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ABC EFFLUX TRANSPORTERS: P-GP, MRP2 AND BCRP – THE 3RD DIMENSION IN KINETICS NOT ONLY OF FLUOROQUINOLONES

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INTRODUCTION: ABC EFFLUX TRANSPORTERS AS DETERMINANTS OF TISSUE PENETRATION AND ELIMINATION OF DRUGS

Drug absorption, distribution and elimination (ADE) are complex processes, which are governed by several factors. While in the past membrane permeability in the course of absorption and distribution was discussed predominantly in relation to physicochemical properties of drugs such as lipophilicity, molecular weight and solubility, it is now evident that transport proteins are one of the major determinants of these processes (Ayrton & Morgan, 2001; Van Bambeke *et al*., 2003; Balayssaca *et al*., 2005). These transmembrane proteins belong to the ATP-binding cassette (ABC) transporter superfamily. Of particular interest are efflux transporters that are expressed at physiological barriers (for example at the blood-brain barrier and the placenta), in the liver and in other tissues responsible for the detoxification and excretion of xenobiotics from the body (Mealey, 2004; Hugnet *et al*., 2004). Hence, efflux transporters influence intestinal absorption of drugs and toxins, distribution as well as excretion of xenobiotics and endogenous metabolites into the urine and bile (for reviews see: Ayrton & Morgan, 2001; Lin & Yamazaki, 2003a). Historically, the first reports on these drug transporters had been devoted to multidrug resistance to chemotherapeutic agents (Juranka *et al*., 1989; Kane *et al*., 1990; Ling, 1997; Klein *et al*., 1999; Miller, 2002), but more recently their impact of ADE of many structurally unrelated drugs, including many antibiotics and particularly the group of fluoroquinolones is gaining increasing attention (Watkins 1997; Ito *et al*., 1998; Greiner *et al*., 1999; Hoffmeyer *et al*., 2000).

At present, out of the group of ABC transporters, comprises more than 49 individual genes in humans, P-gp (P-glycoprotein, encoded by the ABCB1 gene and also referred to multidrug resistance 1), MRP2 (multidrug resistance-associated protein 2, encoded by the ABCC2 gene) and BCRP (breast cancer resistance protein, encoded by the ABCG2 gene) are considered to be the main ABC efflux transporter proteins which are involved in the handling of drugs and toxins.

STRUCTURE, MECHANISM OF FUNCTION AND REGULATION OF EXPRESSION OF ABC TRANSPORTERS

ABC transporters are large membrane proteins. Their basic structure as found in P-gp, consists of 12 transmembrane domains and two ATP-binding sites in a protein of about 1300 amino acids (Jones & George. 1998). In comparison to P-gp, MRP2 consists of 1545 amino acids and has an additional amino-proximal membrane-spanning domain represented by an extension of approximately 200 amino acids (Tusnady *et al*., 1997). In contrast, BCRP is called half-transporter, consisting of a single hydrophobic membrane spanning domain predicted to contain 6 trans membrane (TM) helices preceded by a single nucleotide binding domain (NBD) (Leslie *et al*., 2005) (Fig. 1).

The position and number of substrate-binding sites in ABC transporters remain to be described in more detail. It was suggested that there are at least four substrate-binding sites, which differ in substrate specificity (reviewed by Chan *et al*.,

