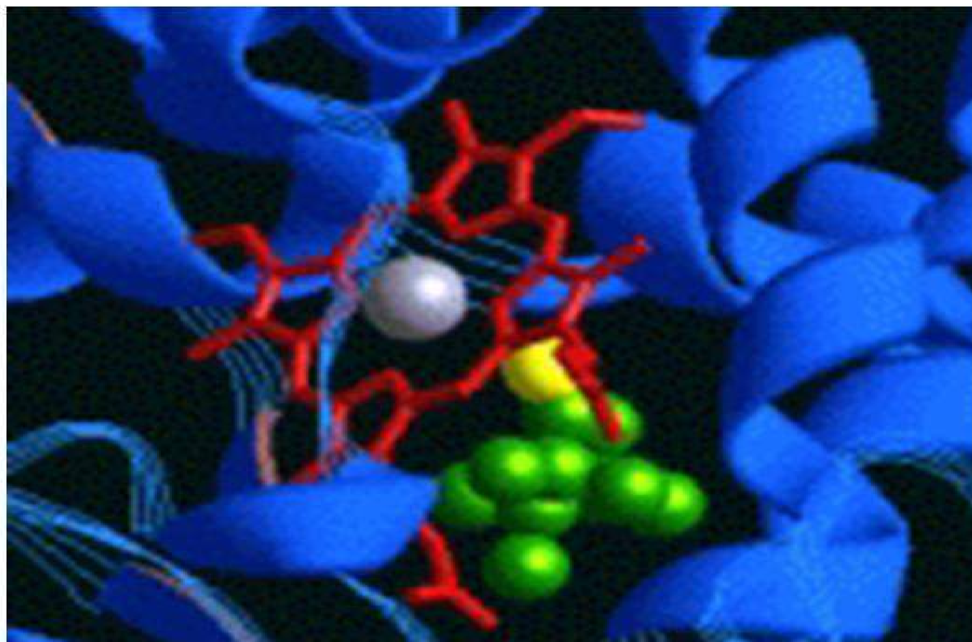
Università degli Studi di Siena
Dipartimento di Scienze Anatomiche e Biomediche

Citocromo P450: aspetti farmacologici, tossicologici e ambientali



12-13 Settembre 2005
Certosa di Pontignano
Siena

Comitato Scientifico:
M Valoti
F De Matteis
MC Fossi
G Sgaragli

Comitato Organizzatore:
S Dragoni
ML Valacchi
L Cavecchia

GENETIC POLYMORPHYSM OF CYTOCROME P450 2D6, 2J2, 1A2 IN AN ITALIAN POPULATION

V. Longo, L. Pucci*, V. Chirulli, S. Nencioni, P.G. Gervasi
*Laboratory of Metabolism and Pharmacogenetic, Clinical Physiology Institute, CNR, Pisa, *Department of Endocrinology and Metabolism, Unit of Diabetes and Metabolism, University of Pisa*

Introduction: Fifty-seven functional cytochrome P450 (CYP) genes have been identified in the human genome. The CYPs play a key role in the metabolism of a variety of xenobiotics and endogenous compounds. Several CYPs exhibit genetic polymorphisms and the relative distribution of the CYP variant alleles differ widely among ethnic groups. The inter-individual variations of the efficacy and the adverse effects of drugs may be the results of CYP polymorphisms. Moreover, many studies have tried to establish a link between the incidence of diseases and CYP polymorphisms. The aim of our study was to evaluate in a group of Italian population: i) the distribution of the CYP2D6-related polymorphism using dextromethorphan (DM) as a phenotype marker; ii) CYP2J2*6 allelic variant frequency and its association with type 1 and type 2 diabetes mellitus; iii) CYP1A2*2 and CYP1A2*11 allelic variant frequencies in a cohort of 200 Italian subjects.
Materials and methods: The determination of the CYP2D6 phenotype was carried out in 110 healthy volunteers treated with a single oral dose of 30 mg of DM. The urines of these subjects were analysed by HPLC for the presence of the parental DM and of the dextrorphan (DEX) metabolite and their logarithm metabolic ratio was determined. Genomic DNA was extracted from peripheral blood cells and analysed for the allelic variants of CYP2J2 and CYP1A2, above mentioned, by the polymerase chain reaction and restriction fragment lenght polymorphism and visualised by polyacrylamide gel electrophoresis.
Results: On the basis of the metabolic ratio values, 6.4% of the subjects were classified as CYP2D6 poor metabolizers (log DM/DEX higher than zero), 80% as CYP2D6 extensive metabolizers (log DM/DEX lower than zero) and 13.6% as rapid or ultrarapid metabolizers (DM was totally metabolized). In the 700 subjects, CYP2J2*6 allele was 0.64 % and the genotype distribution did not show significant differences between controls and patients with type 1 or type 2 diabetes. No homozygotes for CYP2J2*6 allele were found. The CYP1A2*2 and CYP1A2*11 variants, known to be low in Asiatic populations, were absent in our population.
Conclusions: The results of CYP2D6 phenotype of poor and extensive metabolizers in our Italian group were similar to those reported for other Caucasian populations whereas the percentage of rapid/ultrarapid metabolizers was higher than the Caucasian ones The frequency of the CYP2J2*6 allele in our controls and patients with diabetes was found to be very low. The CYP1A2*2 and CYP1A2*11 alleles, studied for the first time in a Caucasian population, were absent, in contrast to what has been reported in Asiatic populations.

CYP3A AND *MDR1* (P-GLYCOPROTEIN) GENE EXPRESSION LEVELS ALONG THE HUMAN GASTROINTESTINAL TRACT.

Roberto Canaparo[1, 2], Anna Nordmark[1], Niklas Finnström[1], Stefan Lundgren[1], Janeric Seidegård[3], Bengt Jeppsson[4], Loredana Serpe[2], Mario Eandi[2], Gian Paolo Zara[2], Anders Rane[1]

[1]Karolinska Institutet, Department of Medicine Laboratory Sciences and Technology, Division of Clinical Pharmacology, Huddinge University Hospital; [2]Department of Anatomy, Pharmacology and Forensic Medicine, University of Torino, Italy; [3]Experimental Medicine, AstraZeneca R&D Lund, Sweden; [4]Department of Surgery, Malmö University Hospital, Sweden.

Our objective was to investigate the expression of *CYP3A4, CYP3A5, CYP3A7* and *MDR1* (P-glycoprotein) genes along the gastrointestinal tract in comparison with the levels in human liver.
Samples of normal stomach, duodenum, jejunum, ileum rectum and colon were collected from 40 patients (14 female and 27 male; age range, 32-93 years) subject to curative surgery after informed consent. We also collected 10 liver samples from patients undergoing hepatobiliary surgery, all but one for the resection of a tumour. The specimens were snap frozen in liquid nitrogen and stored at -80°C.
We used real-time reverse transcriptase-polymerase chain reaction (RT-PCR) to study *CYP3A4*, *CYP3A5*, *CYP3A7*, *MDR1* and 18S rRNA specific mRNA expression. The mRNA expression of 18S rRNA was used as housekeeping gene.
In summary, the *CYP3A* gene subfamilies are expressed in all tissues examined but were most abundant in liver.
CYP3A4 was the major *CYP3A* gene that was expressed in almost all sites examined, but along the gastrointestinal tract our data indicated that the discrepancy between *CY3A4* and *CYP3A5* decreased and this difference, in the relative mRNA expression of *CYP3A4* and *CYP3A5*, may account for organ specific differences in the metabolism of many drugs.
In apparent contrast to the observation for *CYP3A* mRNA expression, *MDR1* mRNA increased from stomach to the small intestine, but at the same level found in the liver.
We have also shown a co-ordinated expression of the *CYP3A* and *MDR1* and between *CYP3A* genes, as well as a large interindividual variation. Such a variation may contribute to the variability in therapeutic response and susceptibility to adverse drug reactions.

LOCALIZATION AND ACTIVITY OF CYP1A WITHIN THE BRAIN OF PIGS

Marvasi L*, Gervasi PG, Chirulli V**, Vaccaro E**, Zaghini A , De Bona C , Giuliani A**
**VETSPIN Srl, Via Tolara di Sopra 50, 40064 Ozzano Emilia; **CNR Pisa; University of Bologna; University of Padova*

Introduction - The domestic pig is increasingly being used as an experimental model for brain studies: PET studies of parkinsonism (1, 2) and pharmacological challenger (Smith *et al,* 1998, 1999)(3, 4). However nothing is known about the presence and the activities of cytochrome P450 in the brain of the pig. The purpose of this study has been to investigate the presence, the activity and the inducibility by betha-naphthoflavone (BNF) of CYP1A1-2 within the brain of pig.
Materials and methods – Six animals, (average weight: 25 kg ± 2) were divided in two group (control and BNF treated group). A daily dose of 30 mg/kg of body weight of BNF in corn oil, given by intra-peritoneal administration for four days, was used to induce CYP1A. After the slaughtering samples from liver, cortex, cerebellum and midbrain was collected and stored in liquid nitrogen until further analyses: RT-PCR for the presence of mRNA of CYP1A1-2 and AhR; EROD and MROD activities (marker of CYP1A1 and CYP1A2) and TST 6β-hydroxylation (marker of CYP3A) and immunocytochemicals assays were performed.
Results – In all brain regions from the BNF treated animals we were able to demonstrate an amplified fragment of 580 pb corresponding to the pig CYP1A1. The EROD, MROD and TST 6β-hydroxylation activities were similar in the brain of treated and untreated animals, whereas, in the liver, EROD and MROD were 50 and 10 times higher in the treated animals, respectively. In the mitochondrial preparations the activities were 2-5 times higher then in the microsomal ones. The immunocytochemicals assays showed the presence of CYP1A within the Purkinje cells of cerebellum, in the astrocyte of grey cortex, and in the endothelium of both, cortex and cerebellum, but there were no differences between treated and untreated animals.
Conclusions – The enzymatic analysis and the immunocytochemicals assays revealed for the first time the presence of CYP1A1-2 within the pig's brain, at comparable levels in both, treated and untreated animals; despite the presence of AhR, the CYP1A1 was resistant to βNF induction in the pig brain, suggesting that there is a different regulation mechanism of this enzyme between the brain and liver. The presence of CYP1A in the blood-brain barrier confirms the defensive role of this enzyme and its presence in the astrocytes and Purkinje's cells further suggests the importance of CYP1A in the brain tissue.

