

International Symposium on Drug Transport and Metabolism
October 13th to 14th, 2009, Buenos Aires, Argentina



PROGRAM AND ABSTRACTS

October 14

Morning

8:30-9:15 **P Steinberg (Germany) – Transport of heterocyclic aromatic amines across the gastrointestinal barrier and colon cancer development**

9:30-10:15 **GF Bramuglia (Argentina) – Effects of transporters on bioavailability of drugs**

10:30-11:00 *Coffee break*

11:00-11:45 **A Lazarowski (Argentina) – ABC transporters and epilepsy**

12:00-12:45 **P Fagiolino (Uruguay) – Theoretical models related to efflux transport. A perspective for individual phenotype characterization**

13:00-14:00 *Lunch*

Afternoon

14:00-15:20 **Oral presentations by invitation**
S. Copsel **Multidrug resistance proteins (MRPs) and cAMP efflux: new potential targets for leukemia differentiation therapy**
C. Cortada **P-glycoprotein activity and therapeutic response in ulcerative colitis**
L. Bartel **Metabolic activation of nifurtimox and benznidazole in rat mammary tissue cellular fractions. Biochemical and ultrastructural alterations**
C. Ghanem **Sub-toxic doses of acetaminophen induce intestinal P-glycoprotein expression and activity**

15:30-16:00 *Coffee break*

16:00-17:20 **Oral presentations by invitation**
A. Brandoni **Expression and function of renal organic anion transporters (oats) in health and disease**
R. Quevedo **Intestinal permeation of zidovudine and zidovudine prodrugs measured in rat intestinal segments**
L. Ruiz **Estrogen receptor- α mediates multidrug resistance-associated protein 3 induction by ethynylestradiol in rat liver and HepG2 cells**
R. Peroni **Interaction between the efflux transporter BCRP (ABCG2) and the anti-HIV drug efavirenz in rats**

18:00-18:30 **Ceremony – Prize for the best oral presentation**

18:30 **J Kapitulnik – Closing remarks**

20:00 *Closing dinner*

Drug transporters: Role in pharmacokinetics and pharmacodynamics

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Although gradient-driven passive diffusion of active, lipophilic molecules through cell membranes often allows them to reach active sites, it has become increasingly evident that active uptake and/or efflux processes play a major role in determining the concentration of drugs and other chemicals at these active sites [1, 2].

Drug transporters are present in intestine, liver and biliary tract, kidney, blood–brain barrier and other organs, and play a critical role in absorption and elimination of many exogenous and endogenous compounds [3-5]. Transport in the liver can result in uptake into hepatocytes followed by excretion of either the parent compound or its metabolites into bile. Conversely, metabolites can be transported from the hepatocyte back into blood. Similarly, metabolites can be excreted across the apical and basolateral membranes of enterocytes and proximal tubule cells of the kidney.

Drug uptake by transporters such as organic anion-transporting polypeptide 1B1 (OATP1B1) may determine the magnitude and rate of elimination even of drugs that undergo extensive metabolism in hepatocytes (e.g., some statins). Transporters can also influence the pharmacological and/or toxicological effects of drugs by limiting their distribution to tissues responsible for their pharmacodynamic effect (e.g., CNS) and/or their toxicity (e.g., fetus, brain, testes).

Uptake of drugs into cells depends largely on the superfamily of solute carrier (SLC) proteins, including the SLC21/SLCO (OATP) and SLC22 (organic anion transporter (OAT) and organic cation transporter (OCT)) subfamilies. Excretion across apical membranes is mediated by members of the ABC superfamily such as ABCB1 (P-glycoprotein), ABCC2 (multidrug resistance protein 2 (MRP2)) and ABCG2 (breast cancer resistance protein (BCRP)). Excretion of drug metabolites back into blood can be mediated by other members of this family located in the basolateral membrane of cells, such as ABCC3 (MRP3), ABCC4 (MRP4) or ABCC5 (MRP5).

Many anionic drugs, as well as glutathione and glucuronide conjugates, require ABCC2 for excretion. This transporter plays a role in the excretion of drugs and anionic conjugates from the enterocytes back into the intestinal lumen as well as in excretion from the proximal tubule cells into the urine. ABCG2 transports anionic and uncharged drugs, while ABCB1 transports uncharged and cationic drugs.

The enterohepatic circulation is an essential factor in the pharmacokinetics of certain drugs, such as ezetimibe that is currently used to reduce cholesterol absorption in the gut. Ezetimibe is glucuronidated in the intestine, and the phenolic glucuronide is more potent than the parent drug at inhibiting cholesterol absorption in rats. The higher potency of the glucuronide results from the increased time it remains in the intestinal lumen as compared with the parent drug; the latter is rapidly glucuronidated and excreted in the bile. In various single and double knockout mouse strains it was shown that the ABC transporters ABCC2, ABCC3 and ABCG2 play a crucial cooperative role in completion of the enterohepatic circulation of this drug.

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Polymorphisms of drug transporters

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The importance of drug transporters in the disposition of, and clinical response to therapeutic drugs is increasingly being recognized. Numerous studies have examined the association between genetic polymorphisms in drug transporters and pharmacokinetic or pharmacodynamic inter-individual variation, often with conflicting results. In my presentation, I will initially show data from our laboratory on the impact of polymorphisms in *ABCB1* and *SLCO1B1* on the pharmacokinetics of the HIV protease inhibitors lopinavir and ritonavir in a cohort of 100 HIV-infected men under stable treatment with Kaletra®. Protease inhibitors are substrates, and may also inhibit and/or induce the P-glycoprotein (*ABCB1*), and have been recently shown to be substrates of OATP/*SLCO1B1*. Univariate and multivariate regression analysis, including non-genetic variables, revealed no influence of *ABCB1* genotypes at three exonic polymorphic loci (1236C>T, 2677G>T/A, and 3435C>T) or their haplotypes on the trough (pre-dosing) concentrations of lopinavir and ritonavir in plasma, semen and saliva (Estrela et al., 2009). Also, no association was observed between the trough concentration of lopinavir or ritonavir in plasma and either the 388A>G or 463C>A SNPs in *SLCO1B1*. By contrast, a significant trend for increasing plasma concentrations of lopinavir (but not ritonavir) from the 521TT to TC and to CC genotypes was observed (Kohlhaus et al., 2009). Pairwise comparison showed that the trough concentrations of lopinavir in the plasma of 521C carriers were significantly higher relative to the wild type genotype. The clinical usefulness of this information is uncertain, in view of both the extensive overlap of the trough concentrations of lopinavir in the plasma across the three *521T>C* genotypes and the low prevalence (<5%) of the homozygous variant genotype (521CC) in the study cohort and worldwide. In another study, we enrolled 302 renal transplant Brazilian patients and investigated the impact of the *ABCB1* polymorphisms on the dose-adjusted trough concentration (C_0/dose) of the immunosuppressants cyclosporine (CSA) and tacrolimus (TAC) during the first three-months post-transplantation (Santoro et al., 2009). The 1236C>T, 2677G>T/A and 3435C>T SNPs and their haplotypes were not associated with the C_0/dose of CSA throughout the study, whereas the number of copies of the *ABCB1* wild-type haplotype was a significant covariate, which in combination with body surface area and prednisone dosing explained 17% of the inter-patient variability at three months after transplantation. At other time points, *ABCB1* polymorphisms were not significant covariates in multiple regression models, and the possibility that the association detected at three months was fortuitous cannot be excluded. The data from our laboratory will be discussed in relation to the impact of *ABCB1* and *SLCO1B1* polymorphisms on the pharmacokinetics and pharmacodynamics of other substrates of OATP1B1 (e.g., hypocholesterolemic statins and meglitinide analog anti-diabetic drugs) and *ABCB1* (anti-convulsants).