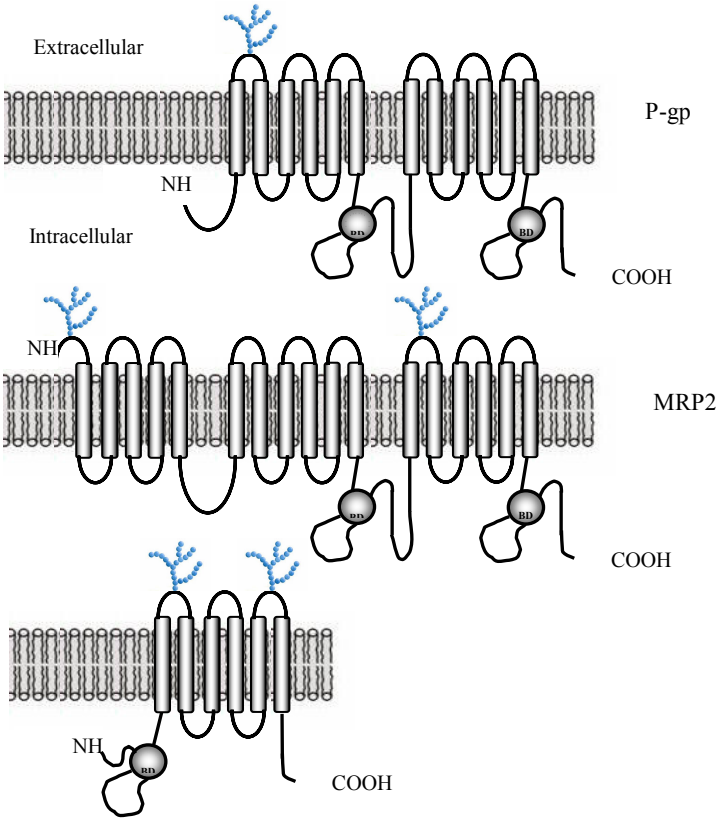

Fig. 1. Schematic structure of P-gp, MRP2 and BCRP with intracellular nucleotide binding sites.

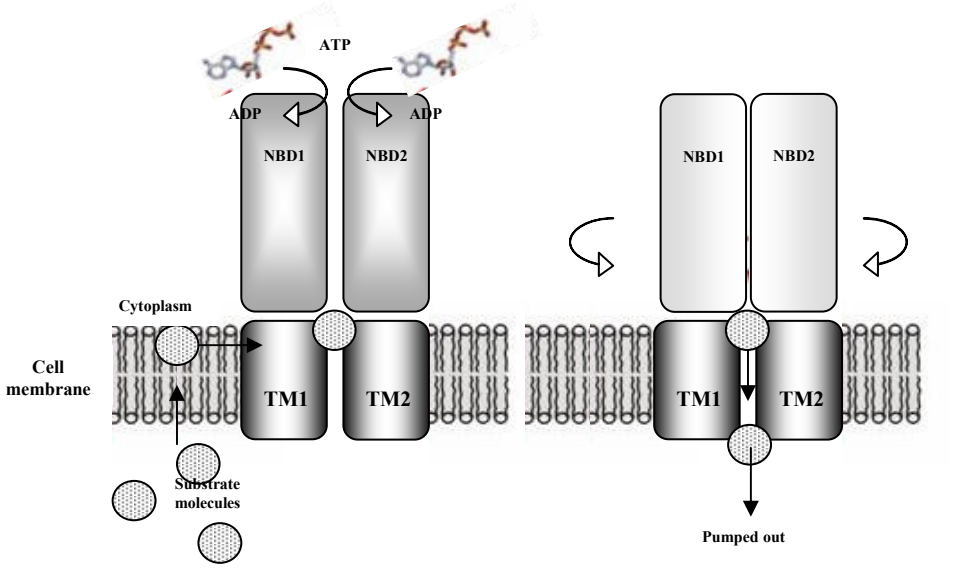

Fig. 2. Schematic model of the mechanism of drug transport by P-gp.

2004). Moreover, it is suggested that the drug-binding transmembrane regions of P-gp could form a single binding site (pocket) that binds more than one compound (Sharom *et al*., 1998; Loo & Clarke, 2005). The ability of a substrate to change the cross-linking pattern is consistent with the ability of transmembrane domains to change their shape to accommodate structurally different compounds (Schuetz *et al*., 1996; Rosenberg *et al*., 2001; Loo *et al*., 2003). In the P-gp two cassettes, each of them contains 6 putative α-helix TM segments, interact cooperatively to form a single functional unit (Loo & Clarke, 1994; Muller *et al*., 1996) (Fig. 2). The nucleotide binding domains are not required for drug binding but it couples ATP hydrolysis to substrate export and is a conservative region for ABC superfamily transporters. ATP binding and subsequent hydrolysis is essential for drug transport (Ambudkar *et al*., 1999; Ambudkar *et al*., 2003).