References
1. Danielsen *et al.*, Cell Transplant, 2000 , 9 , 247-59;
2. Cumming *et al*, Acta Neurol Scand, 2001 , 103 , 309-15;
3. Smith *et al.*, J Neurosci Methods, 1998 , 86 , 17-23; 4) Smith *et al.,* Eur Neuro psych pharmac, 1999 , 9 , 351-9.
Acknowledgements:
This study was supported by Fondazione Del Monte di Bologna e Ravenna

MFO ACTIVITIES AND ANTIOXIDANT ENZYMES IN *Posidonia oceanica*: LABORATORY STUDY AND FIELD APPLICATIONS

Daniela Bucalossi, Claudio Leonzio, Silvia Casini, Maria Cristina Fossi, Letizia Marsili, Stefania Ancora, Wei Wang, Monica Scali
Department of Environmental Sciences, University of Siena, Via Mattioli, 4, 53100, Siena, Italy.

The main aim of this study was to develop and validate a set of sensitive responses (biomarkers) for monitoring conservation status and human impact in *Posidonia oceanica* meadows. Analytical methods were developed for NADPH cytochrome c reductase, ethoxicoumarin-o-deetilase (ECOD), guaiacol peroxidase (GPOX) and superoxide dismutase (SOD) assays. Western blot and a preliminary proteomic approach using 2-DE. were also proposed as biomarkers. These techniques were initially tested on samples of posidonia experimentally exposed to various contaminants. Once validated, this approach was applied to posidonia in a field study. Specimens of the seagrass were collected at four sites with different potential human impact along the northern Tyrrenian coast. The results showed that reductase activity was significally induced in the various sampling areas compared to the reference site. GPOX and SOD showed a similar trend; the highest activities were measured in samples collected in front of a chlor-alkali plant and close to a river estuary. Analysis of residues (heavy metals, PAHs and OCs) measured on leaves showed differences between sites. A significant correlation was found between levels of Hg and GPOX and levels of Cr, Al and As and reductase activity. These results validated the potential use of the biomarkers approach on posidonia for the assessment of ecotoxicological impact on the coastal environment.

FIRST INVESTIGATION ON CYP450 1A1 AND CYP450 2B INDUCTION IN FIBROBLAST CELL CULTURES OF MEDITERRANEAN CETACEANS EXPERIMENTALLY TREATED WITH ORGANOCHLORINE CONTAMINANTS (OCS) AND POLYBROMINATED DIPHENYL ETHERS (PBDES)

M.Cristina Fossi, Letizia Marsili, Silvia Casini, Daniela Bucalossi
Department of Environmental Sciences, Siena University, Via Mattioli 4, 53100 Siena, Italy. fossi@unisi.it

Mediterranean cetaceans, particularly odontocetes, accumulate high concentrations of organochlorine contaminants (OCs) and are therefore exposed to high toxicological risk. Some OCs are known to be endocrine disrupting compounds (EDCs). The possibility that certain Mediterranean cetaceans (*Stenella coeruleoalba, Delphinus delphis, Tursiops truncatus* and *Balaenoptera physalus*) are subject to toxicological risk due to organochlorines and emerging contaminants, such as polybrominated diphenyl ethers (PBDEs) with endocrine disrupting capacity, was investigated using non-lethal “diagnostic” and “prognostic” methods. Benzo(a)pyrene monooxygenase (CYP1A1) activity in skin biopsies was used as a “diagnostic” indicator of exposure to organochlorines in odontocetes and mysticetes and in different populations of *Stenella coeruleoalba*. Marked differences in levels of OCs and CYP1A1 activity were found between fin whales and odontocetes. Organochlorine levels and CYP1A1 activity were significantly higher in the *Stenella coeruleoalba* population of the Mediterranean Whale Sanctuary than in those of two other study areas, suggesting that cetaceans are exposed to high risk in this protected area.
As a new “prognostic” tool we explored interspecies and gender susceptibility to OC-EDCs and PBDEs using qualitative and semi-quantitative evaluation of target proteins, such as CYP450 1A1 and CYP450 2B in cultured cetacean fibroblasts (*Stenella coeruleoalba, Tursiops truncatus* and *Balaenoptera physalus*), by western blot, immunofluorescence technique and real time PCR. The main results of this experiments were: 1) the detection of presence of the cytochromes 1A1-1A2 and 2B4 in striped dolphin, bottlenose dolphin and fin whale fibroblast cells, revealed from the crossreaction of the antibody used and from the presence of fluorescence in the fibroblasts; 2) the increase of fluorescence in relation to the treatment doses of contaminants; 3) the preliminary detection of differences in the induction phenomena between specimens and gender.

INDUCTION OF CYTOCHROME P450 ACTIVITIES AS SENSITIVE BIOMARKER IN THE ECOTOXICOLOGICAL ASSESSMENT OF MEDITERRANEAN OFF-SHORE PLATFORM AREAS : THE BIO-MARE PROJECT

Silvia Casini[1], M. Cristina Fossi[1], Maria Ferraro[2], Letizia Marsili[1], Stefania Ancora[1], Gabriele Mori[1], Daniela Bucalossi[1], Ilaria Caliani[1], Giacomo Stefanini[1], Nicola Bianchi[1]
[1]*Department of Environmental Science, University of Siena, Siena (Italy)*
[2]*ENI S.p.A. – E&P Division, Milan, Italy*

The AGIP "BIO-MARE" pilot project aimed to develop and validate a methodology based on biomarkers in bioindicator organisms to assess ecotoxicological effects related to Mediterranean off-shore activities (Adriatic Sea). The research was articulated in three phases: I) laboratory studies; II) field studies; III) development of a methodological protocol. During phase I, specimens of the flatfish *Solea vulgaris* and barnacles *Balanus trigonius* and *Balanus perforatus* were exposed in the laboratory to increasing concentrations of produced water, obtained from an Adriatic off-shore platform and to a mixture of PAHs, *S. vulgaris* was also exposed to increasing concentrations of gasoline. Several biomarkers (EROD and BPMO activities, Cyp450-reductases, AChE, BChE activities, porphyrins, vitellogenin, zona radiata proteins, antioxidant enzymes and micronuclei) and residues (PAHs and trace elements) were measured in the bioindicator organisms. The main aim of this phase was to identify the most sensitive biomarker responses and build up an ecotoxicological model for the evaluation and interpretation of *in situ* data. Exposure of *S. vulgaris* to gasoline resulted in strong dose-related induction of MFO activities and vitellogenin; exposure to produced water resulted in dose-related induction of MFO activity, slight accumulation of total porphyrins. Barnacles showed a clear induction in BPMO activity in the group treated with PAHs, with respect to control; a clear induction in NADPH-ferrycyanide reductase activity was also found in groups treated with produced water.
Phase II (field monitoring) confirmed the induction of Cytochrome P450 activities as the most sensitive responses. In fact EROD and BPMO activities were found to be induced (with respect to a control site) in fish sampled at different distances from the platform. The highest induction, nevertheless was found in the site D, by the coast of Rimini, 10 miles away from the platform. Results obtained from field investigation suggest that the potential ecotoxicological impact of the platform is not particularly important when compared with the background contamination of the North Adriatic sea

EVALUATION OF ECOTOXICOLOGICAL EFFECTS OF ENDOCRINE DISRUPTERS DURING A FOUR-YEAR SURVEY OF THE MEDITERRANEAN POPULATION OF SWORDFISH (*XIPHIAS GLADIUS*)

Mori G.[1], Porcelloni S.[1], Casini S.[1], Marsili, L.[1], Jimenez B.[2], Ancora S.[1], Ausili A.[3] and Fossi M.C.[1]

[1]*Department of Environmental Science, University of Siena, Siena (Italy)*
[2] *Institute of Organic Chemistry, CSIC, Juan de la Cierva 3, 28006 Madrid (Spain)*
[3]*ICRAM Via di Casalotti 300, Roma (Italy)*

Since 1999 the Department of Environmental Science at Siena University has been involved in research into the ecotoxicological effects of Endocrine Disrupting Compounds (EDCs) in a Mediterranean population of swordfish (*Xiphias gladius*). Using sensitive biomarkers such as Vitellogenin (VTG), Zona Radiata Protein (ZRP), sex hormone pathways and CYP1A activities (EROD, BPMO), exposure and effects of anthropogenic chemicals have been studied in more than 200 Mediterranean specimens. VTG and ZRP were found to be induced in 35% of the adult male specimens. These fishes showed higher levels of both proteins and 17β-estradiol than mean levels in males from reference sites, suggesting high exposure to xenoestrogens in the Mediterranean Sea. A role of Organochlorines in this induction phenomenon is suggested by statistically significant correlations found between VTG and ZRP levels in males and levels of hexachlorobenzene (HCB) in gonads ($p<0.05$), plasma 17β -estradiol and polychlorobiphenyl (PCB) in gonads ($p<0.05$), ZRP and *pp'*DDE in liver ($p<0.06$). These results indicate that the Mediaterranean swordfish could be subjected to reproductive alterations. In order to better understanding the present results, alterations in swordfish liver and gonads (testis and ovary) will be investigated. Histopathological studies including the immunoistochemical detection of VTG, ZRP and CYP1A will be performed. Moreover, the mRNA contents of the aromatase and CYP1A genes in swordfish will be measured. All results obtained will be correlated with the quantitative analysis of TCDD, PCBs and organochlorine pesticides in tissues.

