XXIV. XENOBIOCHEMICKÉ SYMPÓZIUM

Program & Abstract

Edited by
V. Boháčová, A. Breier, D. Zbyňovská

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May 22 – 24, 2007
Hotel Bernard, Liptovský Ján

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TIME SCHEDULE

Tuesday, May 22, 2007

13:30 - 14:00 Opening Ceremony

14:00 - 18:00 LECTURES IN SECTION 3

18:00 - 19:45 Tokay wine tasting

20:00 - 24:00 Diner and Get together party

Wednesday, May 23, 2007

07:30 - 08:30 Breakfast

08:30 - 11:00 LECTURES IN SECTION 4

11:00 - 11:30 LECTURES IN SECTION 5

11:45 - 12:45 Lunch

13:00 - 16:00 Trip to Demänovská ľadová jaskyňa and Jaskyňa Slobody

16:00 - 18:00 POSTER SECTION

20:00 - 24:00 Barbeque

Thursday, May 24, 2007

07:30 - 08:30 Breakfast

08:30 - 10:00 LECTURES IN SECTION 1

11:00 - 12:00 LECTURES IN SECTION 2

12:15 - 13:15 Lunch

13:30 - 14:00 Closing ceremony and final remarks

SCIENTIFIC PROGRAM

Tuesday, May 22, 2007

13:30 - 14:00		**Opening Ceremony**
Section 3 Chairs:		Animal cell as a model for study of xenobiotics Marie Stiborová, Július Brtko
14:00 - 14:30	**L13**	Jan Vondráček, Jiřina Zatloukalová, Zdeněk Andrysík, Lenka Umannová, Soňa Marvanová, Pavlína Polášková, Pavel Krčmář, Jan Topinka, Lenka Skálová, Barbora Szotáková, Alois Jakubík, Miroslav Machala: Liver progenitor cells as an alternative cellular model for studies on effects of AH receptor ligands.
14:30 - 15:00	**L9**	Juraj Kopáček, Jaromír Pastorek, Silvia Pastoreková: Crosstalk between hypoxia and xenobiotic pathways: consequences for expression of carbonic anhydrase IX as marker of tumor hypoxia.
15:00 - 15:30	**L7**	Jana Jakubiková, Juraj Boďo, Ľuba Hunáková, Jozef Duraj, Ivan Chalupa, Ján Sedlák: Molecular mechanisms of isothiocyanates action in potential chemoprevention and therapy of tumors.
15:30 - 16:00		**Coffee Break**
Section 3 Chairs:		Animal cell as a model for study of xenobiotics Juraj Kopáček, Ján Sedlák
16:00 - 16:30	**L12**	Zdena Sulová, Dana Macejová, Mário Šereš, Ján Sedlák, Július Brtko, Albert Breier: Combined treatment of P-gp positive L1210/VCR cells by verapamil and all-trans retinoic acid induced down-regulation of P-glycoprotein expression and transport activity.
16:30 - 16:45	**L11**	Andrej Repický, Soňa Jantová, Ľuboš Čipák: Induction of apoptosis in leukemia cells by benzothiazole derivative AMBAN.
16:45 - 17:00	**L10**	Peter Dočolomanský, Viera Boháčová, Albert Breier, Miroslav Barančík: Structural features of pentoxifylline important for its reversal effects on P-glycoprotein-mediated multidrug resistance.
17:00 - 17:15	**L6**	Viera Boháčová, Miroslav Barančík, Ima Dovinová, Branislav Uhrík, Albert Breier: Multidrug resistance phenotype induced in the L1210 cells by vincristine and doxorubicin is based on the overexpression of the P-glycoprotein.

17:15 – 17:45	**L8**	Stanislav John, Miroslav Červinka, Emil Rudolf: Selenite induces growth inhibition and cell death in hct-116 cells in vitro.
18:00 - 19:45		**Tokay wine tasting**
20:00 - 24:00		**Diner and Get together party**

Wednesday, May 23, 2007

07:30 - 08:30		**Breakfast**

Section 4	Xenobiotics in regulation of animal physiological function
Chairs:	Pavel Anzenbacher, Petr Hodek

08:30 - 09:00	**L17**	Katarína Valachová, Ladislav Šoltés, Peter Gemeiner, Katarína Bauerová: Degradation of hyaluronan samples with the addition of ascorbic acid, Cu (II), Fe (II) and Mn (II) ions.
09:00 - 09:30	**L16**	Bohumila Tarabová, Melinda Drábová, Martina Kurejová, Ľubica Lacinová: Effect of inorganic mercury on neuronal T-type calcium channel.
09:30 - 10:00	**L15**	Ľubica Máleková, Karol Ondriaš: Inhibitory effect of DIDS, NPPB and phloretin on intracellular chloride channels.
10:00 - 10:30		**Coffee break**
10:30 - 11:00	**L14**	Julius Brtko: Nuclear receptors and their cognate biologically active ligands in regulation of physiological functions of organisms.

Section 5	Xenobiotics in metabolism of microorganisms
Chairs:	Albert Breier

11:00 - 11:30	**L18**	Katarína Nigutová, Peter Javorský, Peter Pristaš: Bacteriocins of Gram-positive cocci, characterization, genetics and genomics.
11:45 - 12:45		**Lunch**
13:00 - 16:00		**Excursion in Caves in Demänová**

16:00 - 18:00	**Poster Section**
Chairs:	Miroslav Barančík, Boris Lakatoš, Zdena Sulová, Ľudmila Kameníková

20:00 - 24:00	**Barbeque**

Thursday, May 24, 2007

07:30 - 08:30		**Breakfast**

Section 1 Chairs:	Role of cytochrome P450 in biotransformation Eva Anzenbacherová, Ľudovít Varečka

08:30 - 09:00	**L3**	Marie Stiborová, Eva Frei: Molecular mechanisms of CYP-mediated increase in antitumor activity of ellipticine.
09:00 - 09:30	**L1**	Jiří Hudeček: What role may the side chains of the heme play in cytochrome P450?
09:30 - 10:00	**L2**	Jana Nekvindová, Alena Veinlichová, Pavel Anzenbacher, Eva Anzenbacherová, Juan Antonio Contreras, Damjana Rozman, Antonín Holý: Interactions of antivirals (nucleoside analogs) with cytochromes P450.
10:00 - 10:30		**Coffee break**
10:30 - 11:00		*Commercial presentation of Lambda Life a. s.* Michal Skarupa: Metódy sledovania bunkovej Proliferacie, Viability, Apoptozy, Cytotoxicity a in vitro ADME testy

Section 2 Chairs:	Recognition of xenobiotics by target structure Karol Ondriaš, Jiří Hudeček

11:00 - 11:30	**L4**	Petr Hodek, Miroslav Šulc, Martin Karabec, Jiří Hudeček, Marie Stiborová: Mapping of CYP 2B4 binding sites with diamantane photolabile probe.
11:30 - 12:00	**L5**	Martin Šimkovič, Martina Hunová, Peter Vargovič, Anita Kurucová, Ľudovít Varečka: Unusual features of proteinase secretion from Trichoderma viride submerged mycelia.
12:15 - 13:15		**Lunch**
13:30 - 14:00		**Closing ceremony and final remarks**

LIST OF POSTERS

Section 1 **Role of cytochrome P450 in biotransformation**

P1 D.Aimová, R. Kubík, J. Poljaková, V. Kotrbová, B. Mrázová, E. Frei, M. Stiborová: Regulation of CYP1A1/2 and NADPH:CYP reductase expression by ellipticine and benzo(a)pyrene.

P2 V. Kotrbová, B. Mrázová, L. Svobodová, M. Kořínková, M. Stiborová: Role of cytochrome b5 in regulation of cytochrome P450-dependent pharmacological efficiency of ellipticine.

P3 J. Křížková, K. Burdová, P. Hodek, R. Kizek, M. Stiborová: Cytochrome P450 1A subfamily induction by flavonoids.

P4 J. Matal, P. Řehulka, H.a Řehulková, J. Chmelík, G. Allmeier, E. Anzenbacherová, P. Anzenbacher: Proteomic study of pig liver microsomes

P5 J. Mizerovská, H. Dračínská, M. Stiborová: Kinetics of oxidation of 3-aminobenzanthrone by cytochromes P450.

P6 M. Svobodová, M. Petránková, H. Dračínská, M.Stiborová: Oxidation of carcinogenic 2-nitroanisole and 2-nitrophenol by cytochromes P450.

Section 2 **Recognition of xenobiotics by target structure**

P7 V. Cvilink, V. Kubíček, M. Nobilis, V. Křížová, B. Szotáková, J. Lamka, M. Várady, L. Skálová: Biotransformation of albendazole and flubendazole in Haemonchus contortus: glucose conjugate formation.

P8 V. Cvilink, L. Skálová, J. Lamka, R. Kostiainen, R.A. Ketola: In vitro biotransformation of albendazole and flubendazole in Haemonchus contortus: glucose conjugate formation.

P9 F. Kunc, A. Víšková, A. Libra, H. Radilová, M. Šafářová, M. Bunček: Non-specific immune response after dsRNA application

P10 R. Novotná, L. Škarydová, V.r Wsól: Reduction of anticancer drug doxorubicin by recombinant AKR1C3.

P11 M. Semanská, V. Martínek, M. Dračínský, M. Stiborová: Isolation and characterization of deoxyguanosine adduct formed from Sudan I activated by peroxidase.

P12 B. Szotáková, V. Křížová, V. Cvilink, J. Lamka, L. Skálová: Dicrocoelium dendriticum - biotransformation of anthelmintics and other drugs.

P13 L. Škarydová, R. Novotná, M. Hubálek, V. Wsól: Carboxylesterase 1 - human liver microsomal carbonyl reductase?

P14 Vl. Wsól, R. Novotná, L. Škarydová: Role of AKR1C3 in metabolism of anticancer potential drug oracin.

Section 3 **Animal cell as a model for study of xenobiotics**

P15 M. Barančík, V. Boháčová, J. Sedlák, Z. Sulová, A. Breier: The effects of LY294,002, a specific inhibitor of PI3K/Akt kinase pathway, on P-glycoprotein-mediated multidrug resistance.

P16 J. Brtko, D. Macejová, S. Ondková, M. Ficková, V. Laude: Nuclear Thyroid hormone receptors: Effects of vinclozolin, bisphenol A, and genistein in human MCF-7cells.

P17 L. Dostálová, S. Marvanová, P. Krčmář, Z. Andrysík, J. Topinka, J. Vondráček, M. Machala: DNA adducts formation, induction of oxidative stress and apoptosis in model of rat liver progenitor cells.

P18 H. Farghali, J. Martínek, N. Kutinová-Canová, L. Kameníková: Resveratrol and silymarin pretreatments ameliorate tert-butylhydroxide and D-galactosamine inducedhepatocyte apoptosis and necrosis.

P19 L. Gibalová, M. Šereš, Z. Sulová, M. Barančík, O. Križanová, J. Sedlák, A. Breier: Changes of apoptosis in P-GP positive L1210/VCR cells.

P20 T. Holotňáková, M. Takáčová, M. Baráthová, J. Pastorek, S. Pastoreková, J. Kopáček: Study of ARNT-dependent hypoxic and xenobiotic regulation of carbonic anhydrase 9 promoter in tumor cell lines.

P21 S. Jantová, A. Repický, Ľ. Čipák, M. Janikovicsová: Cytotoxic activity of quinoline derivative AAQ on leukemia cell lines

P22 S. Letašiová, S. Jantová, M. Miko: Cytotoxic and genotoxic effect of 3-(5-nitro-2-thienyl)-9-chloro-5-morpholin-4-yl[1,2,4]triazolo[4,3-]quinazoline in L1210 and Nih-3T3 cells

P23 D. Macejová, S. Ondková, M. Ficková, J. Brtko: Effects of vinclozolin, bisphenol a and genistein on retinoid and rexinoid nuclear receptors and their coregulators expression in human MCF-7 Cells

P24 M. Miko, S. Letašiová: Studies of energy-producing processes in Ehrlich Carcinoma cells by pyridinecarboxylates Cu(II) complexes with biologically active ligands.

P25 S. Ondková, D. Macejová, M. Ficková, J. Brtko: Vinclozolin, bisphenol A, genistein affect expression of nuclear vitamin D_3 receptor and CYP24 and CYP27B1 in human MCF-7cells.

P26 K. Pěnčíková, L. Vykopalová, P. Krčmář, M. Ciganek, J. Neča, J. Vondráček, M. Machala: Modulations of AhR, ER and AR signaling by complex chemical mixtures present in river sediments.

P27 J. Poljaková, J. Hraběta, T. Eckschlager, E. Frei, M. Stiborová: Ellipticine is cytotoxic to neuroblastoma cells: mechanisms of action.

P28 H. Radilová, A. Víšková, F. Kunc, A. Libra, M. Šafářová, M. Bunček: Cyclooxygenase-1 knock-down by shRNA in cell culture: A model systém for gene expression studies.

P29 L. Schröterová, P. Hašková, H. Zmolilová, E. Rudolf, M. Červinka: Anti-proliferatic effect of selenium on malignant colonic cells.

P30 Z. Sulova, Z. Vajcnerova, D. Mislovicova, A. Kovarova, A. Breier: Study of changes in cell surface glycosides associated with over expression of P-glycoprotein in L1210 cells by interaction with lectines.

P31 I. Švarcová, K. Valentová, J. Ulrichová, V. Šimánek: Antioxidant activity of phenolic fraction from Lonicera caerulea L. var. kamtschatica berries.

P32 M. Theiszová, S. Jantová, P. Tchingnabé, A. Repický: Cytotoxical evaluation of new fluor-hydroxyapatite composite on human fibroblast VH10 cells by direct contact.

P33 L. Umannová, M. Machala, A. Kozubík, J. Topinka, Z. Nováková, J. Vondráček: Tumor necrosis factor-alpha potentiates genotoxic effects of benzo(a)pyrene in liver progenitor cells.

P34 K. Valentová, M. Holčapek, E. Vrublová, L. Kolářová, J. Vostálová, J. Ulrichová, V. Šimánek: Hop prenylflavonoids in rat hepatocytes and microsomes: Metabolism and biological activity.