Transcriptional regulation of many mammalian ABC transporters, including MDR1 and MRP2 is under control of so-called orphan nuclear receptors, which also regulate the expression of various biotransformation enzymes (Borst & Elferink, 2002, Di Croce *et al*., 1999). This class of transcription factors comprises among others PXR and SXR, the pregnane- and the steroid-activated (retinoid) receptors, also denoted xenobiotic sensing receptors. SXR, the regulator of MDR1 expression, can be induced by various drugs such as rifampin, phenobarbital, paclitaxel, and HIV protease inhibitors (Xie *et al*., 2000; Synold *et al*., 2001). A typical example is the antibiotic rifampin, which is a known inducer of CYP3A4 and P-gp, but increases also MRP2 mRNA and protein expression

(Fromm *et al*., 2000) via PXR and CAR (constitutive androstane receptor) (Kast *et al*., 2002).

TISSUE DISTRIBUTION OF P-GP, MRP2 AND BCRP

In humans and rodents, **MDR1 mRNA** expression and protein levels of **P-gp** have been found in brain, kidneys, testes, liver, jejunum, colon and lungs, and lower levels in ileum and duodenum (Conrad *et al*., 2001; Langmann *et al*., 2003; Tang *et al*., 2004; Zimmermann *et al*., 2005). Like mammals, chickens show high levels of P-gp expression in the liver, small and large intestine, and in the kidneys (Barnes, 2001), brain, lungs, heart, eyes and follicles (Edelmann *et al*., 1999). Most investigations suggest that the levels of MDR1 mRNA increase progressively from the stomach to the colon, with low levels in the stomach, intermediate levels in the jejunum, and high levels in the colon (Tang *et al*., 2004; Zimmermann *et al*., 2005).

MRP2 is expressed mainly at the apical membranes of epithelial cells in liver, intestines (duodenum, jejunum and ileum), and kidney tubules (Schaub *et al*., 1997; Fromm *et al*., 2000; Gotoh *et al*., 2000; Scheffer *et al*., 2002a). This protein has not been detected in colon, lungs, brain and testes (Conrad *et al*., 2001). Various species differences in the level of expression of MRP2 in individual tissues have been described. For example, a high expression was found in rabbit's kidneys and intestine exceeding those of the liver (Van Aubel *et al*., 2000), whereas in mice the highest levels were detected in the intestines, followed by the liver and the kidneys (Maher *et al*., 2005).

BCRP mRNA is detectable in many tissues: the highest levels were found in the apical membranes of placental syncytiotrophoblasts (Doyle *et al*., 1998; Jonker *et al*., 2000), the ducts and lobules of the mammary gland (Maliepaard *et al*., 2001), venous and capillary endothelial cells of almost all tissues (Maliepaard *et al*., 2001; Scheffer *et al*., 2002b), hepatocytes (Oude Elferink, 2002), and the apical surface of enterocytes (epithelium) of the small intestine and the colon (Dietrich *et al*., 2003). Interspecies differences were described in ABCG2 expression in the kidneys: in human kidneys BCRP was not detected (Maliepaard *et al*., 2001) but functionally it plays important role in the urinary excretion of sulfated compounds (Phase II conjugated metabolites) in mice and rats (Jonker *et al*., 2000; Mizuno *et al*., 2004).

SUBSTRATES OF P-GP, MRP2 AND BCRP

There is limited number of investigations, which try to classify the compounds as substrates and non-substrates of **P-gp** (Sharom, 1997; Didziapetris *et al*., 2003). For example, according to the algorithm used by Didziapetris *et al*. (2003) it can be expected that (fluoro)quinolones are substrates for P-gp because of their molecular weight (close to 400) and the fact that they are zwitterions and posses – COOH groups and ≥8 (N+O) atoms. This hypothesis was confirmed by functional studies in which ciprofloxacin, pefloxacin, grepafloxacin, levafloxacin and danofloxacin were shown to be substrates of P-gp (Seelig, 1998; Seelig & Landwojtowicz, 2000; Schrickx, 2006). Moreover, grepafloxacin, sparfloxacin, and norfloxacin were described not only to be substrates, but also as to be strong inhibitors of the efflux of HSR-903, hence affecting the function of MRP2 (Ito *et al*., 1997a; Mu-

rata *et al*., 1998; Sasabe *et al*., 1998). Additional data show that P-gp transports not only various structurally diverse drugs, but also endogenous steroids (Gruol *et al*., 1999; Johnstone *et al*., 2000) and cytokines (Drach *et al*., 1996; Raghu *et al*., 1996; Veau *et al*., 2002).