References

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Crosstalk between drug metabolizing enzymes, nuclear receptors and drug transporters

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The pharmacokinetic behavior of drugs is determined principally by metabolism (drug-metabolizing enzymes) and transport (uptake and efflux transporters). The role of phase I and II enzymes is quite established, but the role of transporters is less well delineated, although it is quite clear that they are often crucial for drug disposition. Sequential disposition of drugs in hepatocytes includes uptake into the cells followed by excretion of the parent compound and/or metabolites into bile. Metabolites can also be transported back to blood. Similar phenomena occur also in the apical and basolateral membrane of enterocytes and proximal tubular cells of kidneys. Many genes in drug metabolism and transport are regulated principally by three nuclear receptors: aryl hydrocarbon receptor (AhR), constitutive androstane receptor (CAR) and pregnane X receptor (PXR). The flow of most drugs through the hepatocyte is profoundly controlled by the three above mentioned players – enzymes, transporters and regulatory factors – which ensure by a coordinated action that the body gets rid of mainly lipophilic compounds coming from the gut or via circulation from other sites of administration/exposure. It may be worth of stressing that a large number of endogenous substances are also transformed in the liver, essentially via the same system.

Crosstalk (generally used to describe almost any type of interaction between pathways) between these different players can occur at several levels:

- 1) The intracellular concentration of the compound and its metabolites may increase or decrease by the action of uptake or efflux transporters. A change in the parent concentration affects the rate of metabolism by drug-metabolizing enzymes, leading to significant changes in the pharmacokinetics.
- 2) Xenosensing nuclear factors are key regulators of enzymes and transporters. They bind numerous exogenous compounds and broadly regulate adjustment to the chemical environment. CAR and PXR are overlapping both in ligands and target genes. Consequently, exposure to a single drug may elicit a response involving many enzymes and transporters participating in drug disposition.
- 3) Many endogenous substances are handled by drug-metabolizing enzymes and transporters and are also able to activate PXR and CAR receptors. Furthermore, target gene batteries of nuclear receptors extend beyond genes involved in drug metabolism and transport, and include important factors involved in glucose and lipid homeostasis and steroid and bile acid metabolism. Numerous points of interaction between regulation of pharmacokinetics and energy metabolism suggest that energy state-related physiological and pathological conditions such as fasting, diabetes and obesity affect metabolism and transport of drugs. Furthermore, xenobiotics may with this mechanism disturb energy homeostasis and cause yet poorly understood forms of toxicity.

Some recent reviews:

*Pelkonen O, Kapitulnik J, Gundert-Remy U, Boobis AR, Stockis A. Local Kinetics and Dynamics of Xenobiotics. *Crit Rev Toxicol* 2008; 38: 697-720.

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Hepatic/extrahepatic metabolism and intestinal transport of therapeutically-used veterinary drugs

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Livestock animals are exposed to a variety of xenobiotics during their production cycles. These compounds are likely to be metabolized by different enzymatic systems from both hepatic and extra-hepatic tissues. Generally, these enzyme reactions (biotransformations) generate more polar (hydrophilic) and readily eliminated metabolic products. However, metabolism may also give rise to the formation of toxic metabolites. The metabolic activity of different phase 1 (oxidative, reductive and/or hydrolytic) and phase 2 (conjugative) xenobiotic metabolizing enzymes plays a major role in determining the persistence of therapeutically or illegally used drugs in target species, which may additionally impose a risk to the consumers as a consequence of the permanence of drug residue levels in edible tissues, a major concern for public health and consumer's safety. In addition, metabolic interactions with such enzymatic systems may drastically affect the disposition kinetics of different drugs used in animal production.

Undoubtedly, xenobiotic biotransformation takes place predominantly in the liver, although metabolic activity is apparent in extra-hepatic tissues such as the gastrointestinal tract. In this regard, ruminant's nutritional physiology has been widely studied and numerous investigations focused on the metabolic activity of the microflora present in the rumen (the largest forestomach cavity in these species). Ruminants have a symbiotic relationship with their ruminal microflora which allows them to digest fibrous plant materials. Anaerobic conditions predominate in the ruminal environment and reductive and hydrolytic reactions are of primary importance in this organ.

In addition to its major role as an absorptive barrier, the intestinal mucosa has the ability to metabolize xenobiotics by both phase 1 and phase 2 reactions. It is well known that the bioavailability of an enterally administered drug can be reduced by both intestinal and hepatic first pass metabolism. Oxidative metabolism by cytochrome P450 and flavin monooxygenase enzymatic systems has been characterized in both liver and small intestinal mucosa of sheep and cattle ^(1, 2). Both enzymatic systems are involved in the biotransformation of benzimidazole antihelmintics (such as albendazole, fenbendazole and triclabendazole) into their less active sulphoxide metabolites ⁽³⁾. Interestingly, ruminal microorganisms are capable to reduce these sulphoxides back to their parent and more active thioethers ⁽⁴⁾. This liver/ruminal oxido-reductive pathway is relevant for the clinical efficacy of these compounds.

The transport protein P-glycoprotein (P-gp) is located within the brush border of the enterocytes and limits the absorption of orally administered compounds. Besides, P-gp contributes to the active hepatic and intestinal secretion of xenobiotics. For instance, P-gp-mediated intestinal secretion of ivermectin (an endectocide antiparasitic drug) was characterized in sheep (5).

Studies on the relevance of the hepatic/extra-hepatic metabolism and intestinal transport of drugs used in veterinary therapeutics are far from complete. However, it is clear that livestock animals are endowed with the necessary enzyme systems/transport proteins to protect them from xenobiotics to which they are exposed. Future research efforts should be directed to the study of a number of factors (i.e.: breed, diet, age, gender, chronic exposure to drugs, etc.) that may influence drug biotransformation and excretion in species of veterinary interest.