P35 J. Zatloukalova, M. Machala, A. Kozubik, J. Vondraček: Downregulation of Ah receptor expression using RNA interference in a model of liver progenitor cells.

Section 4 **Xenobiotics in regulation of animal physiological function**

P36 M. Bušová: The role of glutathione in detoxification of animal organisms.

P37 A. Dávidová, A. Schreiberová, M. Lacková, D. Kolesár, J. Maršala, N. Lukáčová: The expression of nNOS in the spinal cord after femoral nerve transection.

P38 B. Lakatoš, J. Slováková, Ľ. Varečka: Effect of some effectors of signalling pathways on basal calcium influx in human peripheral blood lymphocytes.

P39 A. Špániková, P. Šimončíková, O. Pecháňová, M. Barančík: The effect of L-name treatment on the levels and activities of regulatory proteins in rat hearts.

Section 5 **Xenobiotics in metabolism of microorganisms**

P40 M. Kaliňák, Z. Ondrušová, D. Hudecová, Ľ. Varečka: Effects of GABA antagonists on growth of *Trichoderma viride.*

P41 M. Mikulášová, L. Birošová, M. Miko, P. Matejov: The antimutagenic effect of vanillin on spontaneous mutations leading to ciprofloxacin resistance.

P42 P. Olejníková, Z. Ondrušová, B. Kaliňáková, L. Krupková, E. Hlavačová, D. Hudecová: Biological activity of new fenamates with metal ion in the molecule.

P43 Z. Ondrušová, H. Paulíková, D. Hudecová, A. Valent, D. Sabolová, M. Kožurková: Ascorbic acid – modulator of antimicrobial effect of N-salicylidene-L-glutamate Cu(II)complexes.

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ABSTRACTS

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LECTURES

Section 1	Role of cytochrome P450 in biotransformation

L1
WHAT ROLE MAY THE SIDE CHAINS OF THE HEME PLAY IN CYTOCHROMES P450 ?

Jiří Hudeček
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Heme enzymes of the cytochrome P450 superfamily, acting as monooxygenases, are involved in foreign compound and drug metabolism. This implies a strong interest in the structure and function of these hemeproteins. One of the most intriguing questions is: how the apoprotein communicates with the heme moiety? Such signaling regulates, in response e.g. to the substrate binding, the redox potential of the heme iron in P450, and thus plays a crucial role in the modulation of enzyme function.

Generally, there are three principally different modes, how the heme iron may "sense" the state of the apoprotein: (i) a direct influence on the heme iron, (ii) distortion of the heme porphyrin skeleton, resulting in its deviation from planarity, and (iii) interactions between the protein and side chains of the heme, chiefly propionates and vinyls. Whereas the role of propionates in the P450 function is somewhat established, the vinyls were mostly overlooked so far.

The differences in mobility and flexibility of heme vinyls in positions 2 and 4 of the heme, found recently by our analysis of P450 crystal structures[1], and the strong change of the heme vinyl vibration upon reduction suggest involvement and role of these side chains in P450 chemistry.

The financial support from the Grant Agency of Czech Republic (Grant No. 303/06/0928) is gratefully acknowledged.

[1] J. Hudeček, P. Hodek, E. Anzenbacherová, P. Anzenbacher: *Biochim. Biophys. Acta* 1770 (2007), 413-416.

L2
INTERACTIONS OF ANTIVIRALS (NUCLEOSIDE ANALOGS) WITH CYTOCHROMES P450

Jana Nekvindová[1], Alena Veinlichová[1], Pavel Anzenbacher[1], Eva Anzenbacherová[2], Juan Antonio Contreras[3], Damjana Rozman[3], Antonín Holý[4]

[1]Department of Pharmacology and [2]Department of Medical Chemistry and Biochemistry, Palacky University, Faculty of Medicine and Dentistry, Olomouc, Czech Republic, [3]Institute of Biochemistry, University of Ljubljana, Slovenia and [4]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic.

Acyclic nucleoside phosphonates are potent antivirals used to treat infections like HIV/AIDS (tenofovir) or hepatitis B (adefovir). We have studied the interactions of adefovir, adefovir dipivoxil (AD), tenofovir and tenofovir disoproxil (TD) with cytochromes P450. Results show that all the compounds studied can affect the activity of the most important isoforms, especially 2C9; adefovir and AD inhibit significatly 3A4, too. TD was shown to influence the activity of 2E1. The character of inhibitions was predominantly competitive and was apparent mostly at higher concentrations (equal or higher than 100 μM). In vivo study on an influence of AD and TD on the expression of individual isoforms did not reveal significant changes in the amount of a particular enzyme on the level of mRNA compared to positive control treated with prototypic CYP inducers. The findings were confirmed also on the protein level using Western blot assays detection of CYPs where antibodies were available. As the concentrations of the compounds studied in human plasma are generally lower than these exhibiting significant inhibition of microsomal enzymes, we don´t expect significant and clinically important effect of adefovir or tenofovir on metabolism of other drugs.

L3
MOLECULAR MECHANISMS OF CYP-MEDIATED INCREASE IN ANTITUMOR ACTIVITY OF ELLIPTICINE

Marie Stiborová, Eva Frei
Department of Biochemistry, Charles University, Albertov 2030, 128 40 Prague

Recently we found a new mode of anticancer drug ellipticine action, formation of covalent DNA adducts mediated by its oxidation with cytochrome P450 (CYP) and peroxidases *in vitro* and *in vivo*. Here, we investigate the formation and persistence of ellipticine-DNA adducts generated in a rat animal model, report the molecular mechanism of ellipticine oxidation by CYPs and identify human, rat and mice CYPs responsible for ellipticine metabolic activation and detoxication. We also present a role of peroxidases in ellipticine oxidation leading to formation of two major ellipticine-DNA adducts. The results shown here allow us to propose species, two carbenium ions, ellipticine-13-ylium and ellipticine-12-ylium, as reactive species generating two major DNA adducts seen *in vivo* in rats and mice treated with ellipticine. It follows from our studies that not only are the patterns of ellipticine-DNA adducts generated in rats very similar to those formed with metabolically activated ellipticine *in vitro*, but the identical CYP enzymes (human, rat and mice CYP3A and CYP1A) also catalyzed formation of DNA adduct 1 that is generated by ellipticine-13-ylium. The contribution of CYP and/or peroxidase enzymes to formation of this adduct and the other ellipticine-DNA adducts *in vivo* in individual organs of rodents is evaluate in this study, too. Mice carrying a deletion in the gene of hepatic NADPH:CYP reductase (*POR* gene), and thus lacking POR and POR-mediated CYP enzyme activity in the liver, are utilized for such experiments. The study forms the basis to further predict the susceptibility of human cancers to ellipticine.

Supported GACR (203/06/0329) and MSMT CR (MSM0021620808).

LECTURES

Section 2	Recognition of Xenobiotics by target structure

L4
MAPPING OF CYP2B4 BINDING SITES WITH DIAMANTANE PHOTOLABILE PROBE

Petr Hodek[1], Miroslav Šulc[2], Martin Karabec[1], Jiří Hudeček[1], Marie Stiborová[1]

[1]Katedra biochemie, Přírodovědecká fakulta Univerzity Karlovy v Praze
[2]Mikrobiologický ústav, v.v.i., Akademie věd České republiky, Praha

Cytochromes P450, enzymes involved in foreign compound and drug metabolism, are extensively studied in the respect of their structure-function relation ships. To determine substrate binding regions, 3-azidiamantane was employed for photoaffinity labeling of cytochrome P450 2B4. Mass spectroscopy and sequencing revealed four diamantane labeled peptides in cytochrome P450 2B4 trypsine digest. Peptide Leu^{359}- Lys^{373}, located between K-K' helices goes through the active center to the protein surface. $Val^{367}$of this peptide makes the close contact (3.5 Å) with diamantene bound in the active center. Interestingly, diamantane docked outside the active center is in contact with Thr^{372} (3.0 Å) of this peptide. The second labeled tryptic peptide Leu^{30}-Arg^{48} spans to the region preceding A-helix. This peptide is likely involved in attracting cytochrome P450 substrates from the membrane. Peptides Phe^{127}-Arg^{140} and Arg^{434}- Arg^{443} belong to C- and L-helices, respectively, which define a possible entrance to the active center from the heme proximal face. Thus, several substrate binding sites outside the enzyme active center were mapped. Their involvement in the substrate access channels is discussed.

The financial support from grants 303/06/0928 and 303/05/2195 from the Grant Agency of Czech Republic, and MSM 0021620808 from the Czech Ministry of Education is highly acknowledged.

L5

UNUSUAL FEATURES OF PROTEINASE SECRETION FROM *TRICHODERMA VIRIDE* SUBMERGED MYCELIA

Martin Šimkovič, Martina Hunová, Peter Vargovič, Anita Kurucová, Ľudovít Varečka

Department of Biochemistry and Mcrobiology, Faculty of Chemical and Food Technology, Slovak University of Technology, Bratislava, Slovakia

Saprophytism is a characteristical feature of filamentous fungi (FFs). In order to cope with the variability of complex substrates FFs are able to secrete many hydrolytic, and other enzymes capable of degrading complex substrates. Families of enzymes for the degradation of cellulose and hemicelluloses are the best examples of versatility and adaptability for the saprophytic strategy. FFs are known to secrete serine- , aspartic- and metallo- proteinases but not cysteine proteinases upon cultivation. The study of the proteinase secretion with deuteromycete *Trichoderma viride* as a model showed that the proteinase secretion could be induced by the yeast autolysate, or the presence of purified proteins (inducers), such as serum albumin, casein or ovalbumin. Proteinase activities induced by individual inducers were different in their properties, such as temperature-, and pH – dependence, and their sensitivities to inhibitors of known proteinase classes were also discernibly different. Proteinase activities were characterised by the SDS – PAGE followed by zymography in the polyacrylamide gel with co-polymerized gelatin. Multiple proteinase bands with m.w. higher than 70 kDa were revealed by this method. Patterns of proteinase bands were inducer-specific. Attempts to purify proteinases also confirmed different behaviour of enzymes induced by different inducers. These results show that *Trichoderma viride* is able to recognize not only the presence of inducer proteins in the medium but also its specific molecular features. In other words, proteins in the medium are not simple nutrients but in addition represent a kind of signalling molecules triggering the specific secretory response. *This work was supported by grants VEGA nr. 1/3251/06 and 1/2335/05*

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ponúka

pre laboratóriá:

- Kolóny pre HPLC a iné materiály pre chromatografiu (TESSEK, SUPELCO, J&W, PHENOMENEX, HYPERSIL, CHROMACOL, CRS, RESTEK),
- HPLC prístoje + príslušenstvo, napr. koncentrátory vzoriek (ECOM, RHEODYNE ...),
- autosamplery, zberače frakcií, peristaltické pumpy a iné prísl. pre chromatografiu (GILSON),
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LECTURES

Section 3	Animal cell as a model for study of xenobiotics

L6
MULTIDRUG-RESISTANCE PHENOTYPE INDUCED IN L1210 CELLS BY VINCRISTINE AND DOXORUBICIN IS BASED ON THE OVEREXPRESSION OF P-GLYCOPROTEIN

Viera Boháčová[1], Miroslav Barančík[2], Ima Dovinová[3],Branislav Uhrík[1], Albert Breier[1]
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Multidrug resistance (MDR) of neoplastic tissue is often associated with the overexpression and increased drug transport activity of plasma membrane transporters like P-glycoprotein (Pgp), multidrug resistance associated proteins (MRPs), as well as with the elevation of the glutathione detoxification pathway.

In the present study, mechanisms of multidrug resistance induced in L1210 cells by vincristine and doxorubicin were analyzed. Compared were resistant sublines obtained by selection pressure of VCR (L1210/VCR) and doxorubicin (L1210/DOX). In both cell lines was observed a dramatic decrease in cell sensitivity to both VCR and DOX. Both L1210/VCR and L1210/DOX cells demonstrated a lack of ability to accumulate Calcein/AM and Fluo-3/AM (fluorescent substrates of Pgp and Mrp). The retention of dyes could be reached in both cell lines by application of Pgp inhibitor (verapamil) but not by inhibitor of anion transporters, including MRP (probenecid). Moreover, in both resistant sublines was detected a massive band interacting with a Pgp specific antibody. It seems that L1210 cells start to overexpress Pgp but not Mrp in both cases i.e., when VCR or DOX was used as selection substance. The activity of GST was found to be similar in both resistant cell lines (L1210/VCR, L1210/DOX) and did not differ significantly from the activity observed in parental cells (L1210). The results suggest that DOX and VCR induce a common multidrug resistance phenotype in L1210 cells, based predominantly on Pgp overexpression.

Supported by Slovak Grant Agency for Science (grant No. 2/7124, 2/6080) and APVV (grant 51-027-404).

L7
MOLECULAR MECHANISMS OF ISOTHIOCYANATES ACTION IN POTENTIAL CHEMOPREVENTION AND THERAPY OF TUMORS

Jana Jakubíková, Juraj Boďo, Ľuba Hunáková, Jozef Duraj, Ivan Chalupa, Ján Sedlák

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The aim of our study was to identify contribution of some intracellular signaling pathways in the cytotoxic effect of isothiocyanates in the tumor cell lines. Flow cytometric analysis and western blotting were utilized to study their modulatory effects. Upregulation of p21cip1/waf1, induction of G2/M cell cycle arrest and apoptosis are common attributes of all isothiocyanates tested, while the activation of ERK1/2, JNK and p38 MAPK was the cell line-dependent event.

Using synchronized leukemic HL60 cells we show that activation of mitogen-activated protein kinases ERK1/2, JNK, and p38 signaling pathways by E-4IB are coupled with delayed transition through the cell cycle and rapid cell cycle arrest culminating in apoptosis. These events were accompanied by histone deacetylase inhibition, increase of double strand DNA breaks detected by histone H2AX phosphorylation and upregulation of cell cycle regulatory protein p21 and phosphorylation of CDC25C phosphatase.