Studies in hepatocytes have shown that **MRP2** is particularly involved in exporting a range of both conjugated and unconjugated anionic compounds into the bile ducts. Among the conjugates are common Phase II metabolites such as glutathione conjugates, sulphate conjugates, and glucuronides, including bilirubin glucuronides as well as glucuronides of fluoroquinolones, such as geprafloxacin (Sasabe *et al*., 1998; Naruhashi *et al*., 2002). MRP2 plays thus an important role in detoxification of xenobiotics (Jedlitschky *et al*., 1997; Madon *et al*., 1997; Keppler *et al*., 1998; Russel *et al*., 2002; Takikawa, 2002). Strong inhibitors of MRP2 are the leukotrien LTC4, phenolphtalein glucuronide and the compounds MK571 and fluorescein methotrexate, which are used as prototypic inhibitors as cyclosporin is a less potent inhibitor (Kawabe *et al*., 1999; Renes *et al*., 2000).

BCRP is a high capacity efflux transporter with wide substrate specificity, recognizing molecules of either negative or positive charge. It can extrude glucuronides and sulfate conjugates formed in enterocytes into the intestinal lumen (Adachi *et al*., 2005), but the most prominent physiological role of BCRP seem to be the mediation of excretion of drugs and xenobiotics into milk, as well as its protective role at the placental barrier.

Finally it is noteworthy to mention that the overlapping substrate specificities of BCRP, MRP2 and P-gp suggests that not only the binding affinity, but also the level of expression in individual tissues accounts for the overall impact these transporters will have on absorption, distribution and excretion of endo- and xenobiotics (Taipalensuu *et al*., 2001).

ROLE OF P-GP, MRP2 AND BCRP IN THE PHARMACOKINETICS OF THE DRUGS

Absorption

Taking into account the current knowledge about the level of expression of ABC transporters in different tissues it is evident that P-gp, MRPs and BCRP are important determinants of the rate of absorption of nutritional compounds as well as orally administered drugs (Hunter & Hirst, 1997; Lin *et al*., 1999a, b; Lin & Yamazaki, 2003b). Cellular uptake of drugs and subsequent efflux by these transporters, also called cycling of drugs, results in a slower rate of absorption and increased exposure to drug-metabolizing enzymes in the intestinal epithelial cells. Their function needs to be added to the commonly assessed factors such as solubility of drugs at different pH, diffusion and the resulting concentration gradient between intestinal lumen and mucosal blood circulation, transit time and intestinal metabolism. The expression of the individual efflux transporters along the gastro-intestinal tract seems to be variable and species-dependent, and requires more investigations. Moreover, the function of various transporters has proven to be readily saturated, already at normal therapeutic concentrations of orally applied drugs. For example, indinavir, ritonavir, quinidine, which are excellent P-gp substrates following *in vitro* studies, show a reasonably good bioavailability between 60 and 80% (Polli *et al*., 2001; Raffanti & Haas, 2001), suggesting saturation of ef-

flux transport. However, in *in vivo* experiments, it might be difficult to discriminate between the rate of absorption from the gut lumen and secretion from the circulation into the gut lumen. This is exemplified by studies with digoxin: despite of the fact that digoxin is a substrate for P-gp, it has apparently a good oral bioavailability of about 60–80% (Ooi & Colucci, 2001). However, Mayer *et al*. (1996) found that orally administered and intravenously injected digoxin is excreted directly into the gut in wild type mice (16% of administered dose in 90 minutes), but hardly at all (2%) in the MDR1 (-/-) mice. Hence, further detailed investigations are required to elucidate the real impact of the level of expression and function of the transport proteins on the absorption and oral bioavailability of individual drugs.