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MRP1 and tissue defense against doxorubicin cardiotoxicity

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Doxorubicin (DOX; adriamycin) is an anthracycline chemotherapeutic drug used in treatment protocols for solid tumors and lymphomas. DOX-induced cardiomyopathy is the main dose-limiting toxicity, and involves oxidative stress. DOX induces lipid peroxidation and generates the highly reactive lipid metabolite 4-hydroxy-2, 3-nonenal (HNE), together with up-regulation of multidrug-resistant associated protein 1 (Mrp1) in cardiac tissue; HNE (10 μ M) also inhibits Mrp1-mediated transport (Mol Cancer Ther 5:2851). In mice treated with DOX (20 mg/kg, ip), Mrp1 expression is increased in heart homogenate (1.6-fold), sarcolemma (2.5-fold) and also in sub-mitochondrial particles (SMP; 5.5-fold) at 24 h (Mol Pharmacol 75:1117-1126, 2009). SMP vesicles from DOX-treated mice mediate ATP-dependent transport of classic MRP1 substrates as well as of the glutathione conjugate of HNE (GS-HNE). GS-HNE transport activities were similar in sarcolemma membrane vesicles from DOX-treated vs. saline control mice, whereas GS-HNE transport activity in SMP was increased 2-fold in DOX vs. saline treated-mice. Importantly, GS-HNE transport in SMP was inhibited by QCRL3, an anti-MRP1 monoclonal antibody, and occurred into an osmotically sensitive space, confirming Mrp1-mediated transport. While expression of Mrp1 was increased at 48 and 72 h after DOX treatment, transport in SMP was inhibited, concomitant with HNE-adduction of Mrp1. These data imply that DOX induces oxidative stress, initiates lipid peroxidation and generates HNE, which alkylates Mrp1 and inhibits its function. We postulate that Mrp1 serves to efflux GS-HNE from cardiomyocytes and thus protect them from oxidative stress. However, under conditions of severe oxidative stress, HNE cannot be completely detoxified by formation of GS-HNE, and inactivates Mrp1. Single nucleotide polymorphisms (SNPs) in MRP1 are associated with differing responses to DOX. In cell lines, the G671V SNP is more sensitive to DOX; this SNP has been associated with DOX-induced acute cardiac toxicity in cancer patients. In contrast, cell lines expressing R433S are more resistant to DOX toxicity. Transport of GS-HNE in HEK293 plasma membrane vesicles showed that G671V-expressing membranes had a markedly decreased capacity to transport GS-HNE relative to vesicles expressing wild-type MRP1 or R433S. Thus, MRP1/Mrp1 may serve to protect the heart by mediating efflux of toxic products of oxidative stress such as HNE from mitochondria and the cardiomyocyte.

Dynamic localization of canalicular transporters in health and disease

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Vesicle-based trafficking of canalicular transporters involves delivery of newly-synthesized carriers from the rough endoplasmic reticulum to either the canalicular membrane or to an endosomal, subapical compartment, followed by its exocytic targeting to the plasma membrane. From there, the transporters can undergo recycling towards and from the subapical endosomal compartment, which serves as a reservoir of pre-existing transporters available on demand.

The balance between exocytic targeting and endocytic internalization is therefore a chief determinant of the overall capability of the hepatocyte to secrete bile and to detoxify endo- and xenobiotics. Hence, it is a highly regulated process. A cAMP-sensitive stimulatory pathway for the apical targeting of transporters exists, which is Ca²⁺-dependent, via Ca²⁺-CaM complex formation. This process is counter-regulated by activation of Ca²⁺-dependent PKCs (cPKC) (1). cAMP-mediated stimulatory effects may also involve PI3K activation, the novel PKC δ isoform being the downstream effector (2). Bile salts also stimulate apical trafficking of transporters by signalling mechanisms. Whereas taurocholate promotes this process by stimulating a PI3K-dependent pathway, the anticholestatic, therapeutic bile salt tauroursodeoxycholate does so by stimulating MAPKs of both p38MAPK and Erk types (2).

Transporters traffic from their synthesis place towards the surroundings of the canalicular membrane in a microtubule-dependent fashion, followed by the microfilament-dependent fusion of transporter-containing vesicles with the apical membrane (3). Actin can regulate transporter localization via binding to plasma membrane-actin cross-linking proteins, such as the ezrin-radixin-moesin (ERM) family of proteins, or by binding to interacting-partner proteins, such as PDZK1 and HAX-1.

Exacerbated internalization of canalicular transporters, resulting in bile secretory failure, occurs in several experimental models of hepatocellular cholestasis (e.g., in 17 β -estradiol glucuronide- and in tauroolithocholate-induced cholestasis). This also occurs in most human cholestatic hepatopathies, including obstructive extrahepatic cholestasis, inflammatory cholestasis associated to autoimmune hepatitis, primary biliary cirrhosis and primary sclerosing cholangitis (2). Sustained internalization of canalicular transporters may lead to delivery to the lysosomal compartment and further degradation, thus explaining the diminished post-transcriptional expression of transporters often associated with these hepatopathies.

The mechanism by which the endocytic internalization of canalicular transporters occurs is poorly known. Most hepatopathies are associated with oxidative stress, and pro-oxidant conditions induce actin disorganization and further transporter internalization (4). In addition, a disturbed colocalization of canalicular transporters and radixin without actin disorganization has been reported both in experimental and in human cholestasis. The participation of "cholestatic" signalling pathways in this effect is being actively studied in experimental settings, as in 17 β -estradiol glucuronide-induced cholestasis, where activation of cPKC was shown to play a key role (5).

Based on the information above, a number of experimental anticholestatic strategies have been tested in experimental settings to restore the normal insertion/internalization balance. They include the stimulation of the exocytic insertion of canalicular transporters (e.g., by cAMP or tauroursodeoxycholate), or the administration of signalling modulators able to block cholestatic signalling pathways (e.g., the cPKC inhibitor Gö6976). Additional strategies are expected to emerge in parallel to the discovery of new molecular mechanisms of transporter internalization, in an attempt to prevent their accelerated degradation by assuring proper localization.

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Transport of heterocyclic aromatic amines across the gastrointestinal barrier and colon cancer development

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A number of epidemiological studies in different countries have shown that a correlation between the consumption of strongly heated meat and the induction of colorectal cancer in humans does in fact exist. By cooking meat at high temperatures for a long time or over an open fire heterocyclic aromatic amines are formed. In this context it has been shown that 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (PhIP) is the most abundant heterocyclic aromatic amine detected in different meat samples. PhIP is known to form DNA adducts and to be mutagenic in bacterial as well as mammalian cell-based genotoxicity assays. Furthermore, it is a carcinogen in the rat colon.