We have confirmed the concentration-dependent switch between apoptosis and necrosis, and recently discovered inhibition of histone deacetylase activity. Caspase inhibitor Z-VAD-fmk decreased sub-G0 fragmentation and extent of apoptosis, while proteasome inhibitor MG132 increased number of apoptotic cells.

We have shown, for the first time that combined isothiocyanate E-4IB and chemotherapeutic drug cisplatin treatment of tumor cells resulted in the synergistic cytotoxic activity. Observed synergistic effects were achievable also at clinically low-toxic concentrations of cisplatin, which can lead to the maximum therapeutic benefit of potential E-4IB/cisplatin combination treatment. Further studies are needed to clarify the useful chemopreventive/chemotherapeutic potencies of phytochemicals and to understand the underlying mechanisms of their action.

Supported by Slovak Grant Agency for Science (grant No. 2/7059, 2/5042) and APVV (grant 51-017505).

L8
SELENITE INDUCES GROWTH INHIBITION AND CELL DEATH IN HCT-116 CELLS IN VITRO

Stanislav John, Miroslav Cervinka, Emil Rudolf

Faculty of Medicine in Hradec Kralove, Charles University in Prague

Selenium is an essential trace element that takes part in many physiological processes in human body. It guards the cell against exogenous and endogenous stresses; however, it can cause cell damage by induced oxidative stress (ROS) and affects intracellular signaling ways leading to cell proliferation arrest and cell death. *In vitro* and *in vivo* studies showed that selenium may play a role in colorectal carcinoma too.

The goal of this work was the characterization of HCT-116 cell line (human colorectal carcinoma) and the examination of the influence of selenium on proliferation and apoptosis. We used sodium selenite in concentrations 1-500 μM during 24-72 hours. Selenium-based inhibitory and cytotoxic effects were studied by WST, Brilliant Blue and BrdU assays along with time lapse videomicroscopy. Furthermore, analysis of the cell cycle (flow cytometry) and the expression specific molecular markers were determined. Pilot experiments with selenite indicate its considerable antiproliferative effects in this cell line, in particular in the concentration rage of 5-10 μM during 24 hours. Selenium stops the cell cycle and induces oxidative stress that is considered to be the main activator of intracellular pathways leading to the cell death. In addition, analyses show the activated apoptotic cell death in this model, but it will have to be further specified. Our results form a basis for further testing of other selenium forms (especially organic ones) in the model of human colorectal carcinoma *in vitro*.

This work was supported by the Ministry of Education of the Czech Republic Research Project MSM0021620820.

L9
CROSSTALK BETWEEN HYPOXIA AND XENOBIOTIC PATHWAYS: CONSEQUENCES FOR EXPRESSION OF CARBONIC ANHYDRASE IX AS MARKER OF TUMOR HYPOXIA

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Carcinogenesis is a complicated process that results from accumulation of genetic alterations, epigenetic changes and abnormal physiological processes in the tumor tissue (related mainly to hypoxia). Genesis and progression of this process are frequently connected with action of unfavorable factors of outer environment called xenobiotics. In general, xenobiotics cause genome destabilization and lead to activation and/or deregulation of several signaling pathways in the cell. Humans are exposed to xenobiotics over the entire lifespan, not only before the tumor initiation but also in the late stages of carcinogenesis.

Main object of our interest is a carbonic anhydrase IX (CA IX), whose expression is strongly induced by hypoxia and its distribution in the tumor tissues significantly correlates with poor prognosis in many tumor types. Our data indicate that CA IX expression could be affected also by xenobiotics. Since CA IX is functionally involved in the carcinogenesis, modulation of its expression by xenobiotics could have a direct influence on the growth parameters of tumor cells. From this point of view, CA IX represents an outstanding molecular model.

In principal, understanding of the crosstalk between xenobiotic and hypoxic pathways has a potential value to reveal mechanism of tumor progression and to develop new therapeutic strategies.

Supported by APVV-51-024905

L10
STRUCTURAL FEATURES OF PENTOXIFYLLINE IMPORTANT FOR ITS REVERSAL EFFECTS ON P-GLYCOPROTEIN - MEDIATED MULTIDRUG RESISTANCE

Peter Dočolomanský, Viera Boháčová, Albert Breier, [1]Miroslav Barančík

Institute of Molecular Physiology and Genetics and [1]Institute for Heart Research, SAS, Bratislava

The resistance of tumor cells to cytotoxic drugs is a problem in cancer chemotherapy. The multidrug resistance (MDR) phenotype associated with the overexpression of membrane P-glycoprotein (P-gp), is defined as a cross-resistance to a wide range of structurally diverse anticancer agents. Several substances (chemosesitizers) can restore the sensitivity of resistant tumor cells against anticancer drugs. This effect was described also for pentoxifylline (PTX) (Breier et al., 1994). To characterize the structural features important for reversal effects of PTX we prepared a set of *N*1-, *N*3-, *N*7- and *C*8-substituted aminoalkylxanthines derived from PTX and tested their effects on vincristine resistance of MDR cell line L1210/VCR.

The results showed that the inhibition of P-gp mediated MDR is increased by: i) prolongation of alkyl chain at the both *N*3 and *N*1 positions, ii) presence of polar substituent on alkyl chain at the position *N*1. On the other hand, the the efficacy of PTX derivatives in reversal of MDR in L1210/VCR cells is decreased by: i) substitution of hydrogen on *C*8 by alkyl chain, ii) shortening of the 5-oxohexyl chain at the *N*1 of xanthine ring.

The results indicate that the effectivity of xanthines in reversal of MDR resistance is dependent on the structure of substance and point to the crucial role of longer polar substituent in position *N*1 in effective reversal of P-gp mediated multidrug resistance.

Breier et al. Neoplasma 41, 1994, 297-303. Supported by grants: VEGA SR 2/415

L11
INDUCTION OF APOPTOSIS IN LEUKEMIA CELLS BY BENZOTHIAZOLE DERIVATIVE AMBAN

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In the present study we have investigated the induction of apoptosis in murine leukemia cell line L1210 and human leukemia cell lines HL60 and U937 by 2-acethyl-3-(6-methoxybenzothiazol-2-ylamino)-acrylonitrile (AMBAN).

Cytotoxic effect of AMBAN after 24-72 h treatment was determined and characterised by IC_{50} and IC_{100} values. L1210 cells were more sensitive than U937 cells whereas HL60 cells were the least sensitive of all tested leukemia cells. Induction of apoptosis after 24 h treatment with AMBAN was detected by agarose gel electrophoresis, analysis of cell cycle by flow cytometry, fluorescence microscopy (Hoechst 33258/propidium iodide) and measurement of caspase-3 activity. The results showed that AMBAN induced apoptotic death of L1210, HL60 and U937 cells by activation of caspase-3 followed by nuclear changes and DNA fragmentation.

Acknowledgements
This study was supported by the Slovak State Committee for Scientific Research VEGA, grant number 1/4305/07 and Science and Technology Assistance Agency under the contract No. APVT-20-007304.

L12
COMBINED TREATMENT OF P-GP-POSITIVE L1210/VCR CELLS BY VERAPAMIL AND ALL-TRANS RETINOIC ACID INDUCES DOWN-REGULATION OF P-GLYCOPROTEIN EXPRESSION AND TRANSPORT ACTIVITY

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P-glycoprotein (P-gp) is a drug efflux pump of plasma membrane with substrate specificity to drugs with different structure. The multidrug resistance (MDR) phenotype based on overexpression of P-gp often results more than hundred times higher cells resistance to several drugs. All-trans retinoic acid (ATRA, ligand of retinoic acid receptors RAR) was described to induce alterations in P-gp expression and/or activity in different cells.

L1210/VCR (R) is a P-gp positive cell line, in which P-gp overexpression was achieved by adaptation of parental L1210 (S) cells to vincristine. The topic of the present paper was the study of relations between regulatory pathways of nuclear receptors for retinoids and P-glycoprotein expression.

When compared R and S cells, the levels of mRNA encoding retinoic acid nuclear receptors RARα and β or RARγ and retinoid X receptors RXRb and RXRγ were increased or decreased, respectively. ATRA did not influence the viability of R cells differently to S cells. ATRA alone did not influence the P-gp expression or the transport activity in R cells. In contrast, when ATRA was applied together with verapamil (P-gp inhibitor), a significant depression of P-gp expression and transport activity were observed. However, any significant differences in [11, 12-3H]-ATRA uptake were observed neither in sensitive nor in resistant cells. Moreover, verapamil did not influence ATRA uptake in any cases.

Thus, we can conclude that the combined treatment of R cells with ATRA and verapamil is able to depress P-gp expression and activity by unknown mechanisms.

Acknowledgements**:** This work was supported from grant APVT-51/027404 and Slovak Grant Agency for Science VEGA grants No. 2/7122/7, 2/6080

L13
LIVER PROGENITOR CELLS AS AN ALTERNATIVE CELLULAR MODEL FOR STUDIES ON EFFECTS OF AH RECEPTOR LIGANDS

Jan Vondráček [1,2], Jiřina Zatloukalová[1], Zdeněk Andrysík[1,2], Lenka Umannová[1,2], Soňa Marvanová[2], Pavlína Polášková[2], Pavel Krčmář[2], Jan Topinka[3], Lenka Skálová[4], Barbora Szotáková[4], Alois Kozubík[1], Miroslav Machala[2]

[1]Institute of Biophysics ASCR, Brno; [2]Veterinary Research Institute, Brno; [3]Institute of Experimental Medicine ASCR, Prague; [4]Faculty of Pharmacy, Charles University, Hradec Králové

In liver, the progenitor oval cells represent cell population which proliferates in response to diverse chemical carcinogens. These cells are able to develop into both hepatocytes and biliary epithelial cells, and they have been suggested to play a significant role in hepatocarcinogenesis. As the ligands of the aryl hydrocarbon receptor (AhR) are known to induce rodent hepatic tumors, which might arise from this cell population, we have used a model rat liver epithelial cell line WB-F344, sharing important phenotypic properties with oval cells, in order to characterize response of these cells to various classes of AhR ligands. This presentation is intended to provide an overview of our recent studies on regulation of the AhR-dependent gene transcription, control of cell proliferation/apoptosis, induction of genotoxic events and deregulation of cell-to-cell communication in this cellular model. We provide evidence that, at least in this experimental model, AhR ligands might induce different types of effects than in mature liver cells – hepatocytes, and this could play a significant role in hepatocarcinogenesis. [This study was supported by the Czech Science Foundation, grant No. 524/06/0517, and the Czech Ministry of Agriculture, Research Plan No. MZE00002716201].

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Prístroje a pomôcky pre dávkovanie kvapalín

Dávkovače a zásobné fľaše, mikropipety a špičky k nim, digitálne byrety, mikrostriekačky, mechanické a elektrické nástavce pre prácu so sklenenými pipetami.

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LECTURES

Section 4	Xenobiotics in regulation of animal physiological function

L14
NUCLEAR RECEPTORS AND THEIR COGNATE BIOLOGICALLY ACTIVE LIGANDS IN REGULATION OF PHYSIOLOGICAL FUNCTIONS OF ORGANISMS

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Nuclear receptors are DNA-binding, trans-acting, transcription-modulating proteins involved in a general molecular mechanisms responsible for transcriptional responses of target genes. They are transcription factors inducible by hormones and/or biologically active ligands involved in a complex arrangement of physiological, metabolic and developmental responses in many tissues of higher organisms. In general, nuclear receptors are essential in embryonic development processes, maintenance of differential cellular phenotypes and cell death. Dysfunction in nuclear receptor signalling pathways leads to proliferative, reproductive and metabolic diseases. Nuclear receptor superfamily contains 24 liganded receptors among the 48 known nuclear receptors in human genome. The response of a given nuclear receptor to a particular ligand in a specific tissue depends also on coregulation proteins (corepressors and coactivators) with which the nuclear receptor is able to interact. At present, it is crucial to understand the role of a variety of more or less specific ligands that affect expression of target genes through their cognate nuclear receptor interaction. Therefore, there is still an urgent call for further investigation of novel biologically active ligands (agonists or antagonists) acting through nuclear receptors in order to understand their role in therapy or chemoprevention of a set of civilization diseases, e.g. cancer, diabetes, or other endocrine or metabolic diseases.

This work was supported by the grant the VEGA grant No. 2/5017/5.

L15
INHIBITORY EFFECT OF DIDS, NPPB AND PHLORETIN ON INTRACELLULAR CHLORIDE CHANNELS

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The chloride channel blockers, DIDS, NPPB and phloretin were reported to prevent apoptosis induced by ischemia-reperfusion in cardiomyocytes. The aim of this work was to study whether the chloride channel blockers inhibit intracellular chloride channels obtained from rat heart mitochondrial and lysosomal membrane vesicles. We characterized the effect of these compounds on single channel properties of the chloride channels incorporated into a bilayer lipid membrane (BLM). The single chloride channel currents were measured in 250/50 mM KCl *cis*/*trans* solutions. The DIDS, NPPB and phloretin inhibited the chloride channels in dose-dependent manner at concentrations 30-100 μM, which were similar as they were reported to protect cardiomyocytes against the reperfusion-induced injury. They inhibited channels by decreasing channel conductance, amplitude, open dwell time and increasing close dwell time, indicating that they could affect the Cl^- selective filter, pore permeability and gating mechanism of the chloride channels. DIDS and NPPB inhibited the channels from the other side than bongkrekic acid and carboxyatractyloside. From the inhibitory effect of the studied compounds we may suggest that inhibition of the heart mitochondrial and heavy membrane vesicle intracellular chloride channels may be involved in their cardioprotective effect.

Supported by APVV grant No. 51-027-404.