Part of the study was financed by ICRAM and Italian Ministry of Environment

BILIRUBIN DEGRADATION BY UNCOUPLED CYTOCHROME P450 COMPARISON WITH A CHEMICAL OXIDATIVE SYSTEM AND CHARACTERIZATION OF THE PRODUCTS BY HPLC -MS

F. De Matteis, G.A.Lord, C.K. Lim and N. Pons
MRC Bioanalytical Science Group, Birkbeck College, University of London, Malet Street, London UK

Introduction

In previous work microsomes from induced animals or the bacterial enzyme CYP102 were shown to degrade bilirubin *in vitro,* when a polyhalogenated substrate analogue was also present. It was suggested that the substrate analogue acted by uncoupling the appropriate CYP enzyme, resulting in increased production of oxidative species and in bilirubin degradation. In order to clarify the mechanism involved we have compared the enzymic oxidation of bilirubin with its chemical oxidation by Fe-EDTA and H_2O_2. We have developed a HPLC system capable of separating birubin fron its degradation products, and these have been characterized by electrospray-Mass Spectrometry.

Results

Following incubation in the chemical system there was a time-dependent decline of bilirubin, accompanied by an increase of biliverdin, its dehydrogenated product, and the appearance of new ions, all eluted separately from bilirubin and probably representing dipyrrolic degradation products. These included 1] a cluster of 4 isomeric products at m/z 301/302; 2] two isomeric products at m/z 333; 3] two isomeric products at m/z 299; and 4] two to four isomeric products at m/z 315.

The structure of dipyrroles has been confirmed for the ions listed under 2] and 4] above, by subjecting to chemical oxidation *meso*-bilirubin, an analogue of bilirubin containing two ethyl side chains (instead of vinyls), and by verifying the increment of two mass units in the corresponding products. A structural assignement for these two ions has been achieved by accurate mass measuraments. The mechanism of their production may involve an initial hydrogen abstraction at the central methene bridge of bilirubin, leaving a carbon-centered radical, followed by oxygen addition and fragmentation.

When microsomes from BNF-induced chick embryos were incubated with NADPH and bilirubin in presence of a planar PCB, an identical pattern of bilirubin degradation was observed suggesting the same or a very similar mechanism of oxidative degradation.

F.De Matteis would like to thank the Royal Society of Edinburgh for an Auber Award

BENZENE BONE MARROW TOXICITY AND EFFECTS ON LIVER ENZYMES OF C57BL/6 MICE

S. Dragoni [a], G. Materozzi[a], G. Curci, N. Volpi, P. Franceschetti [b], D. Doria [b], M.E. Fracasso [b] , M. Valoti [a]

[a]*Dipartimento di Scienze Anatomiche e Biomediche, Università di Siena, Via Aldo Moro 2, 53100 Siena, Italy*

[b]*Dipartimento di Medicina e Salute Pubblica, Sezione di Farmacologia, Università di Verona, Italy.*

Benzene is widely used in industry [1], and can induce aplastic anemia and acute myelogenous leukemia in humans [2] and multiple forms of cancer in rodents [3].
Hepatic cytochrome P450 2E1 catalyses the formation of phenolic metabolites of benzene [4]. Bone marrow mielopeoxidase (MPO), present in the bone marrow, may further convert the phenolic metabolites to free radicals that may contribute to the "in situ" bioactivation of benzene[5]. Nevertheless, the molecular mechanisms underlying the bone marrow toxicity of benzene are incompletely understood.
In order to clarify the mechanisms responsible for the toxicity of benzene and to identify new and more sensible biomarkers of benzene exposure, C57BL/6 mice were treated sub-chronically with either a low or a high dose of benzene (5 and 100 mg/kg b.wt., respectively) . Bone marrow cellularity, apoptotic cell death rate, and DNA damage were then evaluated, as well as the activities of the major phase I and phase II enzymes, involved in benzene metabolism, in both liver and bone marrow.
Bone marrow cellularity, assessed in paraffin embedded sternum, showed that treatment with the low benzene dose markedly decreased the number of bone marrow cells of about 60%. Moreover, the TUNEL test performed on the same tissue preparation, shows an increase of apoptotic cells suggesting that the decreased bone marrow cellularity is due to apoptotic cell death. Similarly, the alkaline comet assay performed on the marrow flushed-out from femurs and tibias of benzene exposed mice, showed a dose-dependent DNA damage.
The low benzene dose gave rise to a significant increase of benzoyloxy-; pentoxy-; ethoxy-resorufin O- dealkylase activities; on the contrary a significant decrease in the activity of the same enzymes was detected after treatment with the high dose. None of the other phase I enzyme activities tested in liver seemed to be affected by both benzene dose. In the same manner sulfotransferase and glutathione S-transferase activities were significantly higher in liver citosol of animals treated only with the low benzene dose, while no differences were detected in the uridine diphosphate-glucuronosyltransferase activity after treatment with both doses. Interestingly enough, the treatment with benzene gave rise to a significant dose-dependent increase in the NAD(P)H:quinone-oxidoreductase (NQO1) activity in liver citosol. On the contrary, in the bone marrow, MPO activity was increased only after treatment with the high dose, while NQO1 activity did not change. The increase of MPO activity, responsible for bioactivation of benzene, not counter-balanced by a corresponding increase of NQO1 activity (detoxifying enzyme) may explain the selective toxicity of benzene on the bone marrow following exposure at high dose.

Acknowledgements

This work was supported by MIUR grant PRIN 2003, N 2003063319_005

References

1. Bauer AK., Faiola B., Abernethy DJ., Marchan R., Pluta LJ., Wong VA., Gonzalez FJ.,Butterworth BE., Borghoff SJ., Everitt JI. and Recio L. (2003)Toxicol Sci.72:201-9.
2. Sanz GF., Sanz MA. and Vallespi T. (1997) Hematol. Cell Ther. 39: 277-94.
3. Whysner J., Reddy MV., Ross PM., Mohan M. and Lax EA. (2004) Mutat. Res. 566: 99-130.
4. Koop DR., Laethem CL. and Schnier GG. (1989) Toxicol. Appl. Pharmacol. 98: 278-88.
5. Ross D. (2005) Chem. Biol. Interact. 30;153-154:137-46.

DRUG-METABOLIZING ENZYMES IN NASAL MUCOSA: RELEVANCE TO TOXICOLOGY

Pier Giovanni Gervasi
Istituto di Fisiologia Clinica, CNR, Area della Ricerca, Via Moruzzi 1, 56100, Pisa.

An increasing number of reports have shown that nasal mucosa of a number of species including mouse, rat, rabbit, cattle, pig and human contains substantial levels of xenobiotic metabolizing enzymes (1). Some of these enzymes are tissue-specific and may produce a selective metabolism of inhaled or blood-born compounds. Indeed, many chemicals of both industrial and environmental importance including, coumarin, 3-methylindole, 1,4-dioxane, nitrosodimethylamine, dichobenil and benzopyrene induce, mostly trough *in situ* bioactivation, nasal lesions or tumours in experimental animals. In human, the incidence of nasal malignant tumours is rare, accounting for only 1% of all cancers. However, in humans the olfactory olfaction is vulnerable to nasal toxicants and nasal cancer is increased in smokers and in Chinese people consuming salted fish rich in dimethylnitrosamine. Studies on the metabolic capacity of nasal tissues are also important as intranasal applications of therapeutic drugs is becoming increasingly common.

My research group has been interested for several years in the expression and metabolic function of phase 1 and 2 enzymes in olfactory and respiratory epithelium of various experimental animals, including fish, bird, frog, monkey and humans (2-11).

It has been observed by us and other authors that the olfactory mucosa of mammals contains a high CYP content (similar to liver) and a specific CYP isoform, namely 2G1 (primarily responsible for hormone metabolism), which is not present in the respiratory mucosa and other hepatic or extrahepatic tissue (2-5). This isoform has not been found in the nose of fish, bird or frog (9-10), indicating a specific role in mammals. CYP 2A, 2B and 3A are constitutively expressed both in olfactory and respiratory epithelium but, unlike liver, are quite resistant to induction. Only CYP 2E1 is promptly inducible by common solvents (ethanol, dioxane, acetone) or fasting in mammals and, along with CYP2A, have been involved in the bioactivation of many nasal toxicants (8-11). In contrast, CYP 1A1, known to be strongly inducible by PAH in many tissues, is hardly inducible in the nose of rodents and frog but it is easily inducible in the fish nose (6;10). Interestingly, also CYP 1A2, constitutively expressed in nasal tissues but not in other extrahepatic tissues, is inducible by fasting in the nasal mucosa of rat (8).

Regarding the phase 2 enzymes, members of the UDP-GT, GST, DT- diaphorase, and aldehyde dehydrogenases families have been generally detected in the nasal epithelium of both mammals and other vertebrates but the expression of specific isoforms remains largely to be determined.

The research in this area is still increasing as it is of considerable importance in view of the constant exposure of human nasal epithelium to inhaled natural or man-made chemicals and thereby the need to assess the risk of potential olfactory toxicants.

References

1. Thornton JR. and Dahl A R, (1997), Mutation Res. 380, 43-59.
2. Gervasi PG, Longo V, Naldi F, Panattoni G, Ursino F (1991) Biochem Pharmacol. 41, 177-185.
3. Longo V, Mazzaccaro A, Naldi F, and Gervasi PG, (1991), J. Biochem. Toxicol., 6, 123-128.
4. Longo V, Mazzaccaro A, and Gervasi PG, (1992), Xenobiotica, 4, 427-431.
5. Longo V, Amato G, Santucci A, and Gervasi PG, (1997), Biochem J.,323, 65-70.