In order to act as a colon carcinogen, PhIP must first be metabolically activated. *N*-hydroxylation is the first step in this process and leads to the formation of 2-hydroxyamino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine (*N*-OH-PhIP). This reaction is catalyzed by cytochrome P450 1A2 (CYP1A2) in the liver, whereas in extrahepatic tissues several other CYPs including CYP1A1 and CYP1B1 may contribute to the activation of PhIP. Thereafter, *N*-OH-PhIP is acetylated, this reaction being preferentially catalyzed by the polymorphic *N*-acetyltransferase 2. *N*-OH-PhIP may also be sulfoconjugated. The acetoxy and the sulfonyloxy esters can then be hydrolyzed and the PhIP nitrenium ion formed can bind to nucleophilic sites in DNA.

Using chamber experiments it was shown that only 1-2% of PhIP present in the mucosal compartment are taken up and that the small intestine, mainly the duodenum and the proximal jejunum, is the most active site in the gut regarding PhIP uptake. The highest amounts of PhIP accumulate in the small intestine and the lowest PhIP levels are detected in the distal colon and rectum. Interestingly, the amount of PhIP released into the serosal compartment is similar in the small intestine (duodenum, distal jejunum and ileum) and in the distal colon and rectum.

The multidrug resistance protein 2 (Mrp2 or Abcc2), initially identified as the canalicular multispecific organic anion transporter in the canalicular membrane of hepatocytes, transports a wide range of compounds conjugated with glucuronic acid, sulfate or glutathione and is expressed in the apical domain of enterocytes present in the small intestine of the rat. When considering the fate of PhIP and its metabolites in the rat intestinal mucosa, one should bear in mind that Mrp2 is able to excrete the above-mentioned compounds into the lumen of the gut, thereby decreasing their bioavailability. The breast cancer resistance protein (Bcrp1/Abcg2) is a member of the ABC family of drug transporters and has been shown to be present in the apical membrane of epithelial cells of the small intestine, colon, caecum and renal proximal tubule, in hepatic bile canalicular membranes, and in placental labyrinth cells of the mouse. Data showing that Mrp2 as well as Bcrp1 are actively involved in the intestinal elimination of PhIP, thereby strongly reducing the tissular bioavailability of PhIP, will be presented.

By taking into account the data presented in this talk, a hypothesis describing the relationship between PhIP transport processes in the gut and colon cancer development will be discussed.

Effects of transporters on bioavailability of drugs

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The ATP-binding cassette (ABC) family of transport proteins represents one of the largest families of proteins in living organisms. P-glycoprotein (P-gp), which is responsible for the efflux of several drug compounds across the cell membrane, obtains the energy required for the vectorial transport of drug substrates across the membrane via the hydrolysis of ATP. P-gp is expressed in the intestine but also in the kidney tubules, adrenal glands, blood-brain barrier, muscle, lung, pancreas, placenta, testis, stomach and liver. P-gp expressed in the enterocyte cells lining the intestine effluxes its substrates across the apical membrane back into the intestine; therefore P-gp-mediated efflux has the potential to decrease intestinal drug absorption and increase drug metabolism in the enterocytes. Large interindividual differences in P-gp expression in tissues such as the small intestine and liver have also been reported.

Indinavir (IDV), a HIV-1 protease inhibitor that showed a large interindividual variability in the plasmatic levels attained after oral administration in healthy volunteers and patients, has been proposed as a substrate of P-gp. Previous reports presented clear evidence for interactions between P-gp and protease inhibitors. Thus, given the tissue distribution of P-gp, it might lower the bioavailability of protease inhibitors and could be responsible for the existence of sanctuary sites, such as the brain and the testes, by limiting the levels of accumulation of protease inhibitors in these tissues. Accordingly, it is reasonable to hypothesize that variability of this transporter could influence drug disposition, as well as antiretroviral treatment efficacy. Different studies have attempted to measure the effect of P-gp-mediated efflux on the oral bioavailability of drugs. However, there are no standardized methodologies to assess P-gp expression or function and its relationship with the bioavailability of P-gp substrates.

Analysis of P-gp by flow cytometry, measuring dye or drug efflux in the presence or absence of a P-gp modulator, is one of the recommended techniques for MDR1 phenotype determination in peripheral blood mononuclear cells and has been used in hematological malignancies as a prognosis marker.

Although there is not enough evidence for a correlation between lymphocitary and intestinal function of P-gp, our results suggest a relationship between the P-gp functionality marker, Rh123 efflux assay in peripheral blood lymphocytes, and the extent of absorption of a P-gp substrate.

Furthermore, various single nucleotide polymorphisms (SNPs) in the MDR1 gene which codifies for P-gp have been identified, including a silent mutation in exon 26 (C3435T), and have been correlated with duodenal expression of P-gp. It was found that genotype-related differences in duodenal P-gp expression between the 3435CC and 3435TT genotypes are approximately 2-fold. These antecedents are in accordance with our results, taking into account that patients with C/T and T/T genotypes showed higher C_{max} levels as compared with C/C carriers.

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ABC transporters and epilepsy

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Epilepsy is a neurological disorder affecting 1-2% of the general population. Despite considerable advances in the pharmacotherapy of epilepsy, about 30% of epilepsy patients fail to achieve good seizure control with the different antiepileptic drugs (AEDs) treatments, developing refractory epilepsy (RE) with a multidrug resistant phenotype. These patients can not control the seizures after use of several AEDs, even when administered maximum tolerated doses (Fig. 1). In most cases, a patient who is resistant to one major AED is also refractory to other AEDs, although these drugs act by different mechanisms.

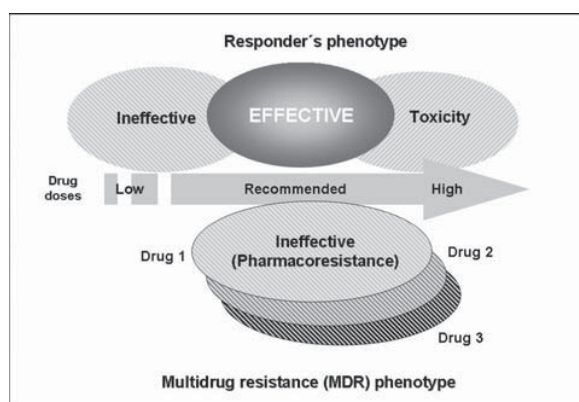


Figure 1: Behavior of responders and refractory patients

Reasoning and making decision in epilepsy: each new diagnosed epilepsy case requires a good identification of the epileptic syndrome, and according with the corresponding classification the treatment is initiated. So, in ~60% of epileptic patients, administration of one or two AEDs is enough to control seizures. However, ~40% of epileptic patients repeatedly fail to control the seizures with one AED given after another or with their combinations, defining the Multidrug Resistance (MDR) Phenotype.