L16
EFFECT OF INORGANIC MERCURY ON NEURONAL T-TYPE CALCIUM CHANNEL

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Mercury is a highly toxic agent that may affects different organs including central nervous system in humans and animals during acute and chronic exposure. The exposure of neuronal cells to organic (methylmercury) or inorganic (Hg^{2+}) mercury may modulate the function of voltage gated ion channels including T-type calcium channels and thus contribute to neuronal symptoms of mercury poisoning. Interaction of mercury with T-type calcium channel was not investigated. We have examined effects of Hg^{2+} on current through the $Ca_V3.1$ calcium channel stably expressed in HEK 293 cells. Hg^{2+} inhibited calcium current through the $Ca_V3.1$ channel in a concentration dependent manner with an IC_{50} 0.63 ± 0.11 μM and a Hill coefficient 0.73 ± 0.08. In the presence of inorganic mercury time constants of current activation, inactivation and deactivation were enhanced and also the peak of current voltage (I-V) relation was shifted towards more depolarised membrane potentials. The activation of an unspecific background current under micromolar concentrations of Hg^{2+} was observed. This current may reflect the increase in the permeability of the cell membrane due to cytotoxic effects of Hg^{2+}. Therefore the viability of HEK 293 cells expressing $Ca_V3.1$ calcium channel was tested using MTT assay and FACS flow cytometry. Minor but significant increase in the cell death was observed only after 4 hours treatment with 1 μM Hg^{2+}. Observed effects of Hg^{2+} on neuronal calcium channels may contribute to the pathology of mercury poisoning in the central nervous system.

Supported by APVV-51-027404.

L17
DEGRADATION OF HYALURONAN SAMPLES WITH THE ADDITION OF ASCORBIC ACID, Cu (II), Fe (II) AND Mn (II) IONS

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[2]Institute of Chemistry, SAS, SK-84538 Bratislava

Hyaluronan is a linear polymer composed of glucuronic acid and of β-*N*-acetylglucosamine linked by β-1,4 bonds. Viscosupplementation in the form of injection application of hyaluronan is performed to heal osteoarthritis.

In our work the efficacy of the action of ascorbic acid to hyaluronan samples by measuring dynamic viscosity during five hours was tested. Ascorbic acid is an excellent antioxidant, but it acts as a prooxidant in the Fenton reaction.

To demonstrate the efficacy of trace amounts of metals present in the samples, the next part of the study was focused on investigating the changes in hyaluronan solutions dynamic viscosity in three systems when added ascorbic acid (100 μM) followed by Cu(II) ions (1.0 and 5.0 μM), Fe(II) ions (0.5 μM and 5.0 μM), Mn(II) ions (0.5 and 5.0 μM). Cu(II) ions degraded hyaluronan more effectively than Fe(II) ions did. Mn(II) ions performed an antioxidant effect. The samples of higher molar mass were more susceptible to degradation compared with low molar mass samples.

Acknowledgement

The Grants 2/5002/5, 2/7028/27 from the Grant Agency for Sciences of the Ministry of Education of Slovak Republic and Slovak Academy of Sciences, Bratislava, the Grants APVV-51-017905 from the Agency for Research and development are gratefully acknowledged.

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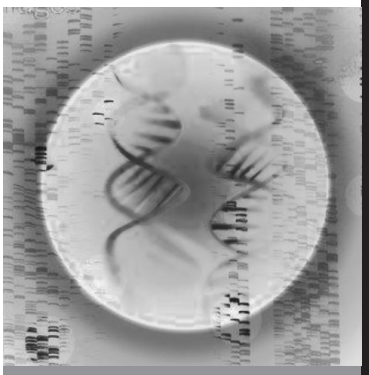

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proteomiky

- technológie na stanovenie protein-proteín (Pull Down) a proteín/DNA, resp. RNA interakcií
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- expresné a fúzne systémy (HIS, FLAG, GST, CAT, GFP, HaloTaq)
- sledovanie expresie, lokalizácie a dynamiky sledovaných proteínov v reálnom čase (HaloTaq)
- syntéza peptidov a tvorba protilátok na požiadanie
- detekcia mutácií v proteínoch (PTT test)
- široká paleta primárnych a sekundárnych protilátok
- reagencie na Western blot, blotovacie membrány
- ultrafiltračné centrifugačné jednotky na zahusťovanie proteínov

LECTURES

Section 5	Xenobiotics in metabolism of microorganisms

L18
BACTERIOCINS OF GRAM-POSITIVE COCCI, CHARACTERIZATION, GENETICS AND GENOMICS

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A term "xenobiotic" is usually used in the context of pollutants and their effect on the biota, however natural compounds can also become xenobiotics if they are taken up by another organisms. It has been known for a long time that many members of the lactic acid bacteria (LAB) produce proteinaceous inhibitors referred to collectively as bacteriocins, which are lethal to bacteria other than the producing strain. In our laboratory two enterococcal bacteriocins were completely characterized. Enterocin BC25, small (4,8 kDa), heat stable, antilisterial bacteriocin with an antimicrobial spectrum towards *Enterococcus*, *Streptococcus* and *Listeria*, an enterocin A homologue; and large (35 kDa), heat labile bacteriocin showing complete identity with enterolysin A isolated from *E. faecalis* LMG2333. Purified or partially purified bacteriocins from LAB could be used as preservatives or for the reduction or elimination of certain pathogens.

The observation that many Gram-positive bacteria are able to synthesize identical bacteriocins indicates possibility of horizontal transfer of enterocin structural genes. For the first time, enterolysin A homologue was identified in genome of enterococcal bacteriophage F4 and our results showed that the bacteriophage participated at the transfer of enlA structural gene. Occurrence of several other bacteriocin structural genes (*entA*, *entB*, *entP*, *enlA* and *cyl*) among enterococcal and streptococcal isolates was tested. The results are consistent with the multitoxicity model of Pagie and Hogeweg, which predicts that natural bacterial populations will show a high occurrence of strains carrying but not expressing genes for bacteriocin production and resistance.

This work was supported by Slovak Research and Development Agency Grant No. 0094-06.

POSTERS

Section 1	Role of cytochrome P450 in biotransformation

P1
REGULATION OF CYP1A1/2 AND NADPH:CYP REDUCTASE EXPRESSION BY ELLIPTICINE AND BENZO(A)PYRENE

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Ellipticine is an antineoplastic agent, whose mode of action is based mainly on DNA intercalation, inhibition of topoisomerase II and formation of covalent DNA adducts mediated by cytochromes P450 (CYP) and peroxidases. Here, this drug was investigated for its ability to induce CYP and NADPH:CYP reductase (POR) enzymes in rat organs and the influence of such induction on DNA adduct formation by the compound. Benzo(a)pyrene (BaP) was used as another model compound, which is known to induce CYP enzymes in several species. In the case of BaP, hepatic POR-null (HRN™) mice and wild-type littermates were used as a model. Both compounds induce expression of CYP1A1 and 1A2 in livers of both animal models. When microsomal fractions from livers of treated animals were incubated with ellipticine or BaP, DNA adduct formation, measured by ^{32}P-postlabelling analysis, was up to 25-fold higher in incubations with microsomes from pretreated animals than with controls. The observed stimulation of DNA adduct formation was attributed to induction of CYP1A1/2 enzymes, which are responsible for oxidative activation of ellipticine to 13-hydroxy- and 12-hydroxyellipticine or that of BaP to benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE), the metabolites generating major DNA adducts by these compounds. Taken together, these results demonstrate that by inducing CYP1A1/2, ellipticine and BaP increase their own enzymatic metabolism leading to an activation of these xenobiotics to reactive species forming DNA adducts, thereby modulating their either pharmacological (ellipticine) and/or genotoxic potential (both chemicals).

Supported GACR (203/06/0329) and MSMT CR (MSM0021620808).

P2
ROLE OF CYTOCHROME b_5 IN REGULATION OF CYTOCHROME P450-DEPENDENT PHARMACOLOGICAL EFFICIENCY OF ELLIPTICINE

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Recently, we found that anticancer drug ellipticine forms DNA adducts *in vitro* and *in vivo*. The formation of the major DNA adduct is dependent on the activation of ellipticine by cytochromes P450 (CYP). Here, not only the molecular mechanism of its activation and detoxication catalyzed by CYPs *in vitro* is resolved, but we also describe for the first time the mechanism, which explain how individual CYPs participate in ellipticine activation *in vivo*. The 9-OH- and 7-OH-ellipticine are the detoxication metabolites. 13-OH- and 12-OH-ellipticine are metabolites forming two ellipticine-DNA adducts. Many CYP-dependent reactions have been shown to be stimulated by another microsomal protein, cytochrome b_5 (cyt b_5). Here we found that this protein in the CYP1A1/2 reconstitution system changed significantly the pattern of ellipticine metabolites as well as kinetics of its oxidation; formation of 9-OH- and 7-OH-ellipticine is decreased, while that of 13-OH- and 12-OH-ellipticine, metabolites generating DNA adducts, is increased. Other proteins (myoglobin, HSA) or hemin were without this effect. CYP3A4, the most abundant CYP in human livers, is affected by cyt b_5 too, but in a different way. Here all five metabolites are created in a higher amount. The results explain clearly why CYPs of a 1A subfamily are one of the predominant enzymes (together with CYP3A) generating ellipticine-DNA adducts *in vivo* even though in *in vitro* incubations they are much less active. Furthermore, the study forms the basis to further predict the susceptibility of human cancers to ellipticine.

Supported GACR (203/06/0329) and MSMT CR (MSM0021620808).

P3

CYTOCHROME P450 1A SUBFAMILY INDUCTION BY FLAVONOIDS

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The increasing incidence of gastrointestinal tract cancer, namely of the intestines and rectum poses a very serious threat. Dietary composition is one of the factors raising the probability of the carcinoma incidence. Compounds in human diet, drugs and dietary supplements are inducers of cytochromes P450, the enzymes responsible for xenobiotic metabolism and carcinogen activation. Many natural flavonoids, which are considered to be chemopreventive compounds, may also exert inductive effects on cytochromes P450. Therefore, the aim of this project is to indicate which flavonoids influence the food carcinogen activation caused by cytochromes P450, mainly CYP1A subfamily.

We investigated the effect of 8 flavonoids (β-naphthoflavone, flavone, baicalin, resveratrol, naringenin, naringin, hesperetin, hesperidin) on CYP1A induction in male rats using Western blotting.

In liver microsomes, we demonstrated the induction of CYP1A1 by flavone and of CYP1A2 by naringin and hesperidin. However, the highest CYP1A1 induction in colon microsomes was observed after administration of naringenin. Elevation of marker activities EROD and MROD for CYP1A1 and CYP1A2, respectively, was in accordance with induction effects of unsubstituted flavonoids, β-naphthoflavone and flavone.

This work was supported by the Czech Science Foundation (Grant No. 303/06/0928 and 303/05/2195) and by the Ministry of Education, Youth and Sports of the Czech Republic (Grant No. MSM 0021620808).

P4
PROTEOMIC STUDY OF PIG LIVER MICROSOMES

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Pig liver microsomal proteome consists of many proteins; our main interest is in characterization of enzymes of biotransformation of foreign substances. As the pig is not only a farm animal but also an experimental model in pharmacology and other medical sciences (e.g. dermatology, physiology), a comparison of the properties of pig systems incl. enzymes with their human counterparts is necessary. Enzymes of biotransformation, mainly cytochromes P450 (CYP450), play a crucial role and their properties may significantly influence the results of studies performed with pigs and their use as sources of tissues and subcellular structures for human medicine.

Isolation of CYP450 isoforms is a multistep proces begining with isolation of liver microsomal fraction. Microsomal fraction was solubilized by cholate and applied onto the octylamino Sepharose CL-4B column. One of the peaks eluted from the octylamino Sepharose column [eluted with buffer (pH 7.25) containing 0.1 M K-phosphate, 1 mM EDTA, 20% (v/v) glycerol, 0.33% Na-cholate, and 0.06% (v/v) Triton X-100 PC] contained CYP1A enzyme together with other P450 forms. This fraction was applied on hydroxylapatite column equilibrated with buffer (pH 7.4) of the following composition: 5mM K-phosphate, 0.05 mM EDTA, 20% (v/v) glycerol. Elution of CYP1A was achieved by a linear gradient of K-phosphate (10 mM to 300 mM). The course of purification has been followed by electrophoresis (Mini Protean apparatus; Bio-Rad) and Western blotting using the goat anti rat CYP1A1 IgG. Electrophoretic separation was performed by SDS-PAGE and gels were subjected to MALDI-TOF spectrometry. Proteomic analysis revealed the presence of CYP1A in the sample obtained. In other samples, also the presence of CYP2A19, 2E1, 2D25 and FMO1 was detected.

Acknowledgment
Financial support from COST861 (1P05OC050) and from Palacky University grant 91110061 is gratefully acknowledged.

P5
INETICS OF OXIDATION OF 3-AMINOBENZANTHRONE BY CYTOCHROMES P450

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The suspected human carcinogen 3-nitrobenzanthrone (3-NBA) is one of the most potent mutagens identified in diesel exhaust and ambient air pollution. The main metabolite of 3-NBA, 3-aminobenzanthrone (3-ABA), was recently detected in the urine of salt mining workers occupationally exposed to diesel emissions, indicating that exposure to 3-NBA can be significant and is detectable. Understanding which enzymes are involved in the activation of 3-ABA is important in the assessment of susceptibility to this molecule. We recently showed that principal enzymes forming DNA adducts from 3-ABA in livers are cytochromes P450 (CYP), especially CYP1A1 and 1A2. Rat hepatic microsomal CYPs oxidize 3-ABA up to three metabolites. These metabolites were separated by HPLC as distinguish product peaks. Using co-chromatography with synthetic standards, two of them were identified to be oxidative metabolites of 3-ABA, *N*-hydroxy-3-ABA and 3-NBA. The structure of the third metabolite (eluted with the retention time of 18 minutes) remains to be characterized. Kinetics of oxidation of 3-ABA by microsomes isolated from livers of rats treated with PB, β-NF and by those of control rats was analyzed. The most effective oxidation of 3-ABA was detected using PB-microsomes, followed by β-NF-microsomes and control microsomes. The values of Michealis constant (K_m), maximum reaction velocities (V_{max}) for 3-ABA oxidation by the microsomal systems were determined. The results suggest that CYP1A1/2 are responsible for the oxidative activation of 3-ABA in livers, whereas CYP2B subfamily might be responsible for detoxication of 3-ABA.

