arterial segments) were examined ultrasonographically and compared with angiography. 286 pathological affections were found. Total accuracy of CDU for prediction of stenosis greater than 50% and obliteration was 95%, sensitivity was 90% and specificity was 97%. Conclusion. CDU is an accurate non-invasive method for determination of haemodynamically significant affections of the arterial system in the aortoiliac, femoropopliteal as well as crural area. CDU shows high accuracy in determination of localisation, character and length of occlusion or stenosis. High agreement between CDU and AG enables to choose patients suitable for PTA on the basis of CDU results, particularly when the overall clinical condition does not enable surgery and diagnostic AG cannot influence planning of further treatment.

Supported by Grant: IGA MZ No. NA/4560-3.

Metabolic and regulatory effect of lipids in artificial nutrition

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The primary goal of this project is to contribute to the optimization of lipid components in artificial nutrition. In this study were particularly emphasized : 1/ Importance of cholesterol and intermediates of cholesterol synthesis in metabolic regulations, 2/ Development of the new and highly sophisticated methods (GCMS) in lipid analysis with special respect to cholesterol synthesis, and to the analysis of cholesterol synthesis intermediates (determination of isoprene, mevalonic acid in plasma and in urine, determination and metabolic role of farnesol, squalene and lathosterol), 3/ Metabolic effect of polyunsaturated fatty acids (n-3 and n-6 family), changes of polyenic fatty acids in the plasma lipoprotein fractions and cellular (erythrocyte) membranes, 4/ Effect of plasma and cell membrane changes in the spectrum of free fatty acids on the lipoperoxidation production of oxygen free radicals (TBARS, dienes, malonyldialdehyd) and antioxidant consumption (alfa-tocopherol, betacaroten). Results of this study are important for rationale application of artificial lipid nutrition and for development of the new disease specific lipid emulsions destined for the treatment of critical patients and severely malnourished cancer patients. References: Zadák Z, Červinková Z. PUFA n-3 lipid emulsion - a promising agent in ARDS treatment. Nutrition, 1997;13(3):232-3. Zadák Z, Bláha V, Kozubík A, Hofmanová J, Žďánský P. New substrates in artificial nutrition - lipids. Progress in Clinical Pharmacy. Proceedings of the 24th European symposium on Clinical Pharmacy, Prague, Czech Republic, 10-13 October 1995.

Supported by Grant: IGA MZ No. IZ/4095-3.

Computer-assisted learning package for medical biophysics

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The aim of the project was to develop a package of computer-assisted teaching materials for a course of medical biophysics. Materials are based on a hypertext structure and they can be used during lectures, for preparing lectures, or for self study. From user's point of view,the basis of this software is a special user interface which is in a form of an electronic textbook. The text is divided into chapters that correspond to specific topics. To simplify work with this software, certain functions are included in the user interface. Those functions are: interactive table of contents, a function for looking up words in a text or keywords. Finally, the most frequently visited pages can be added to, or removed from a list of bookmarks. An important part of this software is a glossary, which can be, under certain conditions, continually updated by users. Documents are in the form of text, pictures, charts, animation, and simulations. The user can perform his own experiments and calculations by means of interactive equations, interactive charts, and simulations. It is possible to do frequency analyses of predefined data or of user's data stored in a text file. The identification of parameters of some pre-defined mathematical models for user's data can be provided. Some parts of materials are separate programs and files. These files can also be used as stand-alone files. The software is designed for operating systems Windows NT and Windows 98/95.

Supported by Grant: FRVŠ No. 1286/1999.

The significance of residual disease in hairy cell leukemia (HCL) for the development of relapse

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The purpose of the project was to determine the content of hairy cells (HCs) in bone marrow trephin biopsies in patients (pts) with HCL in hematological and/or complete remission (HR, CR) and to evaluate its significance for relapse. Methods and pts: Trephine biopsy specimens were fixed and decalcified for 24 hours and then embedded in paraffin. Immunohistochemical method using monoclonal antibody DBA.44 was used for visualization of HCs. DBA.44 + cells with characteristic morphology of HCs were considered leukemic. The quantitative analysis was made on precisely determined area. Three groups of pts were investigated and monitored. In the 1st group of 12 pts the monitoring started before 2-CdA administration. In the 2nd group of 16 pts in CR, monitoring has been started after the 2-CdA administration. Nine pts in HR treated previously with splenectomy or IFN represented the 3rd group. Results: Group 1. 12 pts were investigated 6 months (m), 7 pts 12 m and 2 pts 24 m after 2-CdA. The median of DBA.44+ HCs was 77. 5%(range 21 to 93%) before the therapy with 2-CdA. Ten pts were in CR 6 m after 2-CdA. Minimal residual disease (MRD) was detected in all pts in CR and the median of DBA.44+ cells was 4% (range 1 to 12%). The increase of HCs infiltration from 1 to 12% was observed in 1 pt 24 m after 2-CdA. Group 2. The pts were monitored from 3 to 69m (median 36m) after 2-CdA administration. All pts are in CR. MRD has been detected in 15 pts and the median of DBA.44+ HCs was 4% (range 0 to 18%). The infiltration with HCs inreased in one pt of this group. Group 3. HCs population was detected in all pts. The median of DBA.44+ HCs was 9 % (range 3 to 27%). Conclusion: MRD was detected in 27 of 28 investigated pts in CR. Increasing infiltration with HCs can precede the relapse. The pts are still in HR in spite of an important HCs infiltration.

Supported by Grant: IGA MZ No. IZ/4101-3.