The ABC transporters in refractory epilepsy

The activity of the transporters P-glycoprotein (P-gp), multidrug-resistance-associated proteins (MRPs) and breast cancer resistant protein (BCRP) are directly related with the multidrug resistance (MDR) phenomenon (Fig. 2). According with other authors, we have observed the overexpression of these transporters in the brain of patients with RE. Several experimental epilepsy models have demonstrated the relationship between inducible P-gp overexpression in blood-brain barrier (BBB) and brain parenchyma cells (astrocytes, neurons), the MDR phenotype, and the impairment of brain access of AEDs. Early P-gp detection in vessel-related cells and subsequent additional P-gp detection in neurons correlated with the gradual loss of the protective effect of phenytoin, depending on the intensity and time-constancy of seizure injury. The expression of P-gp and other multidrug transporters in excretory organs suggests that they have a central role in drug elimination. Persistent low levels of AEDs in plasma or increased liver clearance of ^{99m}Tc -MIBI (a P-gp substrate) in RE surgically-treated cases that showed P-gp overexpression in brain, were also documented by our group.

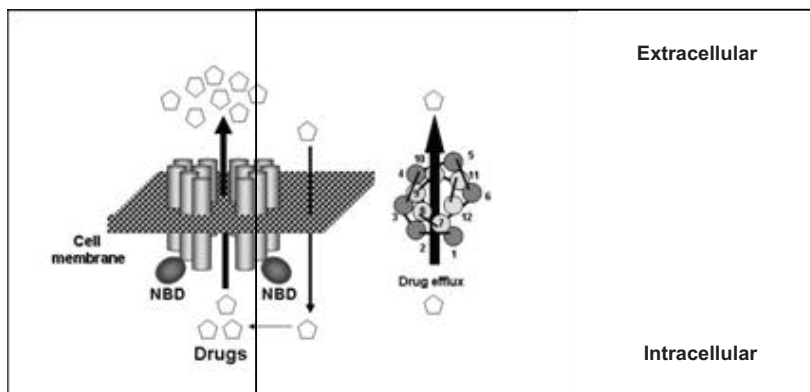


Figure 2: ABC transporter structure and functional activity

Additionally, changes in known AED targets (voltage-gated sodium channels, hyperpolarization-activated current (IH) and GABA receptors) were also reported. The neuronal expression of P-gp described in both clinical and experimental reports suggests an alternative mechanism associated with the lower membrane potential ($E_m = -10$ to -20 mV) observed in P-gp-expressing cells as compared to the normal physiological E_m of -60 mV.

We have observed a progressive membrane potential depolarization of hippocampus from rats with increased P-gp overexpression in brain induced by repetitive experimental seizures, and associated also with a progressive PHT pharmacoresistant phenotype, and finally developing a fatal status epilepticus.

Under these conditions, and irrespective of the P-gp pharmacoresistant property, P-gp+ neurons could increase their sensitivity to new seizures, perhaps as an epileptogenic mechanism, by lowering the convulsive threshold observed by the depolarized membranes. Understanding the properties of these multidrug transporters can offer new tools for better selection of more effective preventive or therapeutic strategies and avoid the invasive surgical treatments for RE.

Theoretical models related to efflux transport. A perspective for individual phenotype characterization

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Drugs travel through our body moved by different forces. In their way, they move through a large number of compartments such as intracellular water, all membranes, interstitial and intravascular fluids, gastrointestinal secretions, etc. These are well known individualized spaces, but inside them many other compartments with homogeneity in drug concentration can coexist.

There are crucial stages where movements of molecules become relevant: entry of substances in the body and in the biophase, removal from the body, etc. In order to simplify the analysis of this complex scenario, multicompartiment models were developed. The transfer of drug among compartments is governed by three main rate determinants ^[1,2]: 1) transfer “surface area”; 2) compartment “volume”; and 3) drug energy in the compartment.

Cytoplasm membranes are normally considered the frontiers between two aqueous compartments, but they should be assumed as non-aqueous compartments by themselves. Located inside them, transporters act not only as membrane gates for transfer but also as solvents providing energy to the solute. Therefore, they efficiently increase the rate of drug transfer among compartments. In this way a minor portion of the membrane, constituted by transporters, could become an important transfer route in relation with other larger portions of the membrane. In some cases transporters drive the drug in both directions, but in others the transfer takes place only in one direction (e.g., influx or efflux pumps).

Still controversial is the role played by efflux transporters, but it is evident that the intracellular concentration of a substrate drug of these transporters can decrease considerably. So, enzyme saturation can be avoided and then the metabolic pathway strongly competes with other kinetic processes. Drug efflux can increase drug removal from the biophase as well as from the body, either by metabolism or excretion. Therefore, overexpression of a transporter in the cell membrane, activated by inducers and by certain pathologies such as epilepsy, or simply by the patient genotype, has a strong impact by reducing treatment efficacy.

Presence of these drug efflux transporters in cells of salivary glands makes saliva a target fluid for studying their expression, by monitoring marker substrate concentration. Some methodological aspects should be taken into account in order to make this concentration a reliable one: sample volumes, physical activity of the individual and sampling time ^[3], lipophilicity of the marker agent, etc.

Recent findings enable us to study antiepileptic drugs as possible markers for efflux transporters located in salivary glands ^[4,5]. Some of them have shown their inducing capacity in overexpressing these transporters not only by showing a correlation between the administered dose and the metabolic capacity of individuals, but also with the index of salivary gland transporter expression. Thus, it can be said that efflux transporters are not only involved in drug metabolism but also in drug distribution. Another anticonvulsant agent showed an interesting association between saliva concentration and clinical status of the patients, which is in agreement with the hypothesis of an induction of these carriers in the presence of a high frequency of seizures.

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Multidrug resistance proteins (MRPs) and cAMP efflux: new potential targets for leukemia differentiation therapy

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Over the last decades the concept of differentiation therapy, whereby immature cells are stimulated to develop into their mature phenotype, aroused considerable interest. Many efforts join to evaluate new differentiation agents for the treatment of leukemia where early hematopoietic progenitors are thought to exhibit maturation arrest. The best evidence for success of this therapy has been the treatment of acute promyelocytic leukemia (APL) with the all-trans retinoic acid or with arsenic trioxide. In addition, agents that increase cyclic AMP (cAMP)-mediated signaling, such as inhibitors of cyclic nucleotide phosphodiesterases (PDEs), augment the ability of these therapies to induce differentiation in APL blast cells¹.

cAMP, one of the most common second messengers, plays an important role in response to hormonal signals for cell proliferation, differentiation, and apoptosis in hematopoietic development. Several mechanisms are involved in the regulation of cAMP levels including its degradation by PDEs and the modulation of G protein-coupled receptor (GPCR) mediated production.