Supported by Grant Agency of Czech Republic (grant 303/05/2195)

P6
OXIDATION OF CARCINOGENIC 2-NITROANISOLE AND 2-NITROPHENOL BY CYTOCHROMES P450

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2-Nitroanisole (2-NA) is an important industrial pollutant. It's used primarily as a precursor in the synthesis of *o*-anisidine (*o*-methoxyaniline), an intermediate in the manufacture of many azo dyes. 2-NA exhibits carcinogenic activity, causing neoplastic transformation in the urinary bladder and, to a lesser extent, in spleen, liver and kidneys in rodents. 2-NA is oxidized by rat, rabbit and human hepatic microsomes to three metabolites, 2-nitrophenol (2-NP), 2,5-dihydroxynitrobenzene (2,5-DNB) and 2,6-dihydroxynitrobenzene (2,6-DNB). 2-NP is the major metabolite generated by rabbit and rat microsomes, but 2,5-DNB is the predominant product in human microsomes. Microsomes of control rats were the most effective in oxidation of 2-NA to 2-NP, followed by microsomes of rats treated with phenobarbital (PB) and β-naphtoflavone (β-NF). 2-NP is metabolized by rat microsomes to its metabolite, 2,5-DNB. Microsomes of rats treated with PB were the most efficient enzymes metabolizing 2-NP, followed by microsomes of control rats and rats treated with β-NF. The effect of four inhibitors - α-naphtoflavone (α-NF), diethyldithiocarbamate (DDTC), diamantane (DIA), sulfaphenazole (SULPH) - on the metabolism 2-NP was investigated. DDTC and DIA inhibit generation of 2,5-DNB, whereas α-NF and SULPH were almost without this effect.

Supported by Grant Agency of Czech Republic (grant 303/05/2195)

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Section 2	Recognition of Xenobiotics by target structure

P7
BIOTRANSFORMATION OF FLUBENDAZOLE AND SELECTED MODEL XENOBIOTICS IN *HAEMONCHUS CONTORTUS*

V. Cvilink[1], V. Kubíček[1], M. Nobilis[1], V. Křížová[1], B. Szotáková[1], J. Lamka[1], M. Várady[2] and L. Skálová[1*]

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Haemonchus contortus is one of the most pathogenic parasites of small ruminants. The treatment of haemonchosis is complicated because of frequent resistance of *H. contortus* to common anthelmintics. The resistance development can be facilitated by action of drug metabolizing enzymes of parasite that can deactivate anthelmintics and thus protect parasite against toxic effect of drugs. The aim of this project was to study the phase I biotransformation of benzimidazole anthelmintic flubendazole and other model xenobiotics in *H. contortus* subcellular fractions. The results showed that cytosolic NADPH-dependent enzymes of *H. contortus* deactivate flubendazole via reduction of its carbonyl group. The apparent kinetic parameters of this reaction were determined (V'_{max} = 39.8 ± 2.1 nM min^{-1}, K'_m = 1.5 ± 0.3 μM). The reduction of flubendazole in *H.contortus* is stereospecific, the ratio of (-) : (+) enantiomers of reduced flubendazole was 90 : 10. Reduced flubendazole was the only phase I metabolite found. Effective reduction of other xenobiotics with carbonyl group (metyrapon, daunorubicin, oracin) was also found. Significant activity of carbonyl reducing enzymes aids to *H.contortus* surviving the attack of anthelmintics or other carbonyl group containing xenobiotics.

This project was supported by Grant Agency of Czech Republic, Grant No. 524/06/1345

P8
BIOTRANSFORMATION OF ALBENDAZOLE AND FLUBENDAZOLE IN *HAEMONCHUS CONTORTUS*: GLUCOSE CONJUGATE FORMATION

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A limited information on biotransformation of anthelmintic drugs in parasitic helminths is available. The aim of our work was to describe the metabolism of albendazole (ABZ) and flubendazole (FLU) in parasitic helminth *Haemonchus contortus*, a worldwide distributed parasite of ruminants. ABZ and FLU are benzimidazole anthelmintics widely used in nematode, trematode and cestode parasitoses treatment. One hundred vivid adults of *H. contortus*, obtained from abomasum of an experimentally infected lamb, were incubated with 10-μM benzimidazole drug (ABZ or FLU) in 5 ml of DMEM medium for 24 hours (37°C, 5% CO_2). The parasite bodies were homogenized, both parasite homogenates and DMEM medium from the incubation were extracted using solid phase extraction. The extracts were analyzed with a liquid chromatograph-triple quadrupole mass spectrometer with electrospray ionization in positive ion mode. The acquired data show that *H. contortus* is able to metabolize ABZ into S-oxide and FLU into its reduced metabolite. Moreover, the glucose conjugates were observed. Detection of all these metabolites signalizes the presence of both phase I and phase II metabolism enzymatic systems in *H. contortus*.

This project was supported by Grant Agency of Czech Republic, grant No. 524/06/1345 and The Fund of Mobility of Charles University.

P9
NON-SPECIFIC IMMUNE RESPONSE AFTER dsRNA APPLICATION

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RNA interference is emerging as a powerful experimental tool for target specific knockdown of gene expression and gene function studies. The potential use of this technology seems to be unlimited, extending to the clinical use and development of new target-specific drugs.

However, recent works demonstrating that there are unanticipated, different nonspecific effects, including activation of the cellular innate immune system, associated with the use of RNA interference has raised concerns about the safety of use of this technique in vivo.

In our study we prepared 3 plasmid constructs with RNA polymerase III promotor (human U6) expressing shRNA for PTGS1 (COX1) knock-down. Hep2 cell line was transfected with these constructs and stable cell line was selected by blasticidin. Cells were harvested 14 days after blasticidin removal, RNA was isolated and cDNA prepared using reverse transcription.

Quantitative RT-PCR was used to measure changes in expression of eight genes involved in cellular innate immune response. Significant changes in expression of selected genes were confirmed in all samples. Project continues with measuring changes in gene expression after siRNA application.

P10
REDUCTION OF ANTICANCER DRUG DOXORUBICIN BY RECOMBINANT AKR1C3

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Anthracyclines are among the most used antitumor drugs and are active against a wide variety of malignancies. Doxorubicin is a highly active agent in the management of patients with solid tumors, including breast cancer. However, its therapeutic use is limited by a number of side effects like acute myelosuppression and cumulative dose-related cardiotoxicity caused by a conversion of doxorubicin to its major metabolite doxorubicinol.

Doxorubicin is also metabolized by a member of aldo-keto reductase superfamily, AKR1C3. This enzyme reduces a weak androgen, androstenedione, to the potent androgen testosterone, and a weak estrogen estrone to the potent estrogen 17β-estradiol, using NADPH as a coenzyme. Estrogens play an important role in progression and development of breast cancer. The local excess of 17β-estradiol is implicated in this disease. Therefore, AKR1C3 can reduce efficacy of anticancer treatment by metabolizing the active cytostatics and also speed up the disease development.

The aim of your work was to evaluate the extent of doxorubicin reduction by recombinant AKR1C3. We used a modified method of Fogli et al. (1999) for incubation of doxorubicin with the enzyme and HPLC determination of its metabolite, doxorubicin. The kinetic parameters of doxorubicin reduction by AKR1C3 indicate that the reaction is not very extensive.

This project was supported by Ministry of Education of the Czech Republic (FRVŠ G/D/1074).

P11
ISOLATION AND CHARACTERIZATION OF DEOXYGUANOSINE ADDUCT FORMED FROM SUDAN I ACTIVATED BY PEROXIDASE

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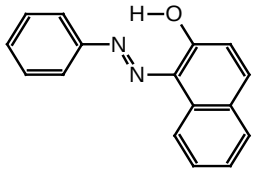

Sudan I

1-Phenylazo-2-hydroxynaphthalen (Sudan I) is a liver and urinary bladder carcinogen in mammals. Peroxidase in presence of H_2O_2 oxidizes Sudan I to reactive intermediates (radicals), which are able to covalently modify DNA, RNA and proteins. These covalent adducts differ from those formed from Sudan I activated by cytochromes P450. The carcinogen oxidized by peroxidases form DNA adducts, which are similar to those formed in the rat urinary bladder *in vivo*, the target tissue for Sudan I carcinogenicity rich in these enzymes. The adduct corresponding to that formed in tRNA was isolated utilizing combination of extraction, TLC and HPLC methods. Mass and NMR spestroscopy were carried out as shown in this work. Sudan I is oxidized by peroxidase to eight products. Two of them were isolated by TLC and HPLC. These metabolites were partially characterized by UV/VIS and mass spectrometry. Both Sudan I metabolites were identified as Sudan I dimers, the characterization of their exact structures remains to be resolved. The major adduct was separated by TLC and/or HPLC and characterized by UV/VIS and mass spectroscopy as well as by NMR. It is evident that the whole Sudan I molecule is bound to (deoxy)guanosine residues. The possible position of Sudan I radical binding to (deoxy)guanosine is proposed.

Supported by GAČR (203/06/0329) and Ministry of education of the ČR (MSM0021620813).

P12
DICROCOELIUM DENDRITICUM – BIOTRANSFORMATION OF ANTHELMINTICS AND OTHER DRUGS

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Dicrocoeliosis – a helminthosis caused by lancet fluke (*Dicrocoelium dendriticum*) – is at present considered a world-widely significant but little investigated illness of farm, domestic and wild animals, and a relatively rare illness of man. The only means so far generally accepted against lancet fluke is pharmacotherapy and pharmacoprophylaxis practised in endangered or attacked breeds with the use of suitable anthelmintics (preference is given to benzimidazole anthelmintics). Biotransformation enzymes can, to a certain extent, protect the parasitic worms against the toxic effects of anthelmintics. The aim of this project was to study the metabolism of benzimidazole anthelmintic albendazole and to assay drug metabolizing enzymes activities in lancet fluke subcellular fractions. *In vitro* sulphoxidation of ABZ in *Dicrocoelium dendriticum* was proven. The highest velocity of ABZ sulphoxide formation was in mitochondria-like fraction, lower in microsomes-like fraction and none ABZ sulphoxide arise in cytosole-like fraction. The apparent kinetic parameters of this reaction were determined (mitochondria-like: V'_{max} = 23.9 nM.min^{-1}, K'_m = 265 nM, microsomes-like: V'_{max} = 8.6 nM.min^{-1}, K'_m = 304 nM). Sulphoxidation of ABZ is stereospecific, formation of (-)-ABZ sulphoxide enantiomer prevail. ABZ sulphoxide was the only phase I metabolite found. New findings about detoxification mechanisms of lancet fluke will contribute to the research of parasites and mechanisms of helminthoresistance.

This project was supported by the Grant Agency of Czech Republic, Grant No. 524/07/0611.

P13
CARBOXYLESTERASE 1 – HUMAN LIVER MICROSOMAL CARBONYL REDUCTASE?

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Carboxylesterases are found in variety of tissues with high activity detected in liver where at least two isoenzymes were detected. Carboxylesterase 1 is a glycoprotein associated with endoplasmic reticulum membrane. It mainly hydrolyzes a substrate with a small alcoholic group and large acyl group, but its wide active pocket allows it to act on structurally distinct compounds.

Microsomal fraction of human liver was solubilized, desalted and loaded onto an anion exchange chromatography column – HiTrap Q FF. The active fraction was applied to isoelectric focusing and tested with nondenaturating PAGE. The native PAGE gel slices were stained with Coomassie blue and for reductive activity with *p*-iodonitrotetrazolium and Meldola´s Blue, and analyzed by MALDI-TOF. The reductive activity was measured via HPLC quantification of the metabolite dihydrooracin after incubation of each enzyme fraction with oracin and an NADPH generating system.

Our results are surprising and indicate that the enzyme with high reductive activity against oracin is carboxylesterase 1. The value of pI of the active enzyme determined by isoelectric focusing was 5.1-5.8 and mainly MALDI-TOF analysis showed only one enzyme present in the active band of native PAGE. All these facts correspond with data for carboxylesterase 1.

This project was supported by Grant Agency of the Czech Republic (GAČR, 303/07/0994).

P14
ROLE OF AKR1C3 IN METABOLISM OF ANTICANCER DRUG ORACIN.

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Human aldo-keto reductases (AKR) of the 1A, 1B, 1C and 1D subfamilies are involved in the pre-receptor regulation of nuclear (steroid hormone and orphan) receptors by regulating of local concentrations of their lipophilic ligands. AKR1C3, also known as type V 17β-hydroxysteroid dehydrogenase (HSD), is one of the most interesting isoforms. It catalyzes the reduction of Δ^4-androstene-3,17-dione to yield testosterone, the reduction of 5α-dihydrotestosterone to yield 3α- and 3β-androstanediol, and the reduction of estrone to yield 17β-estradiol. Relatively high mRNA expression of AKR1C3 was found in human prostate and mammary gland where it is implicated in regulating ligand access to the androgen and estrogen receptor, respectively.

While the most attention is paid to the higher expression of AKR1C3 and its relation to the hormonal level, less attention is given to the biotransformation effect of AKR1C3. Oracin, 6-[2-(2-hydroxyethyl)-aminoethyl]-5,11-dioxo-5,6-dihydro-11*H*-indeno[1,2-*c*]isoquinoline, is one of the prospective anticancer drugs, presently in Phase II clinical trials.

In the present project the extent of deactivation (reduction) of oracin by incubation with recombinant AKR1C3 was tested and its contribution to the cancer therapy resistance was discussed.

This project was supported by Grant Agency of Charles University, Grant No 108/2006/C.