6. Novi S, Pretti C, Cognetti AM, Longo V, Marchetti S, and Gervasi PG, (1998), Aquatic Toxicol. 41, 63-81.
7. Marini S, Longo V, Mazzaccaro A, and Gervasi PG, (1998) Xenobiotica, 28, 923-935.
8. Longo V, Ingelman-Sundberg M, Amato G, Salvetti A, and Gervasi PG, (2000), Biochem. Pharmacol., 59, 1425-1432.
9. Giorgi M, Longo V, Amato G,and Gervasi PG, (2000), Toxicol. Appl. Pharmacol.167, 237-245.
10. Longo V, Marini S, Bucci S, Salvetti A, and Gervasi PG, (2004), Aquatic Toxicol. 69, 259-270.
11. Nannelli A, De Rubertis, Longo V, and Gervasi PG, (2005), Arch. Toxicol.79, 74-82.

THE BIOACTIVATION OF FENTHION BY RECOMBINANT HUMAN CYPS

Claudia Leoni, Franca Maria Buratti and Emanuela Testai
Environment and Primary Prevention Department, Mechanisms of Toxicity Unit
Istituto Superiore di Sanità, -Italy (buratti@iss.it, testai@iss.it)

Fenthion is an organophosphorothionate (OPT) pesticide, widely used in agriculture and characterized by the presence of a second sulfoxidable sulphur atom. Besides oxon, the typical toxic OPT metabolite, fenthion could be therefore biotrasformed by cytochrome P450 to the corresponding sulfoxide and by further oxidation, to the sulfone. The present work has been carried out to detect the human CYPs involved in these reactions at actual level of exposure, within the frame of a research project .aimed to the identification of a possible metabolic biomarker of susceptibility for the entire class of OPTs.
Preliminary study evidenced that the three metabolites inhibit Acetylcholinesterase (AChE) although at different levels (IC_{50} oxon= 3.1μM IC_{50} sulfoxide= 69.4μM IC_{50} sulfone= 9.4μM), showing potential toxicity. c-DNA expressed human CYPs and a specific HPLC method were used to determine the active isoforms and calculate the kinetics parameters V_{max}, K_m and CL_i with a broad range of fenthion concentrations (0.5-100 μM). In the standard incubation conditions the sulfone formation has not been detected. CYPs 1A2, 3A4 and 2C9 formed both oxon and sulfoxide in similar amounts, although the 3 isoforms show different CLi. CYP1A1 produced only the sulfoxide. CYPs 2B6 preferentially formed the oxon, whereas 2C19 catalyzed mainly sulfoxide formation. The other isoforms tested were not active. Considering the average CYPs content in the human liver and CL_i values at low pesticide concentration representative of human exposure levels (1), CYP1A2 gives the highest contribution, 90% and 80% to oxon and sulfoxide formation, respectively. However, it is evident that at fenthion concentrations (≥100μM), representative of accidental or intentional acute intoxication, CYP3A4 role is the more important because of its kinetics and its high average content (40%) in the human liver. These results confirm the data previously obtained on other OPTs (diazinon, chlorpyrifos, azynphos-methyl, parathion, malathion and dimethoate) characterized by linear or aromatic substituents at the thioether sulphur (2, 3). It can therefore be hypothesized that, at actual human exposure levels, independently from the chemical structures of single OPT, this class of pesticide is mainly bioactivated to toxic metabolites by CYP1A2, which can be considered a good candidate as metabolic biomarker of susceptibility to OPT-induced toxic effects.

1. Buratti et al. (2002) Environm. Toxicol and Pharmacol 11 (3-4): 181-190
2. Buratti et al.(2003) Toxicol. and Applied Pharmacol 186 (3): 143-154
3. Buratti et al (2005) Drug Metabolism and Disposition 33 (3) : 1-8

This work has been partially supported by the ISS project 3AAS.

EFFECT OF CYP1A1, GSTM1, GSTP1, GSTT1, AND mEH POLYMORPHISMS ON BENZO(a)PYRENE DIOLEPOXIDE (BPDE)-DNA ADDUCT LEVELS IN SMOKERS

[1] **Maura Lodovici** , [1] **Silvia Caldini** , [1] **Cristina Luceri** , [1] **Victor Akpan** , [2] **Vieri Boddi and** [1] **Piero Dolara** .
[1]Department of Pharmacology, University of Florence and [2] Department of Public Health, University of Florence, Florence, Italy

High-performance liquid chromatography coupled with a fluorimetric detector was utilized to measure DNA adducts of benzo(a)pyrene [B(a)P] diolepoxide (BPDE) in leukocytes of smokers. Comparing subjects with a high exposure to cigarette smoke [B(a)P > 50 ng/day] with a less severely exposed group, we found a slightly higher level of adducts in the more exposed group ($1.70 \pm 0.3 \times 10^{8}$ nt (mean ± SE) vs $1.09 \pm 0.1 \times 10^{8}$ nt (mean ± SE); P = 0.057); however, the correlation between exposure to B(a)P through smoking and BPDE-DNA adduct levels was poor (r = 0.31; n= 41). We also studied the effect of individual variations in genes controlling B(a)P metabolism on BPDE-DNA adducts. The subjects were classified in "high-risk" and "low-risk" genotypes for smoking-related B(a)P DNA damage**.** The first group included subjects characterized by a combination of increased B(a)P activation [cytochrome P450 1A1 (CYP1A1) MspI and/or exon 7 Ile462Val allele variants and microsomal epoxide hydrolase (mEH) fast activity] and decreased deactivation ability [presence of glutathione S-transferase M1 (GSTM1) null allele and wild-type glutathione S-transferase P1 (GSTP1)]. The second group included smokers with lower B(a)P activation (wild-type CYP1A1, low or intermediate mEH activity) and higher deactivation capacity (active GSTM1, GSTP1 Ile105Val allele).The results showed that subjects in the low-risk genotype group, as defined above, had lower levels of BPDE-DNA adducts relative to subjects in the high-risk genotype group; this difference was significant using genotype classification of risk based upon two markers (CYP1A1 and GSTM1; median of adducts ± SD: 0.77 ± 1.16 versus 1.89 ± 0.39, P = 0.03) or three markers (CYP1A1, GSTM1, and GSTP1, median of adducts ± SD: 0.66 ± 0.93 versus 1.43 ± 1.17, P =0.013). Moreover, when mEH was included as an additional marker, these differences became statistically non-significant (P=0.085). In conclusion, CYP1A1, GSTM1, and GSTP1 genotyping seems to be a risk predictor of BPDE-DNA adduct formation in human leukocytes.

LOSS OF MICROSOMAL CYP P450 ACTIVITY DURING INCUBATION WITH NADPH GENERATING SYSTEM: EFFECT OF EDTA ON VARIOUS CYP P450 ISOFORMS

N. Pons, M. Kajbaf*, P. Rossato, R. Tolando
Drug Metabolism and Pharmacokinetic Department, GlaxoSmithKline, Verona, Italy
** CEDD-DMPK, GlaxoSmithKline, Verona, Italy*

An increasing number of drugs have been reported to cause the so-called mechanism-based inhibition (MBI) of cytochrome P450 (CYP) enzymes. The term MBI is conventionally applied to cases where a compound causes irreversible or quasi-irreversible enzyme inhibition, requiring synthesis of new enzyme for recovery of activity (1).
MBI is characterized by NADPH, time and concentration-dependency. It occurs when some drugs are converted by CYPs to chemical species able to inactivate the enzyme. MBI can be detected *in vitro* by pre-incubation (20 min) of microsomes, with inhibitor and cofactor (NADPH). The potency of inhibitor on CYP activity is calculated based on fold changes in IC_{50} after 20 minutes of pre-incubation with and without NADPH.
However, a decrease of the enzyme activities of some CYPs isoforms was observed in human liver microsomes after 20 minutes of NADPH pre-incubation, even in absence of drugs. In particular the loss of enzymatic activity of CYP1A2, CYP2B6 and CYP2C9 was pronounced (loss of 80%, 70% and 60% of the initial activity, respectively). But it was also observed for CYP3A4 and CYP2D6, although at lower level. The reason of this loss of CYP activity is not clear. However it is possible that reactive oxygen species (ROS) play a key role in this phenomenon. In fact, formation of ROS is an inevitable result of NADPH consumption by microsomal CYP in the absence of substrates (2). Many experiments using hepatic microsomes suggested that P450 generation of ROS leads to the loss of haem of cytochrome P450 (3), and ROS may also contribute to the initiation of lipid peroxidation in presence of non haem iron (4).
The aim of this work was to investigate the mechanism(s) underlying the observed CYPs enzymatic activities inactivation at these experimental conditions. Moreover, this work was also aimed to investigate the reason of the observed difference in the level of enzymatic inactivation among different CYP isoforms.

References

1. Shufeng Zhou et al (2004) Current drug metabolism. 5: 415-442.
2. Zangar R.C et al (2004) Toxicology and applied Pharmacology. 199: 316-331.
3. Cantoni A et al (1981) Int J.Biochem. 13: 823-830.
4. De Matteis F et al (1977) Biochem. J. 168: 417-422.