Equipment for basic vital function monitoring in teaching process in physiology

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The follow up of cardiovascular and respiratory apparatus functions is an essential part of the practical exercises in physiology. Some new devices for improvement of the teaching process area were described. The most important new equipment for practical exercise in physiology is OSCARoxyTM. OSCAR is a combined airway gas monitor and pulse oximeter, which measures inspired and expired concentration of CO2, N20 and O2 (by infrared absorption techniques), saturation percentage of arterial hemoglobin (spO2) (paramagnetic oxygen sensor), respiratory and pulse rates. The information is displayed on the video display, both numerically and as continuous, real time wave-forms. The CADISCOPE represents a new generation of diagnostic tools. In one, easy to use device, medical students have the ability to hear heart sounds (stethoscope), while simultaneously visualising heart sounds (phonocardiogram), or the heart's electrical activity (the ECG) in the LCD display. Stethoscope has three operating modes with selective frequency bands: lungs, heart, entero with sensitive amplifiers. The next new device for students is the easy to use tonometer KLOCK for blood pressure and pulse rate measure-

ment from the wrist like a normal wrist watch. Stored measurements (50) can be downloaded to the PC software via infrared transmission and software allows the student to see changes of blood pressure in time. This software was installed into a special PC for medical students. Digital tonometer OMRON served for comparison with classical methods of blood pressure measurement. Cardiovascular and respiratory systems adaptation are demonstrated using a new bicycle ergometer device with an ear pulse rate oscillometric probe. The ergometer allows us to use programmes with various degrees of load. Our program of practical exercises for medical students is ready for use.

Supported by Grant: FRVŠ No. 1283/1999.

Ultrasonographic criteria determinating transjugular intrahepatic portosystemic shunt malfunction

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Purpose: To evaluate the efficacy of Doppler ultrasonography (US) in the long-term follow-up of patients treated with transjugular intrahepatic portosystemic shunts (TIPS). Materials and methods: We performed a retrospective review of 1192 Doppler examinations of TIPS carried out at our institution between 1994 and 1999. No. regular shunt venograms were performed. Sonographic parameters assessed included shunt velocities together with diameter, velocity, flow volume, and congestion index of the main portal vein (MPV). To the best of our knowledge, the congestion index of the MPV was evaluated for the first time in a large group of patients with TIPS. Results: The sensitivity of Doppler US for detection of shunt occlusion was 96% and for shunt stenosis 94%. We encountered 4 false positive stenoses on Doppler US (positive predictive value 96%). Within the course of the study, Doppler US missed a significant shunt stenosis leading to an episode of gastrointestinal bleeding or ascites recurrence only in seven cases. Conclusion: Doppler US is an effective primary imaging method for the follow-up of patients with TIPS. Invasive shunt venography should be reserved only for therapeutic purposes.

Supported by Grant: IGA MZ No. 4109-3.

REVIEW ARTICLE

APOPTOSIS AND CELL DEATH (MECHANISMS, PHARMACOLOGY AND PROMISE FOR THE FUTURE)

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Summary: Rapidly growing body of evidence on cell death mechanisms and its disorders during last five years has replaced old paradigms and opened new horizons in medicine. Identification of different morphological and signaling aspects, as well as variances in requirement for energy enabled us to construct a theory of three main types of cell death: necrosis, apoptosis, and lysosomal cell death. Mitochondria, certain oncoproteins such as Bcl-2 family, and special catabolic enzymes participating in cellular demise might serve as targets for pharmacological manipulation. Upregulation or downregulation of programmed cell death has been implicated in ischemic, neurodegenerative, and autoimmune disorders, as well as in oncology and chronic inflammation. This minireview brings a short overview of genesis and development of theories on programmed cell death and apoptosis, summarizes basic relevant facts on apoptotic mechanisms and draws a new hypothesis on possible implication in medicine and surgery.

Key words: *Cell death; Apoptosis; Oncoprotein; Mitochondria; Caspase; Calcium*

Introduction

Loss of cellular populations is a key limiting factor in many medically and socially high-impact diseases. Refinement of scientific technology in recent decades made detection of various forms of physiological cell death possible. Physiological cell death is very distinct from necrosis. It occurs in two distinct models, and perhaps a whole spectrum between these two. Classic apoptosis, in which most of the early morphological changes occur in the nucleus, and a lysosomal or cytoplasmic cell death, in which the early alterations take place in the cytoplasm (6,46).

Apoptosis, a morphologically defined cellular death, is implicated in removal of cells during ontogenesis, physiological cellular turnover in adults, cytokine-induced tumor involution, clonal selection of T-lymphocytes, gerontopathology, neurodegenerative disorders, immunopathologic states, and even myocardial or cerebral ischemia (6,7,22,70). However, a loss of cell's susceptibility to inducers of physiological cell death is likely an important component in development of malignancy and maintenance of drug resistance (4,68,69). Therefore, after years of academic ignorance, processes of programmed cell death have become a hot issue in scientific world.

Pathways of Cellular Demise

The term 'programmed cell death' appeared in 1960s in works on cell elimination in metamorphosing insect (30,31, 50). This refers to a genetically invoked form of death (6). Morphologically it may assume a picture of either apoptosis or lysosomal cell death. Although morphological descriptions consistent with apoptosis have been present in literature since the 19th century (32), it has not been sooner than in 1970s, when Kerr and associates (22) unequivocally defined its histological appearance. Apoptosis serves as a one of three models of cell death during ontogenesis and its microscopical appearance is less marked than that of necrosis (6). It includes distinctive chromatin condensation, formation of cytoplasmic „finger-like" projections, which detach from the cell body and form apoptotic bodies, and heterophagocytosis of cellular remnants by surrounding tissue. Absence of inflammation, cellular edema and significant subcellular damage is characteristic, but is observed in necrosis. Histological appearance tends to be conserved with respect to type of cell and damage (65). Prompt phagocytosis as well as the absence of inflammatory reaction persuades pathologist to greatly underestimate the contribution of apoptosis in cell removal from population (6,7).

Cytoplasmic cell death may be seen in cells possessing large cytoplasm and is heralded by early and vast changes in cytoplasm. These involve increase in a number of lysosomes along with their redistribution, development of large autophagic vacuoles, and specific sequence of organelle removal. Very late in this process nuclear alterations similar to apoptosis take place (46). Energy resources probably remain generous until the very late stage.

Tab. 1: Principal differences between necrosis and apoptosis.