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Section 3	Animal cell as a model for study of xenobiotics

P15
THE EFFECTS OF LY294,002, A SPECIFIC INHIBITOR OF PI3K/AKT KINASE PATHWAY, ON P-GLYCOPROTEIN-MEDIATED MULTIDRUG RESISTANCE

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P-glycoprotein (P-gp) is transport pump that causes the efflux of chemotherapeutic agents from cells and secures multidrug resistance (MDR) of neoplastic cells. In the present study drug sensitive L1210 and MDR L1210/VCR mouse leukemic cell lines were used as an experimental model. We found that LY294,002 (LY), a specific inhibitor of PI3K/Akt kinase pathway, significantly reduced the degree of vincristine (VCR) resistance in L1210/VCR cells. FACS analysis of proportion of cells in apoptosis or necrosis by annexin-V apoptosis kit showed the following: i.) VCR induced apoptosis in resistant cell to much lower extent as in sensitive cells; ii.) LY alone did not induce apoptosis or necrosis in both sensitive and resistant cells; iii.) LY applied together with VCR significantly increased the number of apoptotic cells. Transport activity of P-gp monitored in cells using calcein/AM as substrate was in resistant cells depressed by LY in concentration dependent manner. Sensitive and resistant cells contain similar amount of uncleaved (unactivated) caspase-3 but in latter cells the activation of caspase-3 by proteolytic cleavage was decreased. The reversal of VCR resistance of L1210/VCR cells by LY was associated with marked activation of caspase-3. All the above findings point to the possible involvement of PI3K/Akt kinase pathway in modulation of P-gp mediated MDR in L1210/VCR cells. Moreover, MDR reversal effect of LY is accompanied with influence of this compound on vincristine-induced apoptosis.

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P16

NUCLEAR THYROID HORMONE RECEPTORS: EFFECTS OF VINCLOZOLIN, BISPHENOL A, AND GENISTEIN IN HUMAN MCF-7 CELLS

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Vinclozolin is a non-systemic fungicide of the dicarboximide group, registered for both pre- and post-harvest use on fruits, vegetables and ornamental plants to control *Botrytis spp., Sclerotinia spp., Monilia fruiticola* and *Gloeosporium spp.* Bisphenol A (BPA) is used in the production of epoxy resins and polycarbonate plastics. Genistein belongs to the isoflavone class of flavonoids, and it is also classified as a phytoestrogen.

The present study was undertaken to investigate the *in vitro* effects of vinclozolin, BPA and genistein i) on thyroid hormone binding to its cognate nuclear receptors in rat liver and ii) on expression of both nuclear thyroid hormone receptor subtypes (TRalpha1, TRbeta) in MCF-7 cell line treated with the above compounds at the concentration of 1 μmol/l for 24 and 72 h. The binding data were evaluated from the competition curves and the expression of thyroid hormone receptors has been analyzed by the RT-PCR technique.

Our data has shown that TRalpha1 expression has been enhanced in MCF-7 cells by both vinclozolin and BPA either after 24 h or 72 h treatment. Genistein caused enhanced expression of TRalpha1 only after treatment of the MCF-7 cells for 72 h. Genistein decreased expression of TRbeta when the MCF-7 cells were treated with the compound for 24 h. On the other hand, this compound exerted enhanced TRbeta expression after 72 h treatment when compared to untreated cells. Similar enhancement of the TRbeta expression has been observed after 72 h treatment of the MCF-7 cells by BPA.

These results from *in vitro* experiments suggest that these tested compounds may play a marked role in modulation of both TRalpha1 and TRbeta expression in MCF-7 cells.

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P17
DNA ADDUCTS FORMATION, INDUCTION OF OXIDATIVE STRESS AND APOPTOSIS IN MODEL OF RAT LIVER PROGENITOR CELLS

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In contrast to hepatocytes or hepatoma cells lines, it has been long proposed that drug-metabolizing enzymes are present at low or undetectable levels in liver progenitor cells. In this study, we determined expression and inducibility of CYP1A1, CYP1B1, AKR1A1and AKR1C9, which are involved in two alternative pathways of oxidative metabolism of PAHs, in rat liver epithelial WB-F344 cell line, an in vitro model of liver progenitor cells. We further measured DNA adduct formation and induction of reactive oxygen species (ROS) as two parameters of genotoxic insult, which may lead to apoptosis and phosphorylation of H2AX and p53 proteins. AKR1A1, although being present at significant levels, was not induced by PAHs. Several carcinogenic PAHs inducing high levels of CYP1A1 and CYP1B1, such as benzo[b]fluoranthene and dibenzo[a,h]anthracene, formed only a limited amount of DNA adducts. Additionally, despite their ability to induce AKR1C9, which may contribute to oxidative stress, no increase in ROS levels, p53 and H2AX phosphorylation and apoptosis was observed. In contrast, dibenzo[a,l]pyrene, benzo[a]pyrene and 7,12-dimethylbenz[a]anthracene formed high levels of DNA adducts, induced arrest in S-phase of cell cycle and apoptosis. Simultaneously, all these potent genotoxins induced ROS production as well, suggesting that the AKR-dependent pathway leading to oxidative DNA damage may contribute to their genotoxic effects. [This work was supported by the Czech Ministry of Agriculture, grant No. MZE0002716201.]

P18
RESVERATROL AND SILYMARIN PRETREATMENTS AMELIORATE TERT-BUTYLHYDROXIDE AND D-GALACTOSAMINE INDUCED HEPATOCYTE APOPTOSIS AND NECROSIS

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Some drug molecules have been reported to be liver protectors including those of natural origin such as, the modified flavonoids and antioxidants in general. The reported experimental and clinical results are encouraging to conduct more extensive comparative experimental studies with specific treatment in various types of liver damage. Resveratrol and silymarin which are polyphenolic compounds do possess antioxidant activity. The aim of this work was to study the effects of resveratrol (RES, trans-3,4',5-trihydroxystilbene) and silymarin (milk thistle, Silybum marianum) pretreatments on D-galactosamine (GalN) and tert-butylhydroxide (TBH) induced apoptotic/necrotic markers in hepatocytes. Hepatocyte in cultures (48 h) and in perifused immobilized agarose threads (5 h) were used as cellular systems. Hepatocyte apoptosis was estimated by caspase-3 activity, cytosolic cytochrome-c DNA fragmentation, and morphologically using Annexin-V/propidium iodide staining. Hepatocyte viability, funtionality and mitochondrial activity were evaluated by ALT, urea synthesis and MTT test. Nitric oxide (NO) levels were examined. Silymarin and resveratrol reduced TBH and GalN induced hepatocyte toxic effects in the short term experiments as measured by reduction in ALT and NO increase produced by TBH. Resveratrol increased urea synthesis that was reduced by GalN. The effects of resveratrol and silymarin were not evidenced in hepatocyte culture at both apoptotic or necrotic levels after 48 h. Morphological findings supported the biochemical ones and where RES reduced apoptotic markers in hepatocytes for short term experiments. Nevertheless both resveratrol and silymarin improved hepatocyte stability in both cellular systems. It may be concluded that resveratrol and silymarin ameliorative effects on GalN and TBH hepatocyte toxicity are comparable and should be re-evaluated in various in vitro experimental conditions. Further studies are needed to differentiate between antiapototic or antinecrotic mechanisms of RES and silymarin against various types of hepatocyte insults.

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P19

CHANGES OF APOPTOSIS IN P-GP POSITIVE L1210/VCR CELLS

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Multidrug resistance (MDR) cells show resistance to a wide variety of structurally and functionally unrelated compounds. MDR is often associated with expression of drug transporting P-glycoprotein (P-gp) in neoplastic cells. MDR cells exhibite a resistance to apoptosis induced by chemotherapeutics (1). Cisplatin (cisPt) – an anticancer drug that is un-transportable by P-gp is also known as inductor of apoptosis. We found that P-gp positive L1210/VCR (R) cells are more sensitive to cisPt as parental L1210 (S) cells. Interestingly, we detected that R cells under application of cisPt were entering the apoptosis in lower extend as S cells. We found predominating amounts of antiapoptotic Bcl-2 protein and proapoptotic Bax protein in Bcl-2/Bax complexes obtained by immunoprecipitation from R and S cells, respectively. Moreover, cancer cells with resistance to cisPt posses reduced inositol 1,4,5- triphosphate receptor (IP_3R) expression that is believed to be connected with reduction of apoptosis (2). IP_3R encoding mRNA level was downregulated only in R cells cultivated on presence of vincristine (VCR) when compared with the R cells cultivated in the absence of VCR and S cells. Calnexin, another endoplasmatic reticulum protein was described to ensure maturation of P-gp (3). However, in our experiments we detected lower levels of calnexin in R as in S cells.

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P20
STUDY OF ARNT-DEPENDENT HYPOXIC AND XENOBIOTIC REGULATION OF CARBONIC ANHYDRASE 9 PROMOTER IN TUMOR CELL LINES

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Tumor-associated expression pattern of carbonic anhydrase IX is mainly regulated by the strong activation of *CA9* gene transcription via hypoxia-inducible factor 1 (HIF-1). HIF-1 binds to the hypoxia responsive element (HRE) localized in the *CA9* promoter. HIF-1 is a heterodimeric transcription factor that consists of an oxygen-sensitive α subunit and a constitutively expressed β subunit, also known as ARNT (aryl-hydrocarbon nuclear translocator). ARNT is not only involved in hypoxia- but also in xenobiotic-induced trancriptional activation. It also binds the liganded aryl-hydrocarbon receptor (AhR) (AhR resides in the cytoplasm and is translocated to the nucleus only after binding to an agonist, e.g. TCDD) and induces the transcription of genes with xenobiotic response elements (XRE) in their promoters. The interplay of these pathways could affect the transcription of *CA9* and many other genes. For this study we have chosen wild-type and mutated mouse hepatoma cell lines (deficient in ARNT or AhR), as well as HeLa cell line, and performed reporter assays with different *CA9* promoter constructs cotransfected with ARNT or AhR or constitutively active AhR. Our results indicate that these pathways crosstalk, and that hypoxic CAIX expression is reduced in the presence of constitutively active AhR or in the cells transfected with the wild-type AhR and treated with TCDD.

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P21
CYTOTOXIC ACTIVITY OF QUINOLINE DERIVATIVE AAQ ON LEUKEMIA CELL LINES

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In our recent studies 4-amino-3-acethylquinoline (AAQ) induced apoptosis in murine leukemia L1210 cells that was confirmed by typical apoptotic DNA fragmentation and caspase-3 activation.

In this study we have elucidated the way of death in human leukemia HL60 and U937 cells treated by AAQ. Cytotoxic effect of AAQ after 24-72 h treatment was determined and characterised by IC_{50} and IC_{100} values. Induction of apoptosis was detected by agarose gel electrophoresis. Results did not confirm the ability of tested concentrations of AAQ to induce apoptosis after 24 - 72 h treatment in both cell line. Cytotoxic concentrations of AAQ were used to monitore necrosis of HL60 and U937 cells by fluorescence microscopy (Hoechst 33258/propidium iodide). Cell membrane damage of treated cells was determined by measurement of LDH activity in culture medium after 24 - 72 h treatment with AAQ in comparison with negative control.

Proapoptotic activity of AAQ was not confirmed in human HL60 and U937 cells. Treatment of human leukemia HL60 and U937 cells with AAQ lead to the loss of cytoplasmic membrane integrity and necrotic cell death.

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P22
CYTOTOXIC AND GENOTOXIC EFFECT OF 3-(5-NITRO-2-THIENYL)-9-CHLORO-5-MORPHOLIN-4-YL [1,2,4]TRIAZOLO[4,3-C]QUINAZOLINE IN L1210 AND NIH-3T3 CELLS

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Quinazolines – 1,3-benzodiazines- are multitarget agents with broad spectrum of biological activity and some of them are now in cancer clinical testing. 3-(5-nitro-2-thienyl)-9-chloro-5-morpholin-4-yl[1,2,4]triazolo[4,3-c]quinazoline (NTCHMTQ) is a new synthetically prepared quinazoline derivative that in our previous studies showed cytotoxic effects on four selected microorganisms and on cancer B16 and HeLa cells. Therefore in the present study we examined the cytotoxic and genotoxic effects of NTCHMTQ on cancer L1210 and non-cancer NIH-3T3 cells. NTCHMTQ induced different cytotoxic/antiproliferative effect dependent on used concentration, time of influence and cell line. While the highest tested NTCHMTQ concentrations induced an acute cytotoxic effect manifested by degeneration (lysis) of L1210 and NIH-3T3 cells, the lowest tested concentrations caused the cytotoxic/antiproliferative effect proportional to used concentration. The formation of DNA damage induced by NTCHMTQ was studied by single cell gel electrophoresis. NTCHMTQ induced weak genotoxic effect that was higher in L1210 than in NIH-3T3 cells. We also found that NTCHMTQ did not induce oxidative DNA damage.

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P23
EFFECTS OF VINCLOZOLIN, BISPHENOL A AND GENISTEIN ON RETINOID AND REXINOID NUCLEAR RECEPTORS AND THEIR COREGULATORS EXPRESSION IN HUMAN MCF-7 CELLS

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Compounds as Vinclozolin, Bisphenol A (BPA) and Genistein, belong to a group of chemicals known as endocrine disruptors. Vinclozolin is a protectant non-systemic dicarboximide fungicide. Bisphenol A is an industrial chemical used primarily to make polycarbonate plastic and epoxy resins. Genistein belongs to the isoflavone class of flavonoids and it is also classified as a phytoestrogen with weak estrogenic and anti-estrogenic properties.

The aim of present study was to investigate the *in vitro* effects of vinclozolin, BPA and genistein i) on retinoic acid binding parameters to its cognate nuclear receptors in rat liver and ii) on expression of nuclear RAR and RXR subtypes (RARalpha, beta, gamma and RXRalpha, beta) a their coregulators (SMRT and SRC-1) in MCF-7 cell line treated with the above compounds at the concentration of 1 μmol/l for 24 and 72 h.

Our data has shown that vinclozolin enhanced expression of RARalpha, RARbeta, RARgamma and both coregulators of nuclear receptors SMRT and SRC-1 in MCF-7 cells after 24 h treatment. On the other hand, 72 h treatment with vinclozolin resulted in enhanced expression of RARgamma and RXRbeta and reduced expression of RXRalpha. BPA enhanced expression of RARbeta and RARgamma after 24 h treatment. We have found enhanced expression of RARalpha, RARbeta, RARgamma, RXRbeta and SMRT and also reduced expression of RXRalpha when treated for 72 h. Treatment of the MCF-7 cells with genistein for 24 h did not affect expression of all studied parameters except SMRT which expression was decreased. Genistein caused enhanced expression of RARbeta, RARgamma, RXRbeta, SMRT and SRC-1 only after treatment of the MCF-7 cells for 72 h.