The human promonocytic leukemic U937 cell line is able to differentiate into monocytes losing their malignant capacity. Thus, these cells are an appropriated model to study hematopoietic cell differentiation mechanisms. Previous reports from our laboratory established an important correlation between duration and intensity of cAMP signaling induced by different agents, and evoked cellular responses such as proliferation and differentiation in U937 cells^{2,3,4}.

Since cAMP levels are critical for leukemic cell differentiation and MRP4, MRP5 and MRP8 are capable to transport cAMP out of the cells⁵, the aim of the present work was to characterize the cAMP efflux mechanism in the U937 cell line and investigate the role of MRPs in the differentiation process. The time course of cAMP accumulation was studied using agents that increase cAMP levels such as amthamine (H2 receptor agonist), isoproterenol (β -adrenergic receptor agonist), or forskolin (an adenylyl cyclase activator). These studies showed that there is cAMP efflux that is independent of the stimuli used. Furthermore, we observed that this process is dependent on both time post-stimuli and cAMP intracellular concentration.

After confirming MRP4, MRP5 and MRP8 expression in U937 cells, we investigated their relevance in the cAMP efflux process using specific inhibitors of MRPs (probenecid, verapamil and MK-571). Inhibition of MRPs significantly increased cAMP intracellular levels and inhibited cell proliferation by 90%. This increase in cAMP levels induced the expression of CD11b and CD88 and augmented the chemotactic response to complement 5a (C5a), which are all markers of monocytic differentiation.

Since cAMP levels are relevant for leukemic cell differentiation and MRPs contribute to its regulation, these proteins may constitute new potential targets for leukemia differentiation therapy.

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P-glycoprotein activity and therapeutic response in ulcerative colitis

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P-glycoprotein, encoded by ABCB1, is a transmembrane efflux pump that is involved in relevant clinical drug transport. First described as overexpressed in cancer refractoriness, it is now proposed to be involved in ADMET (absorption, distribution, metabolism, elimination and toxicity) of many clinically used drugs ¹. Together with the activity of xenobiotic metabolizing enzymes, efflux mechanisms have become the subject of considerable interest in recent studies of gut mucosal defence and pharmacokinetics of drugs ².

Inflammatory bowel diseases are chronically relapsing disorders likely due to a combination of immunological, environmental and genetic factors. Ulcerative colitis (UC), a member of this group, is characterized by a contiguous inflammation of the colonic lamina propria ³. ABCB1 was proposed as a candidate gene for UC therapeutic response and pathogenesis. P-glycoprotein is expressed in lymphocytes and luminal epithelium of colon, the target tissue for treatment and pathogenesis of UC. Increased expression has been reported to be associated with steroid UC refractoriness. Conversely, deficient P-glycoprotein function has been postulated as a UC susceptibility/severity factor.

The aim of the present work was to investigate the role of P-glycoprotein in therapeutic response of ulcerative colitis by studying its functionality in lymphocytes isolated from peripheral blood. Samples were taken from 27 patients with active colitis (Mayo score: severe n = 9; moderate n = 9; mild n = 9) classified clinically as refractory (n = 16) and responders (n = 11) to treatment. These patients were treated with 5-aminosalicylic acid derivatives, glucocorticoids and/or 6-mercaptopurine, depending on their clinical state. Efflux of rhodamine 123 (a fluorescent P-glycoprotein substrate) was studied by flow cytometry in the absence and presence of a P-glycoprotein inhibitor (verapamil, 100 μ M) ⁴. Data were expressed evaluating the behavior of two markers based on % of cells with maximum (M1)/minimum (M2) intracellular fluorescence, reflecting inactivity/activity of the pump. Results were compared with a group of healthy individuals (n = 68). Significant differences were observed in the absence and presence of verapamil inhibition, when comparing refractory vs. responders (p < 0.05) as well as refractory vs. healthy controls (p < 0.01). No differences were observed when comparing responders vs. controls (p > 0.05) (Kruskal-Wallis test and Dunn post-test). Rhodamine efflux assay was also performed in 9 patients who required therapeutic change; a significant reduction of rhodamine transport (p < 0.01) was observed without inhibitor when patients achieved clinical response. In summary, our results suggest a relevant role of P-glycoprotein in ulcerative colitis treatment response and the usefulness of P-glycoprotein functional assay in the early detection of individual therapeutic response.

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Metabolic activation of nifurtimox and benznidazole in rat mammary tissue cellular fractions. Biochemical and ultrastructural alterations observed

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Nifurtimox (NFX) and benznidazole (BZ), two nitroheterocyclic drugs used in the treatment of Chagas' disease, have serious side effects in their clinical use mainly attributed to their nitroreduction to reactive metabolites.

Early NFX long-term toxicity studies showed significant breast tumorigenic effects in male and female SPF Wistar rats, but their mechanism remains to be established. No such reports for BZ were found in the literature, although the drug is known to be a carcinogen in other tissues. Furthermore, it is known that several nitrofurans and nitroimidazoles are widely used in veterinary medicine. Some of them are breast carcinogens in rodents and their mechanism of action is hypothesized to be related to reactive metabolites generated by nitroreduction and/or via oxygen-dependent redox cycling.

We report that NFX and BZ reach the rat mammary tissue and their potential bioactivation in situ. The present work describes the nitroreductive metabolism of NFX and BZ and also of nitrofurazone, nitrofurantoin, furazolidone and metronidazole by the cytosolic and microsomal fractions of rat mammary tissue and the nitroreductase activity of pure xanthine-oxidoreductase (XO).

Both anti-Chagas drugs were detected in mammary tissue from female Sprague-Dawley rats after their intragastric administration. Purified and cytosolic xanthine-oxidoreductase biotransformed NFX but not BZ. These activities were purine-dependent and were inhibited by allopurinol. Microsomes biotransformed NFX, but not BZ, in the presence of NADPH. This process was inhibited by CO and partially by diphenyleneiodonium. All the tested nitrofurans were nitroreduced either by the purified XO or the cytosolic fraction in the presence of hypoxanthine. Furthermore, except for the nitrofurazone, they were nitroreduced by the microsomal fraction in the presence of NADPH suggesting the participation of cytochrome P450 reductase. Metabolism of nitrofurans was significantly more intense than that of NFX and, as well as for BZ, no nitroreductase activity was observed in either subcellular fraction using metronidazole as substrate.

NFX treatment produced: a significant decrease in mammary tissue protein sulfhydryl content after 1, 3 and 6 h; no increases in protein carbonyl content at any time analyzed; a significant increase of *t*-butylhydroperoxide-induced chemiluminescence after 6 h and significantly higher levels of lipid hydroperoxides at 3 and 6 h. Ultrastructural observations after 24 h showed significant differences compared with control epithelial cells. Using the alkaline comet assay a significant presence of DNA comet images were observed in blood of treated animals but only a non-significant tendency to their formation in mammary tissue.