	Necrosis	Apoptosis
Histopathology	Edema Damage to organelles Membrane discontinuity	Cellular shrinkage Chromatin condensation Formation of apoptotic bodies Membrane continuity preserved
DNA cleavage	Random, diffuse	Internucleosomal cleavage
Reaction of surrounding tissue	Inflammation	Phagocytosis, no inflammation

Necrosis is opposed to physiological cell death (Tab. 1). It represents a morphological counterpart of energy resources loss, membrane penetrations, ruined control of ion flow and osmolysis resulting in uncontrolled loss of cellular content (14,61,62).

Using classical staining procedures it is almost impossible to quantify apoptosis due to a very short lifetime of morphologically evident apoptosis, which is believed to be at the level of tens of minutes. Classical microscopic analysis was the first method used to define apoptosis, and it should be kept in mind, that none of modern methods has replaced it. However, a myriad of new methods suitable for detection of certain apoptotic features has emerged, among them annexin V immunohistochemistry (66), multiparameter flow cytometry (11), TUNEL (TdT-mediated dUTP-biotin nick end labeling) staining, and diphenylamine assay of DNA fragmentation. Apoptosis can be in most instances identified by characteristic breakdown of DNA into oligonucleosomal fragments, which give so called laddering appearance on electrophoresis (2). Pioneer observation of this feature should be probably granted to Czech researchers (55). Chemical asymmetry of plasma membrane is a characteristic feature of normal cells. However, early during apoptosis cells export phosphaditylserine residues normally confined to the inner leaflet of the plasma membrane to the outer leaflet, thus flagging apoptotic cell to phagocytes (35). This feature has been recently employed to identify apoptotic cells using immunolabeling techniques against annexin V, cell membrane confined phospholipid binding protein with a high affinity for phosphatidylserine (66).

Morphological manifestation of apoptosis is linked to its terminal stage. Only these latter stages of the whole process are heralded by cell rounding, cytoplasm blebbing, and nuclear condensation and fragmentation. Acquisition of typical apoptotic morphology is dependent on caspase-mediated and energy-dependent rearrangements of cytoskeleton.

Nuclear pyknosis and karyorrhexis are near-to-definite morphological features of apoptosis. Factors responsible for chromatin condensation and pyknosis include DNases, Acinus and AIF (Apoptosis Inducing Factor). Caspase-activated DNase (CAD) is a cytosolic protein inactivated by heterodimerization with its inhibitor ICAD. This heterodimer splits by action of caspase-3 on ICAD and CAD translocates into the nucleus, where it exerts typical internucleosomal chromatin cleavage (12). Acinus (apoptotic chromatin condensation inducer in the nucleus) is newly described chromatin-condensation factor involved in apoptosis. For full activation it requires double caspase cleavage and features an unique peculiarity as it exerts its chromatin-condensing action without any detectable DNase activity (49). Both Acinus and CAD lead to histological appearance of karyorrhexis. Yet another factor, mitochondrial AIF, participates in nuclear changes. However, it produces large scale DNA fragmentation into pieces around 50 kb in length and gives a picture of peripheral chromatin condensation (58).

Caspases have been found to mediate cleavage of many cytoskeleton-associated proteins, among them Gas2 (3), gelsolin (25), and fodrin (35). Detachment of apoptotic cells from plate or from other tissue cells was found to be a consequence of calpain-mediated cleavage of cytoplasmic domain of integrin β3 subunit (40), which is required to maintain cellular adhesion and cytoskeletal association. On the other hand, studies employing microtubule-damaging drugs such vincristine suggest that microtubule damage is an important event in Bcl-2 inactivation via hyperphosphorylation and induction of apoptosis (57). This means that upstream intracellular mediators of apoptosis initiate cytoskeletal rearrangements, which in turn potenciate apoptotic cascade via inhibition of anti-apoptotic function of Bcl-2 oncoprotein. Moreover, initiation of apoptotic cascade at any point may cause self-amplification and inevitable cell death.

Interesting association between inhibition of apoptosis and a gain of metastatic capability was found in cells lacking expression of cytoskeleton-bound Death Associated Protein (DAP) kinase (20). Restoration of normal DAP kinase expression in high-metastatic tumor cells suppressed their metastatic ability. Links among suppression of apoptosis, cytoskeleton rearrangements, and neoplastic immortality and metastatic capability need further elucidation.

Susceptibility of cells to suicide varies significantly. Genetic control of apoptosis is mediated through several gene products. Some of them promote apoptosis (*p53*, *TNF*, *Fas/CD95*, *bax*, *bak* and *bad*), while the others (e.g. *bcl-2*, *bcl-*X_L) block apoptosis and promote cell survival (41, 71).

Subcellular Mechanisms of Apoptosis

Apoptosis seems to be an old and conserved reaction based on a self-scarifying anti-viral defense originally developed in primitive eukaryotes. Execution of this process involves inhibition of protein synthesis at the level of translation initiation, proteolysis specifically involving degradation of DNA repair mechanisms, and polynucleotide degradation. This complex molecular signaling system includes feedback mechanisms tending toward activation of all elements of the execution platform if only one element is initially engaged (67).

Because morphological alteration of nucleus was first observed in apoptotic elements, it was considered a coordinator of whole process. However, studies on enucleated cells have shown they can die with apoptosis as well (52). Although cellular suicide program is genetically encoded, its translation immediately before execution of apoptosis is not required. Apoptosis in certain cellular populations can be prevented with transcriptional and/or translational inhibitors (29,43,60). However, similar approach in different settings may provoke or accelerate apoptosis (5,10). The effector tool of apoptosis is a class of cysteine proteases localized in cytoplasm – caspases, also termed ICE-like enzymes (Interleukin-1β-Converting Enzyme) according to the first discovered member of this family. Two pathways of cell suicide exist. One triggered by signals created within the involved cell (unbalanced oxidative stress, calcium overload) and the other one initiated by signals generated from outside of the cell (TNF, Fas ligand, NO, glucocorticoids, actinomycin D). The intrinsic signals activate caspase-9, while extrinsic engages caspase-8. Both modes converge to sequential activation of other caspases, all process thus resembling limited proteolysis seen with blood clotting. Activated caspases digest structural proteins and degrade chromosomal DNA leading to death of the cell. Cell's decision to commit suicide is driven by death activators and is counterbalanced by the action of trophic factors, among them nerve growth factor, basic fibroblast growth factor, interleukin 2, insulin, and others.