These results from *in vitro* experiments suggest that these compounds may play a marked role in modulation of expression of both retinoid and rexinoid receptors as well as their coregulators in MCF-7 cells. This work was supported by the grant of the European Commission No.: FOOD-CT-2004-506319 and the VEGA grant No.2/5017/5.

P24
STUDIES OF ENERGY-PRODUCING PROCESSES IN EHRLICH CARCINOMA CELLS BY PYRIDINEDICARBOXYLATES Cu(II) COMPLEXES WITH BIOLOGICALLY ACTIVE LIGANDS

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A new class of pyridinedicarboxylates Cu(II) complexes (PDC) with biologically active ligands has been synthesized and tested on energy-producing processes in Ehrlich ascites carcinoma (EAC) cells. The first step in our programme is the primary screening of all complexes soluble in DMSO on aerobic glycolysis of EAC cells after 2 h incubation at 37°C. Complexes can be divided into two groups a) derivatives of 2,6-pyridinedicarboxylic acid (8 complexes) and b) derivatives of 2,3-pyridinedicarboxylic acid (3 complexes). All compounds were tested in unified concentration 600 μmol/L. The consumption of glucose by EAC cells was most inhibited by complexes No. 2 [e.g. diaqua-(2,6-pyridinedicarboxylate Cu(II))] and No. 8 [e.g. nicotineamide-(2,6-pyridinedicarboxylate Cu(II))]. The attachment of cooper (II) ions and nicotineamide essentially increased the inhibitory effect of tested complexes. We selected one of the most effective complexes (No. 2) for detailed studies. We followed effect of complex 2 on aerobic glucose utilization and lactic acid production in time and concentration dependance. At the highest concentration (300 μmol/L) caused a rapid and almost full inhibition of glycolysis. At lower concentration complex 2 decreased consumption of glucose proportional to concentrations. Complex 2 reduced level both total and non-protein thiol groups. At the same time we studied effect of PDC on respiratory processes in EAC cells. Complex 2 was most effective inhibitor of endogenous respiration.

This study was supported by the Grant Agency VEGA No. 1/4305/07.

P25
VINCLOZOLIN, BISPHENOL A, GENISTEIN AFFECT EXPRESSION OF NUCLEAR VITAMIN D_3 RECEPTOR AND CYP24 AND CYP27B1 IN HUMAN MCF-7 CELLS

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The following results were obtained using the human MCF-7 cell line treated with the above compounds at the concentration of 1 μmol/l for 24 h and 72 h. Semiquantitative RT-PCR analysis was used for the determination of relative mRNA expression levels of VDR as well as vitamin D_3 synthesizing enzyme 1α-hydroxylase (CYP27B1) and degrading enzyme 24-hydroxylase (CYP24).

In conclusion, vinclozolin was found to enhance expression of CYP27B1 and to decrease expression of CYP24 after both 24 h or 72 h treatment. BPA caused enhanced expression of VDR and CYP27B1 after 24 h treatment. Genistein treatment of the MCF-7 cells for 72 h resulted in decreased expression of CYP24.

These results from *in vitro* experiments suggest that these compounds may play a marked role in modulation of nuclear vitamin D_3 receptor expression and also in expression of 1α-hydroxylase and 24-hydroxylase in MCF-7 cells.

This work was supported by the grant of the European Commission No.: FOOD-CT-2004-506319 and the VEGA grant No.2/5017/5.

P26
MODULATIONS OF AhR, ER AND AR SIGNALING BY COMPLEX CHEMICAL MIXTURES PRESENT IN RIVER SEDIMENTS

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Toxicity of complex chemical mixtures is one of hotly disputed topics in toxicology. In general, genotoxicity is often considered to be a major mechanism of mixture adverse effects. In this study, nongenotoxic effects induced by extracts of river sediments and their fractions were determined in endpoint-specific cellular models. We analyzed modulations of signaling of aryl hydrocarbon (AhR), estrogen (ER) and androgen (AR) receptors. The highest AhR-mediated activity, determined in the DR-CALUX assay, was found in a fraction of neutral aromatic compounds; polycyclic aromatic hydrocarbons were the most important contributors of this type of effect. In contrast, significant estrogenic activities, determined in the ER-CALUX assay, were observed in polar fraction of river sediment extracts. Sterols of anthropogenic origin and some industrial chemicals, such as dialkyl phthalates, were probably major xenoestrogens present in the sediment extracts. Strong antiandrogenic and a partial androgenic activities were determined in both neutral aromatic and in polar fractions, using the RT-PCR detection of AR-inducible expression of prostate specific antigen (PSA) mRNA in human prostate cancer LNCaP cell line as an endpoint. Our data suggest that disruption of AhR, ER and AR signaling might be important modes of action of environmental chemicals present in river sediments. [This work was supported by the EU FP6 project No. 511237-GOCE Modelkey and by the Czech Ministry of Agriculture, grant No. MZE0002716201.]

P27
ELLIPTICINE IS CYTOTOXIC TO NEUROBLASTOMA CELLS: MECHANISMS OF ACTION

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Ellipticine is an alkaloid exhibiting potent antineoplastic and anti-HIV activities. The inhibition of topoisomerase II after intercalation into DNA, was hitherto considered the most important property for its cytotoxicity. We found that ellipticine also acts as arylation agent, covalently binding to DNA after enzymatic activation. Deoxyguanosine is the target for ellipticine binding to DNA after its activation by enzymes in these cells. The mechanism of formation of these two ellipticine-deoxyguanosine adducts is proposed.

Here we show that two major ellipticine-DNA adducts are generated in human neuroblastoma cell lines in culture (i.e. IMR-32, UKF-NB-3, UKF-NB-4) and their levels correspond to toxicity of ellipticine to these cells. Besides parent neuroblastoma cells, ellipticine is toxic also to lines, which are resistant to doxorubicine, cis-platin and vincristine. The IC_{50} values are in a range of 1 microM for all tumor cells tested in the study, independent of resistance caused by these three individual cytostatics. Expression of enzymes (cytochromes P450, peroxidases) in neuroblastoma cells that might activate ellipticine, is evaluated. Enzymatic activation of ellipticine to a DNA binding species is an interesting finding in view of the compound's activity against human tumors like neuroblastoma cancer.

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P28
CYCLOOXYGENASE-1 KNOCK-DOWN BY shRNA IN CELL CULTURE: A MODEL SYSTEM FOR GENE EXPRESSION STUDIES

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The cyclooxygenase is the key enzyme in the conversion of the arachidonic acid into eicosanoids. The cyclooxygenase has two isoforms, cyclooxygenase-1 (COX-1) and cyclooxygenase-2. COX-1 is continuously expressed in cells and maintains tissue homeostasis. This study was conducted to utilize shRNA specific for COX-1 to establish a model eucaryotic cell system for arachidonic acid metabolism-related and unrelated gene expression studies.

The shRNA sequences were designed, synthesized and cloned into plasmid vector with human U6 promotor. Hep2 cells were transfected by the recombinant plasmid and selected in a cell culture medium with blasticidin. The stably down-regulated COX-1 clones were isolated by the cell cloning method.

The significant COX-1 down-regulation (20%) was confirmed by real-time PCR (on the mRNA level) and Western blot (on the protein level). The PGE2 inhibition was verified by ELISA. The quantification of arachidonic acid metabolism-related genes was performed and the results are discussed.

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P29
ANTI-PROLIFERATIC EFFECT OF SELENUIM ON MALIGNANT COLONIC CELLS

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Colon cancer is a major cause of cancer-associated mortality in the Czech Republic. Therefore it is necessary to broaden chances of anti-cancer therapy. Several selenium compounds have been studied in *in vitro* models as potential anti cancer agents. Induction of apoptosis and inhibition of cell proliferation are considered important cellular events that can account for the cancer preventive effect of selenium.

We studied the effect of sodium selenite, seleno-L-methionine and Se-(Methyl)selenocysteine on proliferation, metabolic activity and apoptosis in three colorectal cell lines with different malignant potential (HT-29, SW 480 and SW 620). Proliferation was measured as BrdU incorporation, total protein amount using Brilliant Blue staining and colorimetric WST-1 assay. Cytotoxicity was assessed by neutral red test. Induction of apoptosis was measured as caspase-3 activity fluorescence assay. Changes in cell morphology were studied by phase-contrast microscopy. Cells were exposed to selenium in concentrations 0-240 uM. All test were arranged in three incubation periods 24, 48 and 72 hours. The most potent compound in induction of apoptosis was Se-(Methyl)selenocysteine.

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P30
EFFECT OF LECTINES WITH DIFFERENT CARBOHYDRATE-BINDING SPECIFICITIES ON LDRUG SENSITIVE L1210 AND MULTIDRUG RESISTANT L1210/VCR CELLS

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Multidrug resistance of murine leukaemic cell line L1210/VCR (obtained by adaptation of parental L1210 cells to vincristine) is associated with overexpression of P glycoprotein (Pgp) – the ATP-dependent drug efflux pump. ^{31}P-NMR spectra of L1210 and L1210/VCR cells (the latter in the presence of vincristine) revealed besides the decrease of ATP level a considerable lower level of UDP-saccharides in L1210/VCR cells. Histochemical staining of negatively charged cell surface binding sites (mostly sialic acid) by ruthenium red (RR) revealed a compact layer of RR bound to the external coat of sensitive cells (S). In resistant cells cultivated in the absence (R) or presence of vincristine (V) the RR layer is either reduced or absent (1).

The effects of lectins with different carbohydrate-binding specificities on S, R and V cells were investigated. Cell viability was estimated by MTT test. *Ricinus communis* lectin (RCA) was exerting highest toxicity on both cell lines from all lectins applied. The N-acetylglucosamine binding *Lycopersicon esculentum* lectin (LEA) and sialic acid binding *Macckia amurensis* (MAA) lectin induced only small effect on viability of both cell lines. We observed higher sensitivity of S cells to ConA as compared with R cells. Consistently with this, we observed differences in interaction of S and R cells with lectins in agglutination experiments.

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P31
ANTIOXIDANT ACTIVITY OF PHENOLIC FRACTION FROM *LONICERA CAERULEA* L. VAR. *KAMTSCHATICA* BERRIES

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Lonicera caerulea L. (blue berry honeysuckle, Caprifoliaceae) fruits are rich in phenolic compounds, including anthocyanins, flavonoids and phenolic acids. The aim of our study was to assess the effect of phenolic fraction *L. caerulea* (var. *kamtschatica*) (LPF) cultured in Central Moravia (CZ) on *terc*-butyl hydroperoxide (*t*BH)-induced lipid peroxidation of rat liver microsomes and Cu^{2+}-induced oxidation of human low-density lipoproteins (LDL).

Rat liver microsomes were prepared by fractional centrifugation. Microsomes were incubated 1 hour at 37 °C in PBS in the presence of LPF (0-1000 $\mu g \cdot ml^{-1}$) and *t*BH. Lipid peroxidation was evaluated by formation of the thiobarbituric acid reactive substances (TBARS) at 532 nm as malondialdehyde. The effect of LPF was expressed as $IC_{50} \pm SD$. LDL were isolated from human serum by stepwise ultracentrifugation at 4 °C and incubated in the presence of 0.0, 0.5, 1.0 and 2.0 $\mu g \cdot ml^{-1}$ LPF or 5 μM ascorbic acid in PBS. LDL oxidation, induced by 10 μM $CuSO_4$, was continously monitored as formation of conjugated diene lipid hydroperoxides at 234 nm, 37 °C. The resistance of LDL to oxidation was estimated in terms of length of the lag phase.

LPF inhibited rat liver microsomes peroxidation with IC_{50} values 160±20 $\mu g \cdot ml^{-1}$. LPF at 0.5, 1.0 and 2.0 $\mu g \cdot ml^{-1}$ and ascorbic acid delayed LDL oxidation by 130±20 %, 200±30 %, 400±10 %, 150±20 %, respectively.

The study of LPF on *t*BH-induced damage of rat hepatocytes and LDL-induced damage of human umbilical vein endothelial cells is the subject of our ongoing research.

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P32

CYTOTOXICAL EVALUATION OF NEW FLUOR-HYDROXYAPATITE COMPOSITE ON HUMAN FIBROBLAST VH10 CELLS BY DIRECT CONTACT

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Fluor-hydroxyapatite composite, fluorapatite and hydroxyapatite discs prepared by precipitate method have been used to investigate the cytotoxic effect on human fibroblast VH10 cells after 24, 48 and 72 h of culturing. The cytotoxicity was established by direct contact. The cell proliferation, morphology, LDH released, protein and DNA content were examined. We found that permanent cell line VH10 was sensitive and suitable for measuring the toxicity of the tested biomaterials. Measuring of cell number, LDH level, cell protein and DNA content showed the same degree of cytotoxicity of tested biomaterials that was in the range of 4.62 – 20.80 %.

Acknowledgements
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P33
TUMOR NECROSIS FACTOR-ALPHA POTENTIATES GENOTOXIC EFFECTS OF BENZO[a]PYRENE IN LIVER PROGENITOR CELLS

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BaP is a ubiquitous environmental pollutant, which is believed to contribute to development of human cancer. Its carcinogenic activity is triggered by the metabolic activation of BaP via cytochrome P450 enzymes (CYPs), such as CYP1B1. The ultimate carcinogenic metabolite of BaP, BPDE, binds covalently to DNA, thereby causing errors in DNA replication leading to mutations, thus initiating process of carcinogenesis. The levels of CYPs in the liver can be modulated by proinflammatory cytokines, such as TNF-α, which are released during various liver diseases; the chronic inflammation is known to be associated with cancer etiology. In this study, we investigated interactions of TNF-α and BaP in regulation of expression of CYP1B1 in rat liver 'stem-like' WB-F344 cell line. TNF-α enhanced induction of CYP1B1, leading to a significantly enhanced formation of DNA adducts. The increased DNA damage corresponded with potentiation of BaP-induced apoptosis induced by BaP, arrest in S-phase of cell cycle, and an increased phosphorylation of p53 tumor suppressor at Ser-15. Our results imply that inflammatory cytokines might significantly enhance genotoxic effects of carcinogenic polycyclic aromatic hydrocarbons in liver progenitor cells. [Supported by grant No. 524/05/0595 from the Czech Science Foundation.]

