MT19

OVEREXPRESSION OF THE GLYCOSYLATED HUMAN SECRETORY COMPONENT IN MONKEY CV-1 CELLS.

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Two vaccinia virus expression systems were used to direct the expression and secretion of the human secretory component (SC), a glycoprotein associated with dimeric IgA in mucosal secretion.
Our data show that whereas both vaccinia virus systems, where the target gene is regulated by different strong promoters, permit the expression of similar amounts of native SC protein, they nevertheless fail in secreting a significant proportion of the recombinant protein which accumulate within the host cell.
Accumulation of the SC protein within the secretory pathway was assayed by indirect immunofluorescence as well as enzymatic deglycosylation. In addition, in the presence of tunicamycin, a general inhibitor of N-linked glycosylation, the secretion of the nonglycosylated SC protein was very weak and delayed in time, suggesting a potential role of the sugar residues of the recombinant protein along the secretory pathway. Pulse-chase experiments were performed in order to assess the rate of the newly translated recombinant protein and whether the accumulated intracellular SC could partially be degraded in the ER compartment.

Pharmacology

P01

CHRONIC ORAL MUSK XYLENE (MX) SPECIFICALLY INDUCES CYTOCHROME P450 1A IN LONG EVANS RATS

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The widely used synthetic fragrance musk xylene, a lipophilic and highly resistant compound, bioaccumulates in fish, human fat and milk.Male and female rats fed with MX 0,1g/kg or 0,03g/kg food pellets for a minimum of 10 weeks were mated and fat concentrations of MX were determined with GC/ECD detection in parents and their 14 day old offspring. Bioaccumulation of MX in the fat of the pups was dependent on the dose: 25mg/kg lipid(MX0,03g/kg) and 130mg/kg lipid(MX0,1g/kg). In the 14 day old pups liver enzyme induction was about in the same order of magnitude as in adult rats, with different enzyme patterns:EROD (ethoxyresorufin-deethylase, Cyp1A1) activity beeing higher in pups and MROD(methoxyresorufin-demethylase, Cyp1A2) activity higher in adults.

P02

α1-ADRENERGIC RECEPTOR ON PRE-B CELL LINES AND BIOLOGICAL OUTCOME

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In previous work we demonstrated that noradrenaline may modulate hematopoiesis via an α1b-adrenergic receptors. We had evidences that these α1b-adrenergic receptors are expressed by pre-B cells. Now we report that two human and one murine pre-B cell lines bear the same receptor with Kds which are similar to that showed in normal pre-B cells. In these cell lines activation of the receptor by noradrenaline caused inhibition of growth and cell death.
We investigated whether Ca^{++} uptake was involved in this effect and whether the α1 antagonist prazosin could reverse the action of noradrenaline. The results showed that Ca^{++} is apparently involved but not via α1-adrenergic receptor while prazosin at low concentration (1pM) counteracted the noradrenaline-induced cell death. Moreover, we found that activation of this receptor induces an overproduction of p53 protein which seems related to the expression of differentiation antigens such as sIgM and CD13.
In conclusion our findings suggest that differentiation and/or apoptosis in hematopoietic cells are not only under cytokines control but seems also to be under a neural adrenergic regulation.

P03

CYSTEINE-227 IS ESSENTIAL FOR ACTIVITY OF HUMAN CARBONYL REDUCTASE

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Carbonyl reductase (EC 1.1.1.184), a member of the short-chain oxidoreductases, catalyzes the NADPH-dependent reduction of a varierty of endogenous and xenobiotic carbonyl compounds. Incubation with one equivalent 4-hydroxymercuribenzoate decreases enzyme activity by about 70% and more than two equivalents almost completely abolish enzyme activity, suggesting the presence of one or more essential cysteine residues. In order to identify the essential residue(s) each of the five cysteines of human carbonyl reductase was converted to alanine by site-directed mutagenesis and the mutant cDNA ligated into the plasmid vector pET-11a and expressed in E.coli. Four mutants, C26A, C122A, C150A and C226A showed normal enzyme activity whereas the activity of C227A was less than 1% of that of the wild type enzyme. Similarly, replacement of Cys 227 by serine abolished enzyme activity. Cys-227 is not conserved in other short-chain oxidoreductases indicating a specific function of this residue in carbonyl reductase.

P04

LONG CHAIN FATTY ACID OXIDATION IN MITOCHONDRIA IS INHIBITED BY CHLOROACETALDEHYDE AND RESTORED BY METHYLENE BLUE

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Chloroacetaldehyde (CAA) is a metabolite of the widely used antineoplastic agent ifosfamide (IFO) and has been implicated as a probable candidate causing the IFO associated neurotoxicity. Clinically, the redox dye methylene blue (MB) is used to prophylactically protect patients from this neurotoxicity although mechanistic information is not yet available. We therefore exposed rat liver mitochondria to CAA [500 μM] and observed a time dependent decrease [85% max.] in state 3u (uncoupling with dinitrophenol) oxidation rates for palmitoyl-L-carnitine as compared to controls. Addition of MB [2.5 μM], following CAA, increased mitochondrial respiratory rates by up to 170%. We conclude that MB is capable of shuttling electrons into the respiratory chain, thereby increasing the rate of oxidative phosphorylation following inhibition by CAA. The direct effect of MB on mitochondria intoxicated with a metabolite of IFO may aid in understanding the related toxicity.