Classical studies have been carried out in a worm *Caenorhabditis elegans* model, which features extensive removal of cell population during ontogenesis. Genes responsible for programmed cell death were identified as cell death genes (ced), and their corresponding proteins were termed CED. The genes ced-3 and ced-4 are essential for cell death; ced-9 antagonizes the activities of ced-3 and ced-4, thus resembling *bcl-2* family in mammals. CED-3 is a counterpart of mammalian caspases, while the function of CED-4 resembles that of mammalian Apaf-1 (Apoptosis Protease-Activating Factor 1). In mammalian cells, Bcl-2 protein drags caspases to mitochondrial membrane and prevents their activation, and blocks release of cytochrome C, which is a potent activator of pro-caspase-3.

Indeed, recent studies provided evidence on rate limiting behavior of mitochondria in early stages of apoptosis (16,41). These have given the experimental basis for three-step model of apoptosis (58):

1. Premitochondrial phase - activation of apoptotic signaling pathways, including so-called upstream caspases (Fig. 1),
2. Mitochondrial phase – loss of mitochondrial inner membrane potential accompanied by a release of proteins activating apoptotic effectors (Fig. 1,2),
3. Postmitochondrial phase – activation and action of apoptotic effectors (catabolic proteases – downstream caspases, nucleases) leading to microscopical appearance of apoptosis (Fig. 1).

Fig. 1: Sequence of main events after injurious stimulus leading to cellular death.

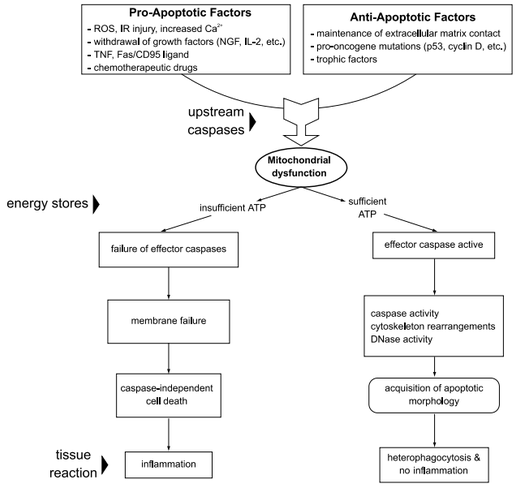
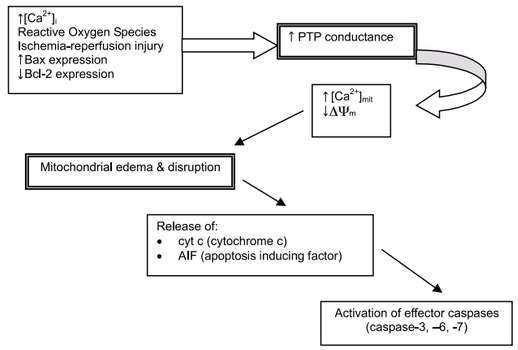


Fig. 2: Mitochondrial dysfunction and opening of permeability transition pore (PTP).



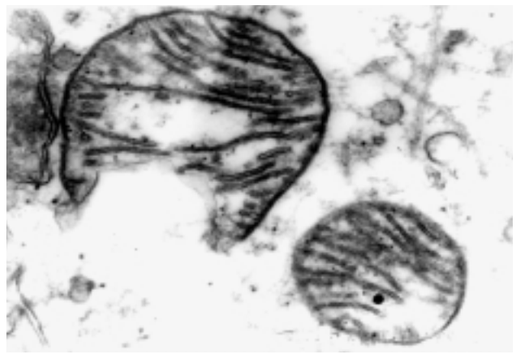
Data published by Zamzami and associates (31,72) document, that lowering of mitochondrial transmembrane potential $\Delta\Psi_m$, and subsequent intramitochondrial production of reactive oxygen species are triggers of apoptosis. Apoptosis has been observed in those cells only, in which depolarization of inner mitochondrial membrane occurred.

It has been reported that disturbances of mitochondrial function and integrity paralleled by disruption of intracellular calcium homeostasis preceded apoptotic changes of nucleus (37,42). Mitochondrial calcium overload initiates opening of a special mitochondrial megachannel and is prerequisite of all further steps of apoptosis (31,42,44).

Loss of electrochemical gradient on inner mitochondrial membrane is mediated by a sudden increase in its permeability. This permeability transition creates a shunt for protons, lowers protonmotive force $\Delta\Psi_m$, and results in a cessation of mitochondrial ATP synthesis. In all likelihood, permeability transition is caused by opening of special proteinaceous multiple conductance channel, or „megapore" or „megachannel". This nonselective channel is probably permeable to any atomic ion as well as water and forms at the junction of inner and outer mitochondrial membranes (45). Its opening gives rise to massive ion movement accompanied by water with resulting edema and rupture of outer mitochondrial membrane. Intermembrane proteins capable of inducing caspases (cytochrome c and apoptosis inducing factor AIF) are thus released to cytosol (16,56,74). Moreover, caspases induce liberation of intermembrane proteins from other mitochondria (36), hence engaging in self perpetuating cycle leading to coordination of proapoptotic behavior among all mitochondria in a given cell. For that reason permeability transition of inner mitochondrial membrane takes on a chain reaction profile and spreads as outbreak affecting entire mitochondrial population (53). Loss of mitochondrial potential $\Delta\Psi_m$, is a common trait of necrosis and apoptosis (72). End result of the mitochondrial dysfunction leads to biological catastrophe culminating in disintegration of plasmatic membrane (necrosis), or to activation of apoptotic proteases with subsequent activation of endonucleases and manifestation of apoptosis. Cell's decision on which morphological presentation will be preferred depends on intensity of initiating factor and energetic charge of the cell (27,28,39). Cells low in energy undergo uncoordinated process of necrosis, yet cells with sufficient energy stores experience apoptosis. Other explanation may be that cells mainly dependent on anaerobic glycolysis (leukocytes) undergo apoptosis, while cells reliant on aerobic glycolysis tend to suffer from necrosis. This is consistent with our findings of necrotic neuronal death in our model of transient seven-minute global cerebral ischemia in dogs, where we repeatedly failed to morphologically identify apoptosis (15,47). However, mitochondrial damage consistent with apoptosis has been observed (Fig. 3).