P34

HOP PRENYLFLAVONOIDS IN RAT HEPATOCYTES AND MICROSOMES: METABOLISM AND BIOLOGICAL ACTIVITY

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Xanthohumol (XN) a principal prenylflavonoid present in hop cone extract is known to be isomerized to isoxanthohumol (IX) during the brewing procedure. IX is subsequently converted into a potent phytoestrogen, 8-prenylnaringenin (8-PN) by human intestinal microflora (Possemiers, J. Nutr. 136, 1862, 2006). The antioxidant activity of prenylflavonoids has been described (Miranda, J. Agric. Food Chem. 48, 3876, 2000; Rodrigez, Food Chem. Toxicol. 39, 437, 2001). In our study, we investigated the biotransformation of XN, IX and 8-PN by liver microsomes from rats treated with phenobarbital or β-naphtoflavone (Yilmazer, Drug Metab. Disp. 29, 223, 2001). More than 10 microsomal phase I metabolites of XN, IX and 8-PN were identified using RP-HPLC/MS/MS, e.g. products of hydroxylation, isomerization, cyclization, epoxidation and dimerization, some of them are described for the first time. In addition, the effect of XN on the oxidative damage to rat hepatocyte primary cultures was studied. After 30 min preincubation with 10 and 100 μg/ml XN protected hepatocytes from *tert*-butyl hydroperoxide (0.5 mM, 1.5 h) induced damage measured by MTT viability test, lactate dehydrogenase leakage and thiobarbituric acid reacting substances (TBARS). Phase II metabolites of prenylflavonoids and the biological activity of IX, 8-PN and their major metabolites both *in vitro* (hepatocytes) and in rats *in vivo* are the subject of our ongoing research.

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P35

DOWNREGULATION OF Ah RECEPTOR EXPRESSION USING RNA INTERFERENCE IN A MODEL OF LIVER PROGENITOR CELLS

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Aryl hydrocarbon receptor (AhR), a member of bHLH/PAS transcription factor family, is known to be responsible for tumorigenic effects of environmental pollutants, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) or benzo[a]pyrene (BaP). Absence or downregulation of AhR expression have been shown to prevent TCDD-induced toxic responses both *in vitro* and *in vivo*. In a previous study, we have successfully inhibited effects of TCDD in rat oval progenitor WB-F344 cells by a transient suppression of AhR expression, which has been mediated by short interfering RNA (siRNA). In the present study, we have designed two distinct short hairpin RNA (shRNA) sequences for stable RNAi-induced downregulation of AhR expression. These were cloned as cDNAs into a commercially-available plasmid vector with tetracycline-regulated promoter and transfected into WB-F344 cells. Tetracycline-inducible decrease in AhR expression might be a useful tool for elucidating roles of Ah receptor and its potential ligands in regulation of xenobiotic metabolism. [This work was supported by grant no. 524/06/0517 from the Czech Science Foundation and grant no. 20061431C0007 from MU Rector‘s Program for Students‘ Creative Activity Support].

POSTERS

Section 4	Xenobiotics in regulation of animal physiological function

P36

THE ROLE OF GLUTATHIONE IN DETOXIFICATION OF ANIMAL ORGANISMS

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Glutathione is a very important tripeptide (gamma-glutamyl-cysteinyl-glycin). It is the key intracellular antioxidant. It is present in all cell in the organism, its concentration varies in different tissues. Its reduced form (GSH) has an irreplaceable role in the organism. Its most important function is to protect cells from the oxidative action of free radicals and cell detoxification by means of conjugation of xenobiotics. GSH protects thiol protein groups (belonging to many important enzymes, receptors and transport proteins), it protects nuclear DNA from oxidative damage. It is a co-enzyme for many antioxidative and detoxification enzymes. A reduction in the GSH concentration can indicate the organism's overload with free radicals, xenobiotics or reduced GSH synthesis. With regard to the important role glutathione has in the organism, my work concentrates on the study of GSH content in select species of freshwater fish. The results of the GSH content analysis in freshwater fish point towards a significant variance of this peptide's contents in the monitored fish species. A higher content of reduced glutathione was found in hepatopancreas samples in Silver Carp (Hypophthalmichthys molitrix) as compared to the Common Carp (Cyprinus carpio) samples. The results may signal the impact of xenobiotic and oxidation product overload of the organism. Also, the influence of other factors, such as nutritional habits, overall health status and species variance of the fish can not be ruled out.

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P37
THE EXPRESSION OF nNOS IN THE SPINAL CORD AFTER FEMORAL NERVE TRANSECTION

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The discovery of nitric oxide (NO) as a messenger molecule in the nervous system has caused a change in understanding of interneuronal communication, because of its gaseous structure. The studies on the role of NO under physiological conditions and in the patophysiology of the spinal cord are based on the activity and/or the expression of nitric oxide synthase (NOS).

We examined the effect of the unilateral femoral nerve transection lasting for 11 days on changes in the level of neuronal NOS (nNOS) protein in the lower lumbar spinal cord segments (L3-L6). Results of our study show that the peripheral axotomy caused significant decrease in the level of nNOS protein in the ventral part of L3-L6 segments on the ipsilateral side, what may result from deprivation of neurotrophic factors produced by peripheral nervous system components. By contrast, in dorsal part the level of nNOS protein was higher on ipsilateral than on contralateral side. This fact may speak in favour of the up-regulation of NOS synthesis, seen after various types of peripheral neuropathies in the relevant dorsal root ganglia. The present study suggests that the motor and sensitive reorganization of the spinal cord after femoral nerve transection seems to be mediated by different mechanisms, including the modulation through the NOS.

The experimental work was supported by the VEGA Grant 2/5134/25 from the SAS and by the APVT 51-013002 and APVV 0314-06.

P38
EFFECT OF SOME EFFECTORS OF SIGNALLING PATHWAYS ON BASAL CALCIUM INFLUX IN HUMAN PERIPHERAL BLOOD LYMPHOCYTES

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Ca^{2+} plays a fundamental role as a second messenger in regulating numerous cellular functions. The mechanism(s) responsible for the increase in $[Ca^{2+}]_i$ during antigen or mitogen induced stimulation of lymphocytes has been widely investigated, by contrast, little is known about the processes underlying Ca^{2+} homeostasis and transport in resting cells in spite of the fact that changes in $[Ca^{2+}]_i$ are associated with variety of diseases. Here we report our observations of regulatory effect of selected effectors of signalling pathways and other compounds influencing calcium homeostasis in human peripheral blood lymphocytes.

This work was supported by the grant VEGA, nr.2/3188/23 and grant APVT 51-013802.

P39
THE EFFECT OF L-NAME TREATMENT ON THE LEVELS AND ACTIVITIES OF REGULATORY PROTEINS IN RAT HEARTS

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Nitric oxide (NO) is an important signaling molecule that acts in many tissues and regulates a diverse range of physiological and cellular processes. We found previously that hearts from rats with chronic NO deficiency (NOD) showed better recovery of contractile function after ischemia/reperfusion. Our aim was to characterize the effects of chronic NOS inhibition by L-NAME treatment on the alterations of regulatory myocardial proteins at the subcellular level. NOD was induced by L-NAME treatment (40/mg daily, 4 weeks). Levels and specific phosforylation (activation) of proteins were determined by Western blot analysis using specific antibodies. The effect of L-NAME treatment was connected with decreased phosphorylation (activation) of Akt kinase and extracellular signaling regulated protein kinases (ERKs). Moreover, L-NAME induced a down-regulation of protein levels of aFGF and H-Ras (possible activators of ERKs). The changes in ERK and Akt kinase pathways correlated with decreased activation of eNOS in L-NAME treated hearts. L-NAME induced also decrease in levels of iNOS. The results point to the role of ERK and Akt kinase pathways in cardiac responses connected with development of chronic NOD and to the relationship between activation of these kinase pathways and eNOS activation. They also show that the reduced vulnerability to ischemic injury in NO deficient rat hearts may be related to inhibition of iNOS expression.

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POSTERS

Section 5	Xenobiotics in metabolism of microorganisms

P40
EFFECTS OF GABA ANTAGONISTS ON GROWTH OF *TRICHODERMA VIRIDE*

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Gamma-aminobutyric acid (GABA) has been shown to be developmentally regulated by filamentous fungi. Its role in filamentous fungi is still a bit unclear although a position in both carbon and nitrogen metabolisms could be used in their regulation. Other possible roles include pH regulation, stress response and pathogen defense. Our former results suggest that glutamate decarboxylase activity is implied in conidia germination and conidiation.

In this work we studied the effects of GABA antagonists on growth and metabolism of *Trichoderma viride*. We have found that Neurontin (gabapentin) and Lamictal (lamotrigine) inhibit growth to 40 % and 70 %, respectively. The conidiation is also inhibited. The NMR analysis of fungal extracts from gabapentin-grown mycelium has not shown significant effect on GABA although concentrations of some intracellular metabolites were changed.

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P41
THE ANTIMUTAGENIC EFFECT OF VANILLIN ON SPONTANEOUS MUTATIONS LEADING TO CIPROFLOXACIN RESISTANCE

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Vanillin (3-methoxy-4-hydroxybenzaldehyde), a naturally occurring compound of vanilla beans has been widely used as a flavoring agent in confectioneries, beverages, foodstuffs, it has also been found in barrel-aged wine and is a by-product of curcumin from turmeric. Vanillin has been reported to be antimutagenic or co-mutagenic in both bacteria and mammalian cells. Treatment of *Salmonella typhimurium* with vanillin inhibited frequency of spontaneous revertants, as well as the frequency of mutations induced by some positive mutagens. It is known, that vanillin is bioantimutagen, which interacts with repair mechanisms in bacterial cells.

Malfunction of genes controlling DNA repair mechanism can drastically affect the spontaneous mutation frequencies, so in this work we have estimated the effect of vanillin on the frequency of spontaneous mutations leading to bacterial resistance to fluoroquinolone ciprofloxacin in wild type, repair proficient strain *Escherichia coli* WP2 and in isogenic or non-isogenic repair-deficient strains.

Vanillin has increased the mutation frequency to ciprofloxacin resistance in WP2*uvr*A strain, which lacks excision repair. Vanillin has strongly decreased the mutation frequency in SOS induction deficient and recombinational deficient strain (by about 80%) and in less degree in SOS induction deficient and recombinational repair proficient strain. Our finding suggests that in the mutagenesis leading to ciprofloxacin resistance vanillin enhance error-free recombinational repair and doesn´t influence the error-prone SOS response.

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P42
BIOLOGICAL ACTIVITY OF NEW FENAMATES WITH METAL ION IN THE MOLECULE

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Coordination compounds with a metal ion in the molecule are still in the center of interest of many biological, chemical and pharmacological sectors. This work presents the biological activity of metal complexes with composition of MX_2 were M is the metal ion (Cu^{2+}, Zn^{2+}, Co^{2+}), X is mefanate, flufenamate, meclofenamate, tolfemate. These bioactive ligands are used especially for treatment of some rheumatic diseases and complexes are classified as non steroid antiflogistics. The antimicrobial effect of new complexes was tested against G^+ (*Staphylococcus aureus*) and G^- (*Escherichia coli)* bacteria; yeasts (*Candida albicans, C. parapsilosis)* and filamentous fungi (*Rhizopus oryzae, Alternaria alternata, Botritys cinerea, Microsporum gypseum, Trichophyton interdigitalis, Aspergillus fumigatus*) and characterized by IC_{50} and MIC values. The highest antibacterial effect was observed in the presence of all meclofenamic complexes. *S. aureus* was more sensitive than *E. coli.* $Co(meclof)_2.(H_2O)$ influenced the growth of both yeasts at the highest level. The most sensitive filamentous fungi were *M. gypseum* and *B. cinerea.* Based on Ames assay results, complexes demonstrated no mutagenic activity. The antioxidant activity (FRAP, TEAC assay) of these complexes was considerably lower than the activity of trolox standard. Some of new metalofenamates had a profound effect on biomembrane permeability. Influence on membrane permeability was observed as: an increase in antocyan efflux in *Beta vulgaris var. rubra,* hemolysis of red blood cells and changes of permeability of plasma membrane of *C. albicas* after incubation with the complexes.

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P43
ASCORBIC ACID – MODULATOR OF ANTIMICROBIAL EFFECT OF N-SALICYLIDENE-L-GLUTAMATE CU(II)COMPLEXES

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Some copper-based drugs catalyze radical formation while others seem to have antioxidant efficacy. Different behavior of Cu-complexes depends upon the chemical environment. Schiff-base copper(II) complexes derived from salicylaldehyde was synthesized for their antimicrobial properties. The product of reaction of N-salicylidene-L-glutamatodiaquacopper(II) monohydrate (CuC) with isoquinoline was prepared, and it was showed that CuC and complex with isoquinoline (IQ-CuC) possessed antimicrobial activity. In this study we showed that both complexes have high affinity to DNA and they are redox active substances consequently, they could have pro-oxidant properties in the presence of reductant. Firstly, the action of the Cu-complex on stability of pDNA has been evaluated. Both complexes in the presence of ascorbic acid (AA) induced single strand and double strand breaks of DNA. To demonstrate that antimicrobial activity of Cu-complexes can be increased by addition of a reductant we have evaluated the effect of complexes on *Candida albicans* viability in the presence of AA (1-25 mM). We found out that both complexes exhibited higher anti-yeast activity in the presence of AA. Antimicrobial effect of CuC was increased about 70 times in the presence of 25 mM AA (IC_{50}=0.05 mM). Presented results suggest that Cu-complexes increased ROS level in the yeast and AA is a potent modulator of their anti-yeast activity.

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