CHANGES IN CYTOCHROME P450 ENZYME ACTIVITIES IN GROWING FISH USED FOR A LAGOON ECOSYSTEM RESTOCKING

Tozzini V., Corsi I., Colafrancesco A.C., Corsolini S., Borghesi N., Schiavone A. and Focardi S.*Department of Environmental Sciences "G. Sarfatti", University of Siena, via Mattioli, 4 53100 Siena, Italy*

The principal component of the MFO system is cytochrome P450 (P450), a superfamily of structurally and functionally related heme proteins which are able to bind and metabolise a wide variety of substrates. Several P450 enzymes are of special interest as biomarkers of environmental pollution in fish including the hepatic 7-ethoxyresorufin O-deethylase (EROD) which is involved in the metabolism of aromatic and chlorinated hydrocarbons such as PCBs (Stegeman and Livingstone, 1998). The aim of the present study was to investigate CYP1A, 2B-like and CYP3A enzymes activity in gilthead seabream (*Sparus aurata*) used for lagoon ecosystem restocking, in order to understand metabolic/detoxifying capabilities correlated to fish growth. In December 2003, farmed juvenile of gilthead seabream (100 g) were released in the Stagnone of Marsala lagoon and some specimens were then re-catched after 3 and 6 months. The following P450 enzyme activities were investigated on liver microsomal fraction: 7-ethoxy-, 7-methoxy- and 7-benzyloxy-, 7-pentoxyphenoxazone (resorufin) O-dealkylation for CYP1A and CYP2B-like respectively (Burke and Mayer, 1984) and also 7-BCF for CYP3A (Miller et al., 2000). Total P450, NAD(P)H P450 and NADH ferricyanide reductases were also measured by the method of Rutten et al., (1987), and Livingstone and Farrar (1984), respectively. Regarding PCBs analyses, samples were analysed following the method of Kannan *et al.,* (1998). Gas chromatography (GC-ECD) was used to identify and quantify the compounds. A significant increase with size of seabream was evident for all P450 activities (EROD, MROD, BROD and PROD and also for the 7-BFC), which also positively correlate with total P450 content ($p<0.05$). Furthermore, they resulted highly positively correlated among them. NAD(P)H P450 and NADH ferricyanide reductase showed significant differences in seabream of different size but any positive correlation was evident with increasing of fish size. PCB levels detected in seabream muscle showed a significant decrease with fish size in particular of those known as CYP1A and CYP2B inducers. The changes discussed aboive of P450 activity and tptal content suggest than in growing fishes the biotrasformation potential increases with age, a compensatory mechanism to the accumulation of toxic inducers of P450 (PAHs, PCBs and dioxins) due to change in diet and larger body size.

Burke, M. D. and Mayer, R. T. (1974). *Drug Metab. Dispo.*, 2:583-588. Kannan K., Imagawa T., Blankenship A.L., Giesy J.P. (1998). *Environ Sci Technol,* 32: 2507-2514. Livingstone, D.R. and S.V. Farrar (1984). *Sci Total Environ*, 39: 209-235. Miller V.P. et al., (2000). *Ann NY Acad Sci*. 919: 26-32. Rutten A. A. J.J.L., ., Falke H.E., Catsburg J.F., Topp R., Blaauboer B.G., van Holsteijn I., Doorn L. and van Leeuwen F.X.R. (1987). *Arch Toxicol*, 61: 27-33. Stegeman, J. J. and Livingstone, D. R. (1998). *Comp Biochem Phys C*, 121, 1–3.

CHARACTERIZATION OF HEPATIC SUBFRACTIONS FROM PHENOBARBITAL-INDUCED CATTLE AND THEIR USE IN THE STUDY OF THE METABOLIC FATE OF BOLDENONE AND ANDROSTA-1,4-DIENE-3,17-DIONE

Cantiello M.*, Nachtmann C.§, Mastrone A.§, Carletti M.*, Gusson F.*, Capolongo F.#, Bellino C. *, Cagnasso A. * and Nebbia C.*
** Dipartimento di Patologia Animale, Università di Torino (Grugliasco)*
§ Istituto Zooprofilattico del Piemonte, Liguria e Valle d'Aosta (Torino)
 # Dipartimento di Sanità Pubblica, Patologia Comparata e Igiene Veterinaria, Università di Padova, Agripolis (Legnaro)

Introduction: Despite the EU ban (1), anabolic steroids such as boldenone (Bol) are currently used illicitly as growth promoters for cattle fattening. Monitoring of the ban requires further knowledge about biotransformation processes to identify a possible biomarker of illegal treatment. In this study hepatic subfractions from phenobarbital (PB)-induced or untreated cattle were first characterized as to their cytochrome P450 (CYP) expression and then used to investigate the metabolic pathways of Bol and its important metabolite/precursor androsta-1,4-diene-3,17-dione (ADD).

Materials and Methods: Male 10 months old-Friesian cattle received either PB (PB; n=4; 18 mg/Kg b.w./day *per os* for 7 days) or remained untreated (C; n=3). Liver subcellular fractions were isolated by differential utracentrifugation, as described earlier (2). Total CYP content and the rate of the *in vitro* metabolism of more than 15 model substrates were assayed as described elsewhere (3). Apoprotein levels were measured by western blotting analysis, according to standard procedures. The metabolic fate of Bol or ADD (1mM final concentration) was studied by *in vitro* incubation, using postmitochondrial fractions supplied with an NADPH generating system. The extracts were analyzed by liquid chromatography-mass spectrometry (LTQ – Thermo Electron Corporation) equipped with an atmospheric pressure chemical ionization (APCI) interface.

Results: As expected, the main isoforms involved in PB induction were CYP2B, CYP2C and CYP3A, showing 1.5 to 15 fold increase in enzyme activity and 2 to 3 fold increase in protein levels. Incubations with 17β-Bol revealed ADD as the major biotransformation product in both C and PB subfractions. Moreover, three hydroxylated metabolites (OH-Bol) were identified and the amount of one of them was 4 times higher in PB than in C group. Incubations with ADD yielded 17α- and 17β-Bol: both in C and PB fractions the β epimer was present to a far greater extent than the α epimer (about 20 fold). Also in this case the amount of one of the 3 OH-Bol formed was about 6 times higher in PB than in C group.

Conclusions: Only the enzymes responsible for OH-Bol generation would appear to be induced by PB; moreover this study demonstrate for the first time the *in vitro* production of 17α-Bol as the result of ADD incubation.

References:

1. Council Directive 96/22/EC, Off. J. Eur. Communities, L125 (1996) 3;
2. Nebbia C., Ceppa L., Dacasto M., Nachtmann C. and Carletti M (2001) J Vet Pharmacol Ther 24:399-403;
3. Nebbia C., Dacasto M., Rossetto Giaccherino A., Giuliano Albo A. and Carletti M. (2003) Vet J. 165: 53-64.

Aknowledgements: This research was funded by the Ministry of Health (Progetto IZSPLV 009/01: "Genesi del boldenone in urina di bovino. Sviluppi e applicazioni inerenti la ricerca del residuo")

NCX 4016 METABOLISM IN SUBCELLULAR FRACTIONS OF HUMAN LIVER. STUDIES ON INHIBITION OF METABOLIC AND NO-RELEASING PROCESSES.

R. Maucci, S. Casagrande, M. Govoni, P. Tocchetti
NicOx Research Institute, Via Ariosto 21, 20091 Bresso, Italy

NCX 4016 (NCX), a NO-donating derivative of aspirin, is a novel antithrombotic and vascular anti-inflammatory agent currently under development for cardiovascular diseases. The purpose of this study was to investigate NCX metabolism in human liver subcellular fractions and, by the use of specific and selective inhibitors, to identify the major metabolising enzyme(s) involved. **Materials and Methods.** Liver mnetabolism was studied adding NCX to S9 and microsomal fractions (1 mg protein/ml, n=3), so as to obtain a range of final concentrations from . 50 to 250 μM. The mixtures were incubated at 37°C up to 1 h in presence or absence of cofactors. To investigate the mechanism by which hepatic GSTs, carboxylesterases, CYPs, and XO could generate NO from NCX, microsomes and cytosol (1 mg/ml, n=3) were incubated at 37°C up to 2 and 4 h, respectively, with increasing conc. (1-5 mM) of various specific inhibitors (EA, SBP, iso-OMPA, NEM, SKF525A and allopurinol), in presence of cofactors and 250 μM NCX. Drug metabolic profiles were assessed by the HPLC/UV-DAD approach while NO release was studied by the increase of inorganic nitrogenous species (NOx) levels as determined by Gas Phase Chemiluminescence.
Results and Discussion. NCX undergoes rapid and extensive hydrolysis in all liver fractions to form mainly SA and NCX 4015. This latter is further metabolized and NO is released in a slow sustained fashion, regardless of incubation conditions. The differences in inhibition of NO release between SBP-treated subcellular fractions (NOx still detectable vs. non-inhibited controls: 15% and 43% in 2.5 mM SBP-treated cytosol and microsomes, respectively); the weak effect on NO release by EA unless at higher conc. in both fractions (NOx detectable vs. controls: 89% and 65% in 5 mM EA-treated cytosol and microsomes, respectively); the maximal NOx reduction of 20% obtained with similar conc. of NEM, suggest that the mechanism of reduction of NO released by NCX probably results from a specific inhibition of a certain enzymatic pathway by GSTs which is not strictly dependent on GSH availability. Furthermore, the activities of SBP and iso-OMPA differ significantly under similar reaction conditions (8% and 15% of NOx detectable in 5 mM SBP-treated cytosol and microsomes, while the corresponding values for 5 mM iso-OMPA were both around 50%), confirming that higher inhibition of NO release in SBP experiments can not be only explained on the basis of a non specific effect due to an interference with carboxylesterases activity. Furthermore, microsomal CYPs and cytosolic XO are unlikely involved in the metabolism of the nitric ester moiety of NCX 4015, SKF525A and allopurinol not reducing significantly NO production under our experimental conditions, even at very high conc.