In agreement with early findings of others who reported NFX ability to promote rat mammary tumors, these results suggest that NFX might produce more deleterious effects to mammary tissue than BZ, and that the nitroreductive metabolism of nitrofurans and the subsequent redox cycling might be involved in the associated mammary tissue carcinogenic effects.

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Sub-toxic doses of acetaminophen induce intestinal P-glycoprotein expression and activity

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The well known analgesic-antipyretic acetaminophen (APAP) is one of the most sold over the counter drugs and is usually co-administered with other therapeutic agents. Previously, we have demonstrated that a toxic dose of APAP (1 g/kg i.p.) induced liver P-glycoprotein (P-gp) expression (1). Moreover, sub-toxic doses of the drug (0.2; 0.3 and 0.6 g/kg i.p., three consecutive days) produced a marked up-regulation of the basolateral transporter Mrp3 relative to apical Mrp2 in the same organ, affecting APAP pharmacokinetics (2) and toxicity (3). Our following aim was to evaluate the effect of sub-toxic doses of APAP on intestinal P-gp expression in rats and their possible interaction with other drugs that are substrates of this transporter.

The sub-toxic treatment increased P-gp expression in duodenum and ileum in treated rats compared to controls (240 and 160%, respectively, $P < 0.05$). The increased expression of P-gp in the treated group, with conservation of its physiological localization (apical lumen of the intestine), was confirmed by confocal immunofluorescence microscopy.

To test if the described protein induction modifies the activity of P-gp in vitro, we evaluated the secretion of rhodamine 123 (R123), a P-gp substrate (4), using intestinal everted sacs in the presence and absence of verapamil (V), a known P-gp inhibitor. The cumulative R123 secretion was 44% higher in APAP-treated rats compared to controls ($P < 0.05$). In the presence of verapamil, R123 secretion decreased P-gp activity in both groups compared to their own activity in the absence of the inhibitor (78 and 72% in APAP and control groups, respectively).

We then studied if sub-toxic APAP treatment modified the in vivo absorption of digoxin, a typical substrate used to study P-gp activity (5). A dose of 0.2 mg/kg of digoxin, including traces of [³H] digoxin, was incorporated into the duodenum, and portal blood was sampled up to 30 min. At the end of the study, the portal digoxin concentration was 67% lower in the APAP-treated group compared to controls (expressed as % of the dose/ml; $P < 0.05$).

Finally, we studied the effect of 12 different concentrations of APAP (0 to 40 mM) on viability of LS174T cells, a human intestinal cell line, to select a sub-toxic dose. LS174T cells were exposed to the sub-toxic dose (5 mM) of APAP for 48 hs. Subsequently, P-gp expression and activity were evaluated. The cells treated with APAP expressed 60% more protein and showed 80% more activity than control cells.

In conclusion, sub-toxic doses of APAP induced intestinal P-gp expression and activity in rats. This increased expression could modify the pharmacokinetics of other drugs, substrates of P-gp that are administered orally. Consistently, preliminary studies have shown induction of P-gp in a human intestinal cell line exposed to sub-toxic concentrations of APAP.

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Expression and function of renal organic anion transporters (oats) in health and disease

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Renal secretion of organic anions plays a critical role in clearing the body of endogenous and exogenous compounds. Several carrier proteins have been cloned and functionally characterized from both membrane domains of renal tubule cells. The organic anion transporters 1 (Oat1), 3 (Oat3) and 5 (Oat5) belong to the SLC22 family and are mainly located in the kidneys. Oat1 and Oat3 represent the key organic anions/ α -ketoglutarate exchangers in the energetically linked basolateral entry of organic anions into the proximal tubule cells. Oat1 and Oat3 mediate renal tubular uptake of several pharmacological agents, such as methotrexate, β -lactam antibiotics and non-steroidal anti-inflammatory drugs as well as p-aminohippurate (PAH, a prototypical organic anion). Oat5 has been cloned and characterized as an organic anions/dicarboxylate exchanger localized at the brush border of proximal tubule straight segment (S3). Oat5 interacts with chemically heterogeneous anionic compounds, such as non-steroidal anti-inflammatory drugs, diuretics, bromosulphthalein, and penicillin G.

An important aspect of Oats regulation concerns the body's response to disease states. We have examined the expression of these transporters in different pathologies by real-time PCR, Western blotting and immunohistochemical techniques, as well as the direct consequences of these effects on organic anion renal elimination.

Modifications in the renal expression of Oat1, Oat3 and Oat5 have been described in renal diseases (chronic and acute renal failure) and in other diseases such as arterial calcinosis and extrahepatic cholestasis.

We have demonstrated the important role of Oat1 expression in the renal elimination of PAH, independently of renal Oat3 expression. In this connection, we have reported that a decrease of Oat1 abundance in basolateral membranes is associated with a diminished PAH renal elimination in experimental models of acute renal failure (ischemia/reperfusion, ureteral obstruction and mercury nephrotoxicity) and in chronic renal failure. The decrease in the expression of Oat1 was inversely correlated with plasma urea levels, suggesting a possible role of uremic toxins in the regulation of this transporter. Meanwhile, Oat3 abundance in renal basolateral membranes decreased, did not change, or increased in these experimental models of pathologies. On the contrary, Oat1 expression was increased in kidneys from rats with extra-renal pathologies (such as extrahepatic cholestasis and arterial calcinosis) and was associated with an increase in renal excretion of organic anions.

Our group pioneered in the detection of Oat5 in urine. Oat5 abundance was increased in urine from rats at an early stage of ischemic acute renal failure suggesting that this protein might be a potential early non-invasive marker of this pathology. We are now evaluating the urinary excretion of Oat5 in the presence of other diseases in order to determine its possible role as an early biomarker of renal tubular damage.

Current experiments performed in our laboratory will increase the knowledge of how these transporters are regulated in the presence of pathological states. Defining the modifications in the expression of these transporters is important both to understand different pathological processes and to identify potential therapeutic targets as well as early biomarkers for new diagnostic tests.