Either preventive or postinsult application of immunomodulant agent cyclosporine A or tacrolimus (FK-506) in settings of disrupted blood-brain barrier protects neurons against apoptosis induced by ischemia-reperfusion injury (18,58,72). These compounds inhibit formation of mitochondrial megapore (54), similarly to performance of intracellular calcium chelators (63,64) and natural inhibitors of apoptosis - some of protein products of *bcl-2* gene family. Employment of free radical scavengers can only retard terminal phase of apoptosis – reduction of cellular volume (73), but not cellular death itself.

Fig. 3: Electronogram of ruptured mitochondria after cerebral ischemia-reperfusion injury in canine neocortical neuron. Original magnification 32 000x.



Immunosuppressant actions of cyclosporine A, tacrolimus and rapamycine are mediated by the drug binding intracellular target - immunophillin. Drug-immunofillin complex binds to and inhibits the phosphatase calcineurin, thus resulting in modification of special proteosynthesis. Immunophillin ligands, including nonimmunosuppressants that do not inhibit calcineurin, stimulate regrowth of damaged peripheral and central neurons (48). Furthermore, tacrolimus inhibits the activity of nitric oxide synthase. Nitric oxide is capable of inducing apoptosis via direct opening of mitochondrial megapore (19).

Cell death activators, both apoptotic and necrotic, may be identical (54). Cell's fate is likely to be defined by the intensity and duration of exposure to initiating event. This is supported by reported succession of necrosis and apoptosis in glutamate-induced model of excitotoxic neuronal death. Early survival of necrotic phase was determined by recovery of mitochondrial energy-producing machinery, and neurons surviving the necrotic phase underwent apoptotic transformation (1).

Conclusion

Known associations of apoptosis controlling and cellular growth gene mutations include familial adenomatous polyposis (APC gene), hereditary malignant melanoma (regulators of cyclin-dependent kinases), Lynch syndromes (microsatelit DNA mutations) and others (8). Mutations in the p53 tumor suppressor gene are amongst the most frequent genetic abnormalities identified in human solid neoplasms. Besides malignancy, downregulation of apoptosis controlling mechanism is seen with inability to handle some forms of chronic inflammation (26,51), e.g. ulcerative colitis and rheumatic arthritis. Upregulation of apoptosis has been implicated in many autoimmune, neurodegenerative, and ischemic disorders (21,23,67).

Current immediate clinical applications of apoptosis-related research constitute estimation of anticancer chemotherapy effectiveness (33), survival prediction in acute leukemia (13), or reduction of allograft reperfusion injury after transplantation (24). Basic research has also recently questioned usage of lactated Ringer solution for acute shock therapy (9).

New knowledge of cellular death control is to be conceived by basic research. Thereafter, applied research should extend our pharmacological armamentarium with new approaches for therapy of ischemic disorders, malignant diseases, chronic inflammation and many others. Sufficient understanding of apoptosis and cell growth regulation yet requires more years of investigation. Nevertheless, new millenium may bring a significant breakthrough in treatment of many incurable and incapacitating diseases.

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ORIGINAL ARTICLE

DOSE DEPENDENT BIOLOGICAL EFFECTS OF IDARUBICIN IN HL-60 CELLS: ALTERATIONS OF THE CELL-CYCLE AND APOPTOSIS

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Summary: TP-53 deficient cells of human leukaemia HL-60 die by massive apoptosis after treatment by high (50-100 nmol/l) doses of DNA damaging agent Idarubicin, regardless of the cell-cycle phase, in which they are affected. In contrary, after relatively low dose 10 nmol/l the cells die after cell-cycle arrest in G_2 phase. The results show, that apoptosis induced by idarubicin could appear independently of the cell-cycle phase and that period in which apoptosis is observed is related to the dose of Idarubicin.

Key words: *HL-60; Idarubicin; Apoptosis; G_2 cell-cycle arrest*

Introduction

Acute myeloid leukaemia (AML) accounts for over 80% of all adult acute leukaemias (7) and is a characterised by a clonal expansion of immature myeloid cells in all haematopoietic tissues. Many patients progress to AML from preleukaemic myelodysplastic syndrome (MDS) or from chronic myelogenous leukaemia (CML). AMLs show varied morphologic, cytochemic, immunologic and cytogenetic characteristics and varied sensitivity to conventional chemotherapeutic regimes. Sixty percent to 70% of patients with de novo AML initially achieve complete remission. However, the majority of these patients relapse and eventually die of the disease. The first described and best characterized mechanism of resistance is mdr1 gene product, P-glycoprotein. This molecule spans the cell membrane and act as an efflux pump for toxins, including chemotherapy drugs such as anthracyclines, vinca alkaloids and topoisomerase II inhibitors. The biological bases of drug resistance and relapse in AML are not understood and prognoses are still largely based on descriptive parameters. Several lines of evidence indicated that apoptosis plays roles in responses of AML patients to chemotherapy. Aldrige and Radford (1) showed that differences between human haematopoietic cell lines, in the rate of induction of apoptosis after irradiation were generally related to the functioning of cell cycle checkpoints. Whereas the rapidly dying and radiosensitive HSB-2 cell line underwent apoptosis at different points in the cell cycle, the more slowly dying cell lines showed a variety of cell cycle arrest profiles and initiated apoptosis after accumulation of cells in the G_2 phase. HL-60 cells showed a markedly longer G_2 arrest that correlated with their greater radioresistance. The result suggest that the total length of time available for DNA damage repair (regard less of whether this time occures as arrest in G_1, S or G_2), prior to potential activation of apoptosis, is a critical determinant of radiosensitivity in human haematopoietic cell lines.