Abbreviations: GSTs, glutathione S-transferase; XO, xanthine oxidase; EA, ethacrynic acid; SBP, sulphobromophtalein. NOTE: SA,DAD, NEM and OMPA are not defined among the asbbreviations

N-GLUCURONIDATION OF ANGIOTENSIN II RECEPTOR ANTAGONIST (AT_1), CR 3210, IN RAT LIVER MICROSOMES

N. Caradonna, S. Dragoni, M. Valoti, S. Vomero, M. Anzini, A. Cappelli
Dipartimento di Scienze Biomediche, Dipartimento Farmaco Chimico Tecnologico, Università di Siena

In vitro studies have shown that the recently synthesized (4-[4-[(2-ethyl-5,7-dimethylimidazo[4,5-b]pyridin-3-yl)methyl]phenyl]-3-(2H-tetrazol-5-yl)quinoline, CR3210, is an angiotensin II receptor antagonist, AT_1 , with an affinity value similar to the structurally related compounds, losartan and L158,809 [1]
Pharmacokinetic studies showed, however, that the intravenous administration of CR3210 gave rise to a significant plasma concentration, that rapidly decreased within 15 min after injection It is well known that the tetrazole derivatives undergo metabolism by glucuronidation that confers them a rapid bile excretion [2]
In order to explain the poor pharmacokinetic properties of CR3210 we have studied its glucuronyl-transferase-dependent metabolism, comparing it to that of the structurally related biphenyl-derivative L158,809, which presents more favourable pharmacokinetic properties.
The compounds, at variable concentrations (0-500 μM), were incubated with 1 mg/ml microsomal proteins, 3 mM UDP-glucuronic acid (UDPGA), in 25 mM phosphate buffer, pH 6.2, at 37°C. The metabolites and parent compounds were extracted in methanol and analysed by HPLC, monitoring their elution by their absorbance at 280 nm. A standard curve was constructed by using different concentrations of a synthetic glucuronide derivative of L158,809.
The HPLC-MS analysis revealed that the CR3210, as well as, L158,809 were glucuronidated on the tetrazole moiety and that the corresponding glucuronyl derivatives were the only metabolites detected in our experimental conditions.
CR3210 presented an atypical kinetic profile characteristic of inhibition at high-substrate-concentrations. On the contrary L 158,809 followed a hyperbolic, Michealis-Menten, dependence on substrate concentration, suggesting that only one glucuronyl transferase isozymes is involved in its metabolism. The ratio V_{max}/K_m, taken as an index of intrinsic clearance, was higher in the case of *CR3210* N-glucuronidation compared to that of L158,809 glucuronidation, suggesting a more efficient metabolism of CR3210.
The evidence that the CR3210 underwent extensive metabolism by N-glucuronidation indicates that this metabolic pathway greatly influences its pharmacokinetic properties. Although CR3210 is characterized by a greater lipophylicity than L158,809, its rapid N-glucuronidation metabolism may give rise to an extensive bile excretion and explain the rapid excretion phase.

This work was supported by University of Siena and Ministero degli Affari Esteri, legge 401/99.

1. Cappelli A, Pericot Mohr Gl G, Gallelli A, Rizzo M, Anzini M, Vomero S, Mennuni L, Ferrari F, Makovec F, Menziani MC, De Benedetti PG, Giorgi G. J Med Chem. 2004; 47:2574-86.
2. Rizzo M, Anzini M, Cappelli A, Vomero S, Ventrice D, De Sarro G, Procopio S, Costa N, Makovec F. Determination of a novel angiotensin-AT1 antagonist CR 3210 in biological samples by HPLC. Farmaco. 2003;58:837-44.

EFFECTS OF THE ANTINEOPLASTIC DEIDROTARPLATIN ON CYP AND OXIDATIVE STRESS ENZYMES IN LIVER AND KIDNEY OF RAT

Annalisa Nannelli, Emilia Vaccaro, Sandra Marini*, Silvia Trasciatti* and Pier Giovanni Gervasi
*Istituto di Fisiologia Clinica CNR Pisa; *Abiogen Pharma Pisa*

Introduction: Cis-platinum (Cis-Pt) is a widely used antineoplastic drug, highly efficient against several human tumours such as bladder, testis and ovary ones. Nevertheless, Cis-Pt induces serious side effects including nausea, nephrotoxicity, neurotoxicity, depletion of circulating testosterone and a drastic variation of the hormone regulated CYPs expression. In male rats, in fact, a decrease in the hepatic levels of male-specific isoforms (CYP2C11, 3A2, 2A2, 4A2) and an increase of female-predominant ones (CYP2A1, 2C7) have been observed. Rat treatments with Cis-Pt induced, in renal cortex, oxidative stress, lipid peroxidation and depletion of antioxidant defence system (Glutathione (GSH), Glutathione-S-Transferase (GST), Superoxide Dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase (CAT)). In the last few years, many attempts have been made to produce Cis-Pt analogues with the same antineoplastic activity but with reduced side effects. In this study, we have evaluated in rats the modulation of CYP activities and the induction of oxidative-stress caused by Deidrotarplatinum (DTP), a newly developed antineoplastic drug.
Materials and methods: Three groups of six rats were used: the first and the second groups were injected with DTP 25 mg/Kg and sacrificed after 3 days and 7 days, respectively; the third one was kept as the control group. Liver and kidney were excised; microsomes and cytosols were prepared using a standard procedure. Testosterone plasma content, GSH level of liver homogenate, and renal and hepatic CYP contents were determined according to literature. Aniline hydroxylase and lauric acid hydroxylase activities (markers for CYP2E1 and 4A) were determined in liver and renal microsomes, whereas testosterone hydroxylase activity (marker for CYP2B, 2C and 3A) was assayed only in the liver microsomes. CAT, GST, GSH-Px and GSSG-Reductase activities were performed in renal and hepatic cytosols.
Results: DTP treatments, unlike Cis-Pt, did not cause a reduction of total CYP contents in either liver or renal microsomes. However, a decrease in circulating testosterone levels was observed after DTP administration, (though less severe than with Cis-Pt) , and a consequent inhibition of hepatic CYP2C11-dependent 2α and 16α testosterone hydroxylase activities. Preliminary data showed that DTP also caused some change in the hepatic aniline hydroxylase activity but did not enhance the hepatic lauric acid hydroxylase activity. In renal microsomes, DTP did not influence the CYP2E1-linked aniline hydroxylase activity, in contrast with Cis-Pt that strongly inhibits it; moreover, DTP, unlike Cis-Pt, induced significantly renal lauric acid hydroxylase activity. Treatments of rats with DTP did not deplete the liver homogenate GSH level and only marginally affected hepatic GSSG-Reductase, GST, GSH-Px and CAT activities. At renal level, DTP did not decrease, unlike Cis-Pt, the GSSG-Reductase, GST, GSH-Px and CAT activities.
Conclusions: We have shown that DTP had only a weak effect on renal and hepatic antioxidant enzymatic defence system Moreover, DTP caused a lower reduction of testosterone plasmatic level and hormone regulated CYPs activities than Cis-Pt. Thus, our results suggest that DTP may have reduced side effects compared to Cis-Pt.

ANALYSIS OF CYP1A1 IN DIFFERENT BRAIN REGIONS OF CONTROL AND βNF-TREATED PIGS

Chirulli V.[1], Gervasi P.G.[1], Zaghini A.[2], Vaccaro E.[1], Fiorio R.[1], Longo V.[1] and Marvasi L.[2]

[1] *Istituto di Fisiologia Clinica, CNR di Pisa;* [2] *Dipartimento di Sanita Pubblica e Patologia Animale, Università di Bologna*

Introduction: Many studies have recently been conducted on the biotransformation of foreign compounds and drugs in food-producing animals, such as pigs and cattle. Pig The pig has been considered as the best donor of hepatocytes to be used in bioartificial liver for human application (1). Pigs and minipig have recently been used to study Parkinson disease by PET (position emission tomography) investigations of the SNC (2). To better understand the biotransformation in brain we have began a characterization of cytochrome P450 in the most important cerebral regions for the motor functions, such as the frontal cortex, the cerebellum and the midbrain. Here we report on the expression of the aryl hydrocarbon receptor (AhR), the P450 1A1 and related activities in the brain from control and β-naphthoflavone (βNF) treated pigs.

Materials and methods: Two groups of three pigs (about 25-30 kg) were used: the first group was injected with 30 mg/kg of βNF for 4 days while the second one was kept as the control; 24 hours after the last treatment, pigs were sacrificed; liver and brain regions were excised and microsomes and mitochondria prepared according to Novi et al. (1998) and Sims (1990), respectively. Total RNA was extracted from each tissue, treated with DNAse, retrotranscripted and amplified by PCR using specific primers. The activities of ethoxyresorufin O-deethylase (EROD) and methoxyresorufin O-demethylase (MEROD), known markers for the CYP1A1/2, were determined in liver microsomes and microsomal and mitochondrial cerebral regions from control and βNF treated pigs.

Results: The βNF treatment enhanced the expression of CYP1A1 mRNA in the liver. In contrtast, in the cerebral regions examined the CYP1A1 transcript was detected only after βNF treatment. Regarding the AhR mRNA, it was expressed, although at different extent, in the liver, cerebellum and midbrain from both control and βNF-treated pig; it was not detected in the control cortex, possibly due to a very low and cell type specific constitutive expression. Unlikely CYP1A1 mRNA expression, the βNF treatment did not induce the EROD and MEROD activities in the microsomes and mitochondria of the brain regions examined. As previously observed in the rat brain, the EROD and MEROD activities were higher (about 3-4 folds) in mitochondrial than in microsomal fractions obtained from the cerebral regions. On the contrary, in the liver EROD and MEROD activities were induced about 15-folds by the βNF treatment.

Conclusions: CYP1A1 expression was observed in the cortex, cerebellum and midbrain from treated pigs suggesting a trascriptional activation of this gene by βNF in brain. However an induction of EROD and MEROD, as CYP1A1 marker activities, were observed in the liver but not in either the microsomal or mitochondrial fraction fron the brain regions, suggesting a different mechanism in the CYP1A1 transcription processes in the liver and brain tissues.