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Intestinal permeation of zidovudine and zidovudine prodrugs measured in rat intestinal segments

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Zidovudine (AZT) was the first drug approved to treat the AIDS syndrome, and is still part of the Highly Active Antiretroviral Therapy (HAART) regimen.¹ Although its clinical efficacy has been vastly demonstrated, its oral absorption and biodistribution properties still need to be fully understood and optimized.² As part of our studies dealing with the design and development of novel prodrugs of AZT with potential application to HAART, this work deals with the evaluation of intestinal permeation properties of AZT and two novel prodrugs of AZT,^{3,4} by applying the intestinal gut sac technique. With this aim, the corresponding apparent permeability coefficients (P_{app}) were determined in the basal-to-apical and apical-to-basal direction, analyzing different small intestine portions and drug concentrations. Our findings demonstrated that AZT exhibited no difference in its apical-to-basal permeability in proximal segments of small intestine at all concentrations assayed, while a lower permeability was found in distal ileum at higher concentrations. Also a significant decrease in the amount of drug transported was found when the time of exposure of the tissue to the drug was prolonged. The decrease in AZT transport in the apical-to-basal direction in distal ileum was not observed in presence of verapamil, thus providing evidence for a P-gp mediated mechanism. When the permeability in the basal-to-apical direction was evaluated, an increase in the amount of drug transported with respect to time at high drug concentrations was observed, clearly suggesting an efflux pattern which may reduce AZT bioavailability. On the basis of the above results, a rapid P-gp activity up-regulation is proposed. Two novel prodrugs of AZT (AZT-Iso and AZT-Ac),^{3,4} currently under development in our research group, were evaluated by means of this assay. AZT-Iso exhibited an enhanced lipophilicity with respect to that of AZT, but was completely hydrolyzed during its permeation through the enterocyte, with identical permeation patterns to those determined for AZT. On the other hand, AZT-Ac, a highly hydrophilic prodrug of zidovudine,⁵ was able to permeate through the intestinal tissue without significant enzymatic or chemical hydrolysis. When AZT-Ac apical-to-basal transport was evaluated, lower P_{app} values were obtained compared to those of AZT, but remarkably no decrease in the amount of permeated drug was observed as time of exposure of the intestinal tissue to this compound increased, suggesting that there is no up-regulation of P-gp. In contrast to what was observed for AZT, a significant enhancement in AZT-Ac permeability was observed as the drug concentration increased in the mucosal solution. Considering the results presented, the role of P-gp in AZT intestinal efflux needs to be reconsidered, addressing also the dynamic nature of expression and regulation of this transporter. Also, the elucidation of the molecular basis of P-gp substrate specificity may contribute to the development of new prodrugs of AZT with enhanced bioavailability and biodistribution.

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Estrogen receptor- α mediates multidrug resistance-associated protein 3 induction by ethynylestradiol in rat liver and HepG2 cells

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Multidrug resistance associated protein 3 (Mrp3) is a basolateral transporter that pumps anionic substrates such as sulphated bile salts, bilirubin glucuronides, 17 β -glucuronosyl estradiol, some anti-cancer drugs, etc., from hepatocytes into blood (1,2). In normal conditions, these substrates are preferentially excreted into the canaliculus via multidrug resistance associated protein 2 (Mrp2). The extent of constitutive Mrp3 expression varies between species. It is weakly expressed in rat compared to mouse and human liver. However, Mrp3 is markedly up-regulated in naturally multidrug resistance associated protein 2 (Mrp2)-deficient animals (3), in animals with obstructive cholestasis (4), and in patients with Dubin-Johnson syndrome (2). It was suggested that Mrp3 functions to extrude bilirubin and bile salt conjugates from the hepatocytes when bile secretion is impaired. We previously demonstrated that Mrp3 expression and activity is up-regulated in rat liver in response to administration of ethynylestradiol (EE), a synthetic estrogen that causes cholestasis (5). The first aim of the present study was to determine if Mrp3 induction in rats results from a direct effect of EE, requiring participation of estrogen receptor- α (ER- α), and independently of any accumulation of common endogenous Mrp2-3 substrates, due to impaired biliary secretion. Interactions of nuclear receptors with selective ligands may vary between species. As a second aim, we explored if EE is able to induce MRP3 in HepG2 human hepatoma cell line, as occurs in rats, and the potential participation of ER- α . For the first aim, two experimental models were used. *In vivo* studies: a single dose of EE (5 mg/kg) was injected to male rats, and 6 h later basal bile flow and biliary excretion rate of bile salts and glutathione were measured. This dose, which demonstrated to be non-cholestatic, induced Mrp3 mRNA levels, as tested by real time PCR. *In vitro* studies: rat hepatocytes in primary culture were incubated with EE (1-10 μ M) for 4 h, resulting in increased Mrp3 mRNA at the 10 μ M concentration. When the hepatocytes were pre-incubated for 30 min with an estrogen receptor antagonist (ICI182/780, 1 mM), induction of Mrp3 mRNA was abolished, suggesting that the estrogen receptor mediates up-regulation of Mrp3. Experiments in HepG2 cells, which express very low levels of estrogen receptor constitutively, showed absence of induction of MRP3 by EE (1, 10 or 50 μ M, for 48 h). When HepG2 cells were transfected with ER- α , MRP3 was up-regulated by EE at a 50 μ M concentration. This increment was not observed in HepG2 cells transfected with the empty vector. In conclusion, the present data demonstrate for the first time that induction of Mrp3 by EE in rat liver and of MRP3 in HepG2 cells occurs in a direct way, independent of any endogenous substrate accumulation, requiring participation of ER- α .

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Interaction between the efflux transporter BCRP (ABCG2) and the anti-HIV drug EFAVIRENZ in rats

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OBJECTIVES: Breast cancer resistant protein (BCRP/ABCG2) is a member of the superfamily of ABC transporters that affects drug disposition (1). The substantial expression of BCRP in organs important for drug disposition, such as small intestine (2) and blood-brain barrier (3), implies that BCRP could affect the absorption and distribution of drugs that are BCRP substrates. The non-nucleoside reverse transcriptase inhibitor efavirenz (EFV) is an anti-HIV drug, used in combination therapy, which significantly inhibits BCRP in vitro (4). This study was conducted to investigate the role of BCRP in the intestinal absorption and in the delivery to the central nervous system (CNS) of EFV and the influence of the chronic treatment with EFV on the expression of BCRP in adult male rats. **METHODS:** Adult male Sprague-Dawley rats were used to perform all the experiments. The intestinal permeation rate and the delivery to CNS of EFV were investigated by using ileum everted sacs and by CNS microdialysis techniques, respectively. BCRP expression was assessed by Western Blot after oral administration by gavage of 25 mg/kg EFV or its vehicle (Pluronic F127 10%, pH 5.0), daily during five days (5). **RESULTS:** In control rats, the concentration-dependent efflux of EFV (1-10 mM) observed in ileum everted sacs was almost completely blocked by pretreatment with the specific BCRP inhibitor fumitremorgin C (10 μ M). Moreover, the delivery of EFV to CNS after a single i.v. administration of 20 mg/kg was increased two-fold after treatment with the BCRP inhibitor gefitinib (20 mg/kg, i.p.). Furthermore, an increase in the expression of BCRP in the small intestine and in the blood brain barrier was observed in both tissues after a 5-day treatment with EFV. **CONCLUSIONS:** Our study demonstrates that the intestinal absorption and the distribution into the CNS of EFV was modulated by BCRP in rats, and that EFV itself could modulate BCRP expression which in turn could influence the bioavailability and delivery of this drug to target or sanctuary HIV-sites.

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