The mode of induction of apoptosis is dependent upon the cell type and the type and concentration of cytostatic drug used. Three different routes to the induction of apoptosis, presumably reflecting differences in nature of the initiating lesion, were identified for lymphoid and myeloid cell lines: 1. Rapid interphase apoptosis, where death occurred soon after death stimulus and in different phases of cell cycle. 2. Delayed interphase apoptosis, where death occurred following arrest in G_2. 3. Mitotic/delayed mitotic death, where death occurred after one or more cell division. (6).

To investigate whether the sensitivity of leukaemias to chemotherapeutic agents depends on the abilities of leukaemia cells to respond to therapeutic insult by initiating apoptosis is an important task. In our work idarubicin was chosen as it is an anthracycline which is extensively used in the treatment of leukaemia's (10) and because it is less affected by P-glycoprotein expression (which can be responsible for cytostatic resistance) than other anthracyclines (2). Idarubicin also has the advantage of being available for oral administration, which is often appropriate for the treatment of elderly patients with AML and MDS, as part of combination treatment regimens. Idarubicin is a DNA intercalating agent, which interacts with topoisomerase II and has an inhibitory effect on nucleic acid synthesis.

Variable response of malignant cells to cytostatic therapy could be explained first by proliferation status of these cells, second by the ability of the cytostatic to induce cell-cycle arrest in a specified cell-cycle phase and third by the ability to induce apoptosis. In our work we analysed cell cycle status of HL-60 line, duration and intensity of cell-cycle arrest and the ability of damage repair or apoptosis initiation after low-doses idarubicin treatment using flow-cytometric DNA analysis.

Materials and Methods

Cell culture and culture conditions

Human leukaemia HL-60 cells were obtained from the European Collection of Animal Cell Cultures (Porton Down, Salisbury, UK) and were cultured in Iscove's medium (Sigma Inc.) supplemented with 20% fetal calf serum (FCS) in a humidified incubator at 37°C and controlled 5% CO_2 atmosphere. The cultures were divided every 3rd day by dilution to a concentration of $2x10^5$ cells/ml. Cell count was performed with a haemocytometer, cell membrane integrity was determined using the Trypan blue exclusion technique. HL-60 cells in the maximal range of 20 passages were used for this study.

Cell treatments

Exponentially growing HL-60 cells were suspended at a concentration of $2x10^5$ cells/ml in complete medium. 10 ml of aliquots were plated into 25 cm^2 flasks (Nunc) and mixed with idarubicin (Zavedos, Pharmacia Upjohn S.p.A. Laboratories) at desired concentrations. After 4 hours idarubicin-containing medium was removed and replaced with fresh culture medium without idarubicin. Following 6, 24,48 and 72 hours the cells were counted and cell viability determined with the Trypan blue exclusion assay.

Cell morphology

For calculation of the percentage of cells showing morphology of apoptosis, aliquots were removed from control and drug-treated cell cultures at various times of incubation and usually 400 cells were counted on Diff-Quik (DADE BEHRING) stained cytospin preparations. Apoptotic cells were identified by the condensed and fragmented state of their nuclei and focal protrusions of the cell surface.

Cell surface markers and cell size analysis

Flow cytometry was used for cell surface antigen analysis and for apoptosis monitoring. Cells were washed twice with PBS containing 5% FCS. Then, $1x10^5$ cells were suspended in 0.5 ml PBS with 5% FCS and 0.02% NaN_3 and incubated with mAb APO2.7 for 30 min at 4°C.

For apoptosis detection the mouse phycoerythrin (PE)-conjugated mAb APO2.7 (clone 2.7 A6A3) (obtained from Immunotech) for detecting 7A6 antigen expressed by cells undergoing apoptosis has been used. We used this method without cell permeabilisation.

Flow cytometric analysis was performed on a Coulter Epics XL flow cytometr. A minimum of 10 000 cells was collected for each sample in a list mode file format. List mode data were analysed using Epics XL System II software colour eventing (Coulter Electronic, Hialeah, FL, USA).

Cell cycle analysis

Following 6, 24, 48 and 72 hours of incubation, the cells were washed with cold PBS, fixed by 70% ethanol and stained with propidium iodide (PI) in Vindelov's solution for 30 minutes at 37°C. Fluorescence (DNA content) was measured with Coulter Electronic, Hialeah, FL, USA apparatus. A minimum of 10 000 cells analysed in each sample served to determine the percentage of cells in each phase of cell cycle, using Multicycle AV software. Three independent experiments were performed.

Results

Cell growth and viability

Fig. 1 shows the effects of idarubicin on the proliferative rate of HL-60 cell line. Cultivation with 5 nmol/l idarubicin induced high inhibition of the rate of HL-60 cell growth. The decrease of the proliferative rate observed in HL-60 cells after addition of higher concentration of idarubicin was observed. After 48 hours all cells with 100 and 50 nmol/l idarubicin were dead.

Morphologic changes

HL-60 cells were incubated in the presence 5, 10, 20, 50 and 100 nmol/l idarubicin for 72 hours. After 1, 6, 24, 48 and 72 hours cell morphology was examined on Diff-Quik stained cytospin preparations. Morphologic evidence of apoptosis was found in cells treated with idarubicin. After addition of idarubicin dose dependent increase in the proportion of apoptotic cells was detected in cultures exposed to 5 -100 nmol/l idarubicin. The maximal percentage of apoptotic cells was observed in cultures incubated for 6 or 24 hours treated with 100 or 50 nmol/l idarubicin respectively. (Fig. 2). During in vitro studies, where apoptotic cells cannot be removed by fagocytosis, secondary necrosis can be observed in later intervals (24 to 72 hours in Fig.2).