References:

1. Szotakova B. et al., 2004, Comparison of in vitro activities of biotrasformation enzymes in pig, cattle, goat and sheep. Research in Veterinary Science 76: 43-51
2. Cumming P. et al., 2001, Normalitation of markers for dopamine innervation in striatum of MPTP-lesioned miniature pigs with intrastrial grafts. Acta Neurol Scand, 103:309-315

3. Novi S. et al., 1998, Biotrasformation enzymes and their inductin by β-naphtoflavone in adult sea bass (Dicentrarchus labrex). Aquatic Toxicol. 41, 63-81
4. Sims N.R., 1990, Rapid isolation of metabolically active mitochondria from rat brai and subregions using Percoll density gradient centrifugation. J Neurochem. 55, 698-707

THE HUMAN FOETAL P450 ISOFORM CYP3A7 IS ABLE TO BIOACTIVATE ORGANOPHOSHOROTHIONATE PESTICIDES : A COMPARISON WITH THE ADULT ISOFORMS 3A4 AND 3A5.

Franca Maria Buratti and Emanuela Testai
Environment and Primary Prevention Department, Mechanisms of Toxicity Unit
Istituto Superiore di Sanità, -Italy (buratti@iss.it, testai@iss.it)

Some experimental evidences have associated *in utero* exposure to organophorothionate pesticides (OPT) to adverse neurodevelopmental adverse effects in the offspring. Many OPT have been shown to pass through the placenta to the foetus after oral administration to the dam in the rat. On the other hand, in humans OPT metabolites can be detected in postpartum meconium, representing the intestinal content of the foetus indicating the occurrence of prenatal exposure. OPTs are not toxic *per se*, since the actual inhibitor of AChE is the corresponding oxon, the product of their bioactivation catalysed by different P450 isoform. Therefore, in order to provide valuable data for the estimation of the developmental toxicity risk subsequent to prenatal exposure to OPTs, the catalytic activity of the CYP3A7, the major foetal P450 isoform, very likely responsible for *in situ* oxon has been assessed. In addition a comparison with CYP3A4 and 3A5 has been carried out. CYP3A4 is the most abundant hepatic and intestinal isoform while CYP3A5 is present to a variable extent in the adult livers, present in about 20% of Caucasians and being the predominant 3A form in more than 50% African-Americans.
The formation of the toxic metabolite oxon from chlorpyrifos (CPF), parathion (PAR) and malathion (MAL) has been characterized in c-DNA expressed human CYPs with a spectrofotometric method, based on AChE inhibition, able to detect nM levels of oxon. CYP3A7 is able to produce significant levels of oxon from the 3 OPT tested in a range of concentrations (1-10μM), representative of actual exposure conditions. The kinetics of the reaction is linear up to the highest concentration tested (50μM) with CPF and MAL, while a typical saturation curve was obtained with PAR. The Cl_i values were quite similar (0.007, 0.008 and 0.01 nmoloxon/(nmolP450 * min * μM) for PAR, CPF and MAL, respectively). In the range of tested concentrations (0.01-50 μM) also CYP3A4 and 3A5 shows linear kinetics. The Cl_i values show that with CPF the efficiency of the reaction is similar for 3A5 and 3A4 but lower for 3A7. With PAR, the ranking of Cl_i is 3A5 $\ll$ 3A7 $<$ 3A4 (0.0009, 0.007 and 0.03 nmoloxon/(nmolP450 * min * μM), respectively).With MAL the CLi is similar for 3A4 and 3A7 and lower for 3A5.
Considering these results, it is possible to hypothesized that the foetus is able to catalyse oxon formation *in situ* when exposed *in utero* to different OPTs, with similar efficiency. However, the metabolic capability of the foetal isoform is usually lower than the predominant adult isoform, that is CYP3A4.

This work has been partially supported by the ISS project 3AAS.

EFFECTS OF 17-β ESTRADIOL, 4-NONYLPHENOL AND PCB 126 ON THE ESTROGENIC ACTIVITY AND PHASE 1 AND 2 BIOTRANSFORMATION ENZYMES IN MALE SEA BASS (DICENTRARCHUS LABRAX).

Emilia Vaccaro[a], Domenica Di Bello[b], Valentina Meucci[c], Vincenzo Longo[a], Luigi Intorre[b], Giulio Soldani[b], Pier Giovanni Gervasi[a], Carlo Pretti[b,*].
[a] *Istituto di Fisiologia Clinica, via G. Moruzzi 1, Area della Ricerca CNR, 56100 Pisa, Italy.*
[b]*Dipartimento di Patologia Animale Profilassi ed Igiene degli Alimenti, Università di Pisa, Viale delle Piagge 2, 56124 Pisa, Italy.*
[c]*Dipartimento di Clinica Veterinaria, Università di Pisa, Viale delle Piagge 2, 56124 Pisa, Italy.*

Introduction
The endocrine systems of wildlife are exposed to a wide variety of natural and man-made chemicals which may lead to damage of reproduction and other adverse effects including alteration of the drug-metabolising enzymes. In the present study, we investigated the effects of *in vivo* exposure to a natural (17β-estradiol: E2) or a synthetic (4-nonylphenol: NP) estrogen or an anti-estrogen (3,3',4.4',5-pentachlorobiphenyl: PCB 126) upon the vitellogenin (Vtg) synthesis and the hepatic phase 1 and 2 enzymes in adult male sea bass.
Materials and Methods
Different groups of fish were injected i.p (in corn oil as vehicle) with.:1) 17-β-estradiol 0.1; 0.5; 2.5; and 5 mg/kg for 3, 7, 14 and 28 days; 2) NP 5 and 50 mg/kg for 3, 7, 14 and 28 days; 3) PCB126 10 and 100 μg/kg for 3 , 7 , 14 and 28 days; 4) PCB126 10 and 100 μg/kg + 17-β-estradiol 0.5 mg/kg for 3, 7, 14 and 28 days; 5) Only corn oil as control. From each fish, blood samples were collected, livers excised and microsomes immediately prepared according to (1). Vtg of sea bass was purified according to (2) and used for the polyclonal antibody production and as standard in the ELISA assay. Western blot analysis were performed using both heterologous antibodies anti-seabream/anti-turbot/anti-salmon/anti-carpVtg from commercial source (Biosense, Usa) and purified anti sea bass Vtg. The immunodetections for CYPs in the control and treated microsomes were performed using polyclonal antibodies raised against rat P4501A1 and trout hepatic P4503A27 as previously described (1). The activities of erythromycin N-demethylase (ErD) ethoxyresorufin-O-deethylase (EROD), testosterone hydroxylase, (ω)- and (ω-1)-lauric acid hydroxylase, glutathione S-transferase and DT-diaphorase activity were determined as previous described (1).
Results
The sea bass sensitivity to Vtg induction was found to be similar to those of other fish species but with a delay of maximum response. E2 treatments induced in plasma an increase of Vtg levels showing a dose- and time-dependent effect. A rapid formation of Vtg in plasma was found in qall groups dosed wiyh E2, with a peak of increase at day 14. The E2 treatments also caused a selective time and dose dependent inhibition of hepatic CYP1A-linked EROD and GST activities without affecting the activities of the CYP3A-linked 6β-testosterone hydroxylase, the (ω)- and (ω-1)-lauric acid hydroxylases and DT-diaphorase. A similar selective inhibition on CYP1A was also observed in the fish treated with 50 mg/kg NP. The enzymatic results regarding CYP1A and CYP3A were also confirmed by the western blots analysis. When the sea bass were treated with either 10 or 100 μg/kg PCB 126 alone, an AhR ligand, a modest and selective induction of EROD and DT-diaphorase activities was observed. Interestingly, both these activities were restored to their control levels in the sea bass co-treated with 0.5 mg/kg E2 and 10 or 100 μg/kg PCB 126, suggesting a cross-talk mechanism between the estrogen receptor and AhR.

Furthermore, PCB 126 exhibited a potent anti-estrogenic activity *in vivo* in the sea bass, as it inhibited the E2-induced Vtg synthesis with an IC_{50} of 28 μg/kg.

Conclusions

In conclusion, the present study clearly documents: i) the male adult sea bass is sensitive to natural and synthetic estrogens, ii) the heterologous commercial antibodies anti Vtg cannot be used, in place of a species-specific anti-Vtg, for monitoring Vtg as a marker of sea bass exposure to endocrine disrupting chemicals, iii) PCB126 is a potent anti-estrogenic compound in adult male sea bass with a IC_{50} of 28 μg/kg. Furthermore, it was demonstrated that the natural or xeno-estrogens depress selectively the activity associated to CYP 1A and GST and this may compromise the detoxification capability of sea bass exposed to environmental pollutants such as polycyclic aromatic hydrocarbons.

References

Novi S., Pretti C., Cognetti A.M., Longo V., Marchetti S., Gervasi P.G., 1998. Biotransformation enzymes and their induction by β-naphtoflavone in adult sea bass (*Dicentrarchus labrax*). Aquatic Toxicol. 41, 63-81.

Mañanós E., Zanuy S., Le Menn F., Carrillo M., Núñez J., 1994. Sea bass (*Dicentrarchus labrax* L.) vitellogenin. I-Induction, purification and partial characterization. Comp. Biochem. Physiol. 107B, 205-216.

DOSE-RESPONSE INCREASE OF CYTOCHROME P450 CATALYTIC ENZYMES ACTIVITY IN THE MEDITERRANEAN LIMPET *PATELLA CAERULEA* LABORATORY EXPOSED TO BENZO(A)PYRENE

Iacocca Annalisa, Corsi Ilaria, Ghezzo Claudia, Focardi Silvano
Department of Environmental Sciences "G. Sarfatti", University of Siena, Italy.