P05

A METHODOLOGY FOR VOLUMETRIC ANALYSIS OF THE LIVER FROM RATS EXPOSED TO PCBs

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Accuracy is required to make decisions in predicting risks for human beings to PCBs (polychlorinated biphenyls), persistent pollutants. Sprague-Dawley rats of both genders received 50 ppm PCB 153 mixed in corn oil in diet; animals that served as controls had oil-contained diets without the PCB. Following 13 weeks of feeding, rats were killed and liver samples were harvested from 12 animals (3 of each gender) in dosed and control groups. Thick resin-embedded sections were prepared by conventional methods to estimate average zone 3 hepatocyte (up to six cells from terminal venule) and nucleus volumes in μm^3 (Gundersen & Jensen, 1983). A "Bioquant" system, and a double square lattice (Mathieu *et al.*, 1981) whose position was randomized, was superimposed on monitor screen for measurement of images at 2593x (obtained by calibration). The intercepts through coarse points that hit cells and nuclei hit by fine points were measured. Each recorded microscopic field was randomized within the cell. PCB 153 qualitative studies data are in harmony with our measurements.

P06

Partitioning of Drugs in a Phosphatidylcholine-Liposome/Buffer System

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Lipophilicity is an important parameter for drug design as it determines the pharmacokinetic behavior of a drug. It is expressed as the partition coefficient PC, which is defined as the concentration ratio between a lipophilic and a hydrophilic phase. In vivo biological membranes represent the lipophilic and aqueous compartments the hydrophilic phase. To mimic biological membranes, a liposome/buffer system has been developed for the determination of PC. Phosphatidylcholine liposomes are prepared with either the detergent dialysis or extrusion technique. Partition studies are performed with an equilibrium dialysis chamber under standard conditions. The partition behaviour of selected drugs (acidic, basic, neutral) has been tested in the range of pH 2 to 12. The resulting data could be fitted with superposition of dissociation equations (Henderson-Hasselbalch) of all components involved, i.e. of drug and lipid. Fit values for pK_a correspond perfectly well to the values obtained by titration, which means that all tested compounds show an ideal partition behavior.

P07

IDENTIFICATION OF CYTOCHROME P450 ISOZYMES INVOLVED IN N-DEMETHYLATION OF CITALOPRAM IN HUMAN MICROSOMES.

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The selective serotonin reuptake inhibitor properties of the antidepressant citalopram reside mainly in the S-isomer with the R-isomer probably contributing little to the therapeutic effect. Stereoselective methods have shown that in citalopram treated patients, mean plasma concentrations of the active S-isomer were 35% of those of total citalopram. Using human liver microsomes, the present study shows that CYP2D6, 2C19 and 3A4 are involved in N-demethylation (a major pathway) of citalopram enantiomers. Intrinsic clearence values (Vm/Km) for S- and R-citalopram were within a small range for these 3 isozymes: 0.25 to 0.39 µl/h x pmol of CYP. Taking into account that CYP isozymes are expressed at various levels, CYP2D6, which is expressed lower than CYP2C19 and CYP3A4, plays a minor role in the metabolism of citalopram enantiomers. Nevertheless, CYP2D6 has an opposite stereoselectivity in the biotransformation of citalopram enantiomers than CYP2C19 and 3A4; the S/R ratio's of the intrinsic clearence were 0.71, 1.57 and 1.37, respectively. These results help to predict clearence modifications of citalopram enantiomers especially in PM patients of CYP2C19 or patients comedicated with CYP2C19 or 3A4 inhibitor(s).

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P08

EXPERIMENTAL GLUTARIC ACIDURIA IN RATS: A NEW ANIMAL MODEL FOR MITOCHONDRIAL IFOSFAMIDE TOXICITY IN MAN

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Ifosfamide is an important drug in alkylating chemotherapy and its neuro- and nephrotoxic side effects are currently under investigation. We have reported glutaric aciduria (GAU) in a patient with ifosfamide overdose (Küpfer et al., Lancet 343: 763-764, 1994) but an animal model for the clinically observed organic aciduria was not available at that time. Ifosfamide and ifosfamide metabolites (aziridino-ifosfamide, chloroethylamine, chloroacetaldehyde, chloroacetic acid and carboxymethylcysteine) at single i.p. doses of up to 1 mmol/kg, are unable to produce GAU in rats. However, when bromoethylamine hydrobromide (1 mmol/kg i.p.) was administered to rats, glutaric acid (GA) was found in urine and its identity was confirmed by GC/MS after silylation. Quantitative analysis was carried out by GLC of the methylesters (Carbowax 20 M, 180°C) using 3,3'-thiodipropionic acid as internal standard. At days one and two, 50 and 7 µmol/24h of GA, respectively, were excreted by these rats. From these data we conclude that 1) bromoethylamine is able to provoke GAU in rats, 2) this newly available animal model may allow for the development of future antidotal strategies against mitochondrial toxicities in ifosfamide treated patients.