Analysis of cell-cycle and sub-diploid DNA content

We assessed DNA cleavage in the afore mentioned 5 and 10nmol/l idarubicin-treated tumour cells. We have observed that after 6 hours of incubation most of the live idarubicin-treated cells were in S phase of cell-cycle (62% or 71%, respectively), after 24 hours most of them moved to G_2 phase (61% or 75%) and after 48 hours the percentage of cells in various cell-cycle phases was comparable to control untreated cells (Fig.3). Results of one representative experiment with idarubicin concentration 5 nmol/l are shown in Fig. 4.

Fig. 1: Kinetics of idarubicin effect on the proliferative rate of HL-60 cell line. HL-60 cells were exposed to various idarubicin concentrations (5-100 nmol/l) for 4 hours and then cultivated in idarubicin-free medium. Number of viable cells was determined by Trypan blue staining.

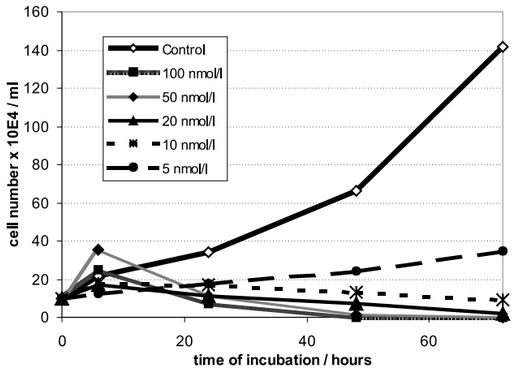


Fig. 2: Time course of apoptosis in HL-60 cells exposed to idarubicin as determined by cell morphology examined on Diff-Quik stained cytospin preparations. Data represent medium values from 3 independent experiments.

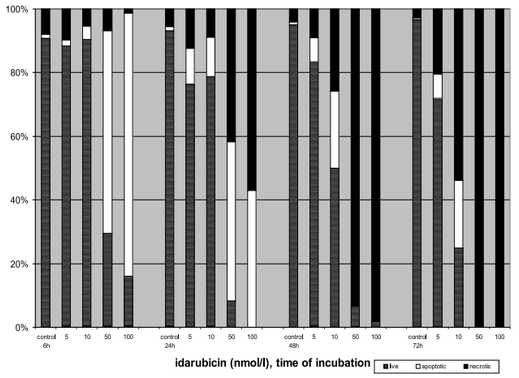


Fig. 3: Flow cytometric analysis of DNA content and cell-cycle after treatment with 5 and 10 nmol/l idarubicin.

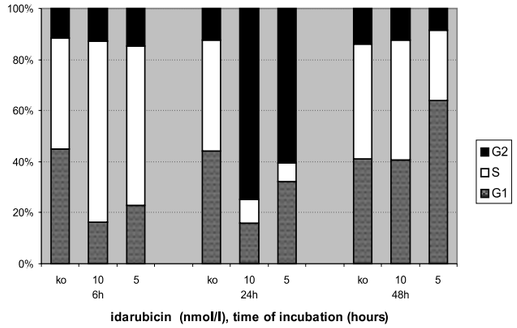
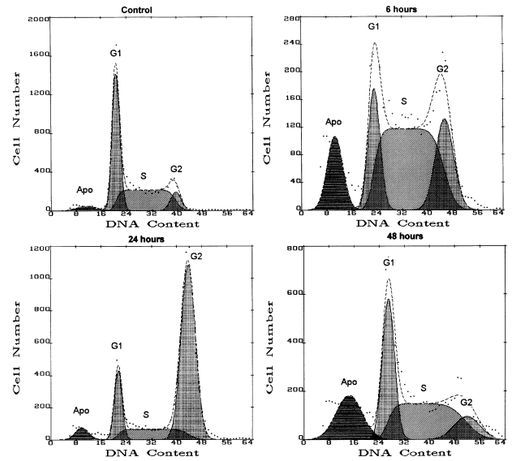


Fig. 4: Flow cytometric analysis of DNA content and cell-cycle after treatment of HL-60 cells with 5 nmol/l idarubicin. Apoptotic cells are identified as cells with subdiploid DNA content (lower DNA content than cells in G_0/G_1 phase), i.e. subG_1 peak. Representative results for single experiment are shown.



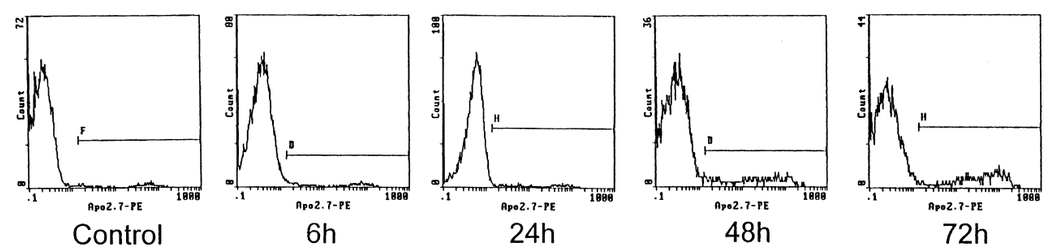


Fig. 5: Histograms for cell number versus APO2.7-PE fluorescence intensity of unprocessed HL-60 cells after treatment with 5 nmol/l idarubicin. Representative results for single experiment are shown.

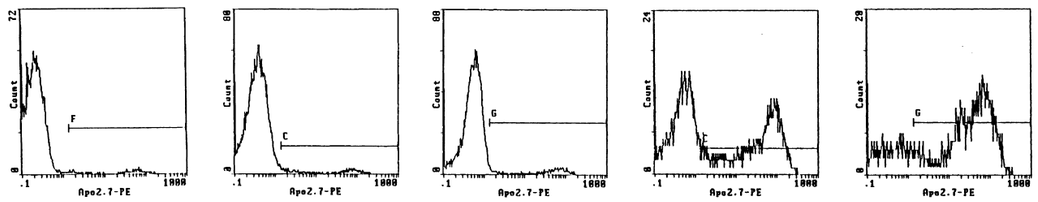


Fig. 6: Histograms for cell number versus APO2.7-PE fluorescence intensity of unprocessed HL-60 cells after treatment with 10 nmol/l idarubicin. Representative results for single experiment are shown.