The increasing contamination by xenobiotics of aquatic ecosystems has made it necessaryled to the need of assess the impact of these contaminants on living organisms. In this context, cytochrome P450 enzymes, which are involved in detoxification processes, have been extensively used as biomarker of exposure in marine pollution monitoring programmes (Livingstone, 1998). Many studies have reported that in molluscs the concentration of total cytochrome P450 and its activities are increased by exposure to xenobiotics (Livingstone, 1989; Peters et al, 1998). Nevertheless, this biomarker has been validated in only a small number of invertebrate species, mainly bivalve molluscs(Snyder, 2000). The gastropod limpet *Patella caerulea* is a sedentary species, widely distributed in rocky coastal areas and capable of accumulating aquatic contaminants; for these reasons it has been widely used as a sentinel species in many biomonitoring studies (Campanella et al, 2001). In the present research cytochrome P450 catalytic phase I enzymes were investigated in the Mediterranean limpet by laboratory exposure in order to validate their application as suitable biomarkers of exposure in biomonitoring studies of marine coastal areas. Limpets of homogeneous size (2-3 cm in length) were exposed for 24h to water spiked with benzo(a)pyrene (B(a)P), at 0.1, 1, 5, 10 ppm using DMSO (0.1‰) as a carrier. Microsomal fractions were prepared from the digestive gland and NADH ferricyanide reductase and NAD(P)H cytochrome c reductase activities were measured according to Livingstone and Farrar (1984) and total cytochrome P450 as described by Rutten et al (1987). A dose-dependent significant increase was observed for all enzyme activities except for the highest dose of 10 ppm of B(a)P for which a significant decrease was observed. Positive correlations were observed among reductase activities: r=0.68 between NADH cyt c and ferricyanide and r=0.95 between NADPH cyt c and ferricyanide. A less clear increasing trend was observed for total P450 levels. A molecular investigation of CYP encoding gene by testing several primers for invertebrates by PCR was performed in order to identify P450 gene expression in this species. The results obtained seem to indicate that P450 catalytic enzyme activities are modulated/induced by B(a)P in the Mediterranean limpet, thus suggesting their application as a suitable biomarker of polycyclic hydrocarbons exposure in biomonitoring studies of marine coastal areas.

References: Livingstone D.R. (1998) Comp. Biochem. Physiol. Part A 120: 43-49. Livingstone D.R. (1989) Xenobiotica, 19: 1041-1062. Peters L. D., Nasci C. and Livingstone D. R. (1998) Mar. Environ. Res. 46: 295-299. Snyder M.J. (2000) Aquat. Toxicol. 48: 529-547. Campanella L., Conti M.E., Cubadda F. and Sucapane C. (2001) Environ. Pollut. 111: 117-126. Livingstone D.R. and Farrar S. (1984) Sci. Total Environ. 39: 209-235. Rutten A. A., Falke H.E., Catsburg, J.F., Topp R., Blaauboer B.J. van Holsteijn I., Doom L. and van Leeuwen F.X. (1987) Arch. Toxicol. 61: 27-33.

CYTOCHROME P450-DEPENDENT METABOLISM OF PF9601N, A NOVEL MAO-B INHIBITOR, BY HUMAN AND MOUSE LIVER MICROSOMES

Stefania Dragoni, Claudio Polloni, Maria Frosini, Giampietro Sgaragli, Mercedes Unzeta*and Massimo Valoti
*Dipartimento di Scienze Anatomiche e Biomediche, Università di Siena, Siena, Italy and *Department de Bioquimica y Biologia Molecular, Universitat Autónoma de Barcelona, Bellaterra, Barcelona, Spain*

The acetylenic monoamine oxidase inhibitor (MAOI) PF9601N may prove an effective therapeutic agent for improving early symptoms of idiopathic Parkinson's disease (Perez and Unzeta, 2003) The inhibitors of MAO have been reported to inhibit enzymes such as diphosphopyridine nucleotidase, succinic dehydrogenase, guanidine deaminase, spermine oxidase and histaminase . Nevertheless the interaction of such inhibitors (for example l-deprenyl) with the microsomal monooxygenase system has already been reported (Valoti et al, 2000). In order to investigate whether PF701N can interact with the CYP system, we have treated C57BL/6 mice with PF9601N (10 mg Kg^{-1} by i.p. injection in normal saline) for 4 days. CYP content and related activities were assayed in liver microsomal preparations. Metabolite identification and kinetic analysis were performed on the same preparations. In another series of experiments kinetic studies were performed in vitro using human liver microsomes. The results obtained indicate that treatment with PF9601N did not significantly affect the total content of CYP and cytochrome b_5, the activities of NADPH-cytochrome P450 reductase and of several monooxygenases assayed**,** at variance to what is observed with the structural related MAOI, l-deprenyl (Valoti et al, 2000). Kinetic analysis of PF9601N was performed in 5 different liver microsomal fractions prepared either from control and treated animals. In control animals, at all the concentrations tested, the N-dealkylated derivative (namely FA72) turned out to be the only metabolite, and its formation followed a hyperbolic, Michealis-Menten, behaviour. Similar results were observed when kinetic analysis was performed using microsomes from PF9601N-treated mice. Inhibition studies showed that only ketoconazole significatively inhibited the FA72 formation rate, suggesting that an isoenzyme of the CPY3A subfamily may be involved.
When kinetic analysis was performed in pooled human liver microsomes the apparent K_m and V_{max} values resulted similar to those observed with mouse microsomes. Significative inhibition was again obtained with ketoconazole indicating that CYP3A4 is involved in PF9601N metabolism in humans.
In conclusion the present study show that PF9601N does not interfere with the liver pattern of CYP-dependent drug-metabolizing activities and presents a better metabolic profile than the corresponding MAOI, l-deprenyl. Furthermore the similarity of results obtained with preparations from mice and humans suggests that the C57BL/6 mouse may be a good model to study the metabolic fate of PF9601N.

This work was supported by University of Siena, Fondazione Monte dei Paschi Siena and COST working group: D13/0018/01 and Ministero degli Affari Esteri, legge 401/99.

Dragoni S, Bellik l, Frosini M, Sgaragli G, Marini S, Gervasi PG, Valoti M.(2003), Xenobiotica, 33, 181-195
Perez V and Unzeta M, (2003) Neurochem Int, 42, 221-9.
Valoti M, Fusi F, Frosini M, Pessina F, Tipton KF, Sgaragli GP, (2000) Eur J Pharmacol, 391, 199-206

EFFECTS OF VARIOUS DP-DERIVATIVES ON RAT CYP450 ISOFORMS: A STRUCTURE-ACTIVITY CORRELATION STUDY

D'Elia P., Dragoni S., Valoti M., Sgaragli GP.
Dipartimento di Scienze Biomediche, Università degli Studi di Siena, Italia

Chemotherapy is often the favourite treatment against cancer. Tumor cells may exhibit multidrug resistance (MDR) to several cytotoxic drugs by overexpression of ATP-binding cassette membrane transporters, including P-glycoprotein (P-gp). These transporters pump out the cells the drugs. Finding compounds that inhibit P-gp action (MDR reverters) is a possible way to overcome MDR.
In a recent study (1), novel 3,5-diacetyl- (DP1-DP5) and 3,5-dibenzoyl-1,4 dihydropyridines derivatives (DP6-DP11) were assessed for their ability to inhibit P-gp-dependent MDR. Their effects on rat vascular system were tested and it was found that DP7 [3,5-dibenzoyl-4-(3-phenoxyphenyl)-1,4-dihydro-2,6-dimethylpyridine] was devoid of vascular activity at a concentration (10^{-5} M) two order of magnitude higher than its IC_{50} as a P-gp inhibitor (10^{-7}).
This is not sufficient to guarantee for DP7 safety and further experiments on other physiological systems are needed before clinical investigations on DP7 can be addressed .
Some dihydropyridine compounds interact with the cytochrome-P450 system (CYP450). So, we have noiw investigated whether our DP-derivatives can affect rat CYP isoforms.
METHODS. Inhibition by DP7 and other DP-derivatives of CYP-dependent activities was tested on liver microsomal preparations from male rat. We evaluated inhibition by DP-derivatives of methoxy- (MTR), ethoxy- (ETR), pentoxy- (PTR) and benzyloxy- (BZR) resorufin-O-dealkylase reactions catalyzed by the CYP system. Each one of the resorufin-substrates is a marker for a specific CYPisoform as follows: PTR (CYP2B1/2), MTR (CYP1A2), ETR (CYP1A1), BZR (CYP1A1/2-2A1/2-3A1/2). Concentration of each resorufin-substrate was kept constant, while DP7 concentration was changed (0.15-15 µM) to obtain the corresponding IC_{50} value. The other DP-derivatives were only tested at a concentration of 1.5 µM. Rat liver microsomes were suspended in a TRIS buffer reaction mixture containing the resorufin-substrate (PTR 10 µM, MTR 5 µM , ETR 2µM or BZR 5 µM), dicumarol, and a DP-derivative; NADPH was added to start the reaction.
RESULTS. DP7, inhibited all the 7-alkoxyresorufins-O-dealkylase activities. At the highest tested concentration (1.5 µM) it inhibited PTR-, MTR- and ETR-O-dealkylation with percent values of inhibition of about 50, 20 and 45, respectively.
The effect on BZR-O-dealkylation was the strongest with percent inhibition value of about 70, at DP7 concentration of 1.5 µM. The inhibition was of a mixed type, with inhibition constant values (K_i e K'_i):of 0,1357 and 0,8606 µM, respectively.
A study of the structure-activity relationship revealed that the 3,5-diacetyl (DP1-DP5) were less effective in inhibiting the various CYP isoforms.
than the 3,5-dibenzoyl derivatives (DP6-DP11).
This work was supported by the University of Siena and Ministero degli Affari Esteri, legge 401/99, Programma di cooperazione Scientifica e Tecnologica tra Italia e Ungheria

Saponara S., Kawase M., Shah A., Motohashi N., Molnar J., Ugosai K., Sgaragli G. and Fusi F. (2004) Br. J. Pharmacol. 141: 415-422.