Flow cytometric detection of apoptotic cells using monoclonal antibody APO2.7

Since 7A6 antigen is selectively expressed on the mitochondrial membrane in cells undergoing apoptosis, we attempted to detect apoptotic cells using APO2.7 monoclonal antibody after 5 and 10nmol/l idarubicin treatment of HL-60 cells. As shown in Fig.5 in unpermeabilised cells APO2.7 antibody staining significantly increased from 6% at 6h to 65% at 72h after treatment with 10nmol/l idarubicin, while increased only slightly after treatment with 5 nmol/l idarubicin (from 6% at 6h to 16% at 72h) (Fig. 6).

Discussion and Conclusions

HL-60 line has amplified myc and activated ras oncogene, it is p53 negative and does not contain the characteristic t(15;17) translocation seen in acute promyelocytic leukaemia (3, 4). HL-60 cells were negative for expression of CD34 and AC133 antigens (0.6/0.7%), the antigens usually used for separation of haematological progenitors from mobilised peripheral blood patients for autologous transplantation. HL-60 cells expressed high levels of CD15 (93%) and CD33 (84%) antigens, 82% cells were $CD15^{+}/CD33^{+}$ (5). Haematopoietic progenitor cells giving rise to monocytic and granulocytic lineages express numerous surface antigens to varying degrees depending on their developmental stage: CD33 antigen is expressed prior to myeloid commitment and CD15 is expressed at later stages in myelomonocytic development. Hofmanová et al. (3) described that 90% HL-60 cells were promyelocytes and 8% myelocytes with no expression CD14/CD11b antigens.

We show in this study that relatively high doses of idarubicin (50-100 nmol/l) induce as soon as after 6 hours in leukemic cell-line HL-60 cell shrinkage, membrane blebbing and cytoplasmic and nuclear fragmentation leading to the formation of apoptotic bodies, as determined by evaluation of Diff-Quik stained cytospin preparations using standard light microscopy at 1000 x magnification, as well as by flow-cytometric analysis of cell DNA content. During apoptosis DNA becomes fragmented by endonucleases and these small DNA fragments can break out from the cells, resulting in a reduced total DNA content and hence a sub-G_1 fluorescence peak representing apoptotic cells. Our results show that high doses of idarubicin induce apoptosis soon after treatment (6 hours). We presume that the cells die by rapid interphase apoptosis, where the apoptosis is triggered in all phases of cell cycle. Some studies (8) suggest that apoptosis induction and G_1 or G_2 cell cycle arrest are two separate phenomenons in Jurkat cells (T-cell line, mutated gene TP53, undetectable levels of TP53 protein) It has been shown (in accordance with thesis that cells with mutated TP53 are radioresistant) that apoptosis occurs in these cells during 24 hours after irradiation by high doses of ionising radiation(10-20 Gy). Regardless of the cell cycle phase 20% of apoptotic cells have been detected 6 hours after irradiation dose 20 Gy. Apoptosis was lower when early G_1 subpopulation has been irradiated in comparison to other cell cycle phases. It seems that after irradiation of cells in G_1 phase apoptosis occurs 2 hours later in comparison with cells in other cell-cycle phases. However, 24 hours following irradiation by 20 Gy all cells were apoptotic regardless of the cell-cycle phase, in which they were irradiated.

Relatively lower doses of idarubicin (5-10 nmol/l) first inhibit proliferation of the cells and induce changes in cell cycle. We have observed that after 6 hours of incubation most of the live cells accumulate in S phase of cell-cycle, after 24 hours we observed arrest in G_2 phase. After 48 h following 10 nmol/l idarubicin treatment we observed significant apoptosis and the cells did not proliferate during 72 hours. Lower dose (5 nmol/l) induces only small percentage of apoptosis after cell-cycle arrest in G_2 phase and the cells slightly proliferate during 72 hours following idarubicin treatment. We suppose that after 10 nmol/l the cells die by delayed interphase apoptosis, which typically occurs after cell-cycle arrest in G_2 phase. Similar results have been reported after irradiation of HL-60 cells by 5-10Gy, where the apoptosis occurs following cell-cycle arrest in G_2 phase 36-48 hours after irradiation (9). Cell-cycle arrest in G_2/M phase has been observed in Jurkat cells (8) following irradiation by relatively low dose 2 Gy, regardless of cell-cycle phase, in which they were irradiated. Apoptotic cells were cumulated 22-50 hours after irradiation. It is interesting, that apoptosis occurred sooner in population of cells irradiated in G_2 phase in comparison to other cell-cycle phases.

It can be concluded that apoptosis induced by low doses of idarubicin in TP-53 negative cells HL-60 as well as apoptosis induced by ionizing radiation in dose 2-10 Gy in various TP-53 negative haematopoietic cell lines (Jurkat, HL-60) occurs following G_2 cell-cycle arrest. Apoptosis was observed at HL-60 cells as soon as 6 hour after treatment with high idarubicin concentrations (50-100 nmol/l), similarly to Jurkat cells irradiated by supralethal doses (10-20 Gy), which also underwent apoptosis after 6 hours. Syljuasen and McBridge (8) proved that Jurkat cells treated by ionizing radiation could undergo apoptosis independently on cell-cycle arrest and that period in which apoptosis is observed is related to the dose of radiation. Similar results result from our experiments on idarubicin influence on HL-60 cells.

Acknowledgment

This work was supported by grants from the Czech Ministry of Defence (MO66020398129) and from Grant Agency of Charles University (GA UK 279/98) and in part by research project J13/98: 111500001.

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Submitted February 2000.
Accepted March 2000.

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