Distribution

P-gp has a key function in the penetration of drugs through the blood-brain barrier (BBB) and in the removal of substances out of the brain into the cerebral blood circulation. With this it plays a decisive effect on the clinical usage of drugs (Ayrton & Morgan, 2001; Lin & Yamazaki, 2003a; Fromm, 2004). A classical example demonstrating the pivotal role of PgP at the blood-brain barrier is loperamide, an opioid agent widely used in the treatment of diarrhoea. It can be safely administered without undesirable neurological side effects, as it is a strong P-gp substrate and hence P-gp prevents brain penetration by efflux the drug back into the circulation. In turn, undesirable side effects are observable in the MDR1a (-/-) mice, at common dose levels that showed no side effects in the MDR1a (+/ +) mice (Sadeque *et al*. 2000). Loperamide neurotoxicity following the application of a standard dose can be incidentally observed in Collies, a breed of dogs, in which mutations of the MDR1 gene accompanied with functional losses of P-gp, is common (Sartor et al., 2004). Similarly, functional blocking of P-gp by the co-administration of a potent P-gp inhibitors such as for example quinidine, increases the passage of loperamide into the brain, resulting in serious neurotoxicity (Lin & Yamazaki, 2003a).

In a model with porcine brain capillaries, which expressed P-gp and MRP2, it could be shown that both transporters contribute substantially to the active barrier function (Fricker *et al*., 2002). With this model, the inhibitory activity of ivermectin on P-gp activity could be demonstrated (Rose *et al*., 1998). The importance of P-gp function in BBB for penetration of ivermectin into the brain was already demonstrated in MDR1 (-/-) mice, in which the unlimited diffusion of ivermectin into the central nervous system is fatal (Schinkel *et al*., 1994). It is worthwhile to mention that for other compounds, such as anti-epileptics and drugs for the treatment of psychiatric disorders, the ability to pass the BBB and to reach appropriate concentrations in certain brain compartments, is a prerequisite for the therapeutic use (Löscher & Potschka, 2005). P-gp function at BBB can explain few CNS side effects of second generation H1-antagonists, which are good P-gp substrates (Chishty *et al*., 2001).

In mammals, placental P-gp functions protect fetus from harmful compounds. Fetal ivermectin concentrations were 10-fold higher in P-gp deficient mice than in wild type mice (Lankas *et al*., 1998).

Of additional therapeutic interest is the distribution of antibiotics, including fluoroquinolones, between the extracellular and intracellular space. It has been proposed that the intracellular concentration

(and hence the activity) of ciprofloxacin and azithromycin could be improved by inhibiting their efflux out of macrophages. Inhibition of efflux pumps may be a useful strategy to improve the antibiotic efficacy of a wide range of antibiotics against intracellular bacteria (Seral *et al*., 2003). Among the compounds for which drug distribution is regulated by P-gp are grepafloxacin and a new fluoroquinolone compound HSR-903 (Tamai & Tsuji, 2000).

Metabolism

Efflux transporters play a dual role in drug metabolism. Accumulating evidence indicates that Phase I biotransformation enzymes and efflux transporters are regulated in their expression by the same class of transcription factors, as mentioned above. The most prominent example is Cytochrome P450 3A4 (the major cytochrome in human liver and intestines) that is likely to play a synergistic role with P-gp in suppressing the systemic bioavailability of many orally ingested compounds (Guengerich, 1995; Benet *et al*., 2004). P-gp could increase presystemic metabolism by CYP3A4 by repeated cycling of the drug at the apical membrane of the intestine (Cummins *et al*., 2002). Dexamethasone induced P-gp in the intestine of rats and thus increased the intestinal metabolism of indinavir (Lin *et al*., 1999a). In contrast, extent of metabolism of K77 (a cysteine protease inhibitor; P-gp and CYP3A4 substrate) in Caco-2 cells was lower when P-gp was inhibited because of increased absorption and thus decreased total metabolism (Cummins *et al*., 2002).

Moreover, Phase II conjugation products (glucuronides, glutathion conjugates, sulfate conjugates) have limited membrane permeability and hence efflux transporters have to facilitate their transport into the bile ducts, as well as central veins. The prominent role of BCRP and MRP2 in the transport of conjugated metabolites (mentioned above) is a matter of increasing interest (Sasabe *et al*., 1998; reviewed by Köning *et al*., 1999; Adachi *et al*., 2005).

Excretion

The liver and kidneys play an important role in the excretion of unchanged drugs and their metabolites. The early finding that the biliary and renal clearance of digoxin is greater in MDR1a (+/+) mice than in MDR1a (-/-) mice was the first example to demonstrate the important role efflux transporters play in drug excretion (Kawahara *et al*., 1999). Studies with MDR1 (-/-) double knockout mice have demonstrated also that P-gp significantly contributes to the biliary secretion of both doxorubicin and vinblastine (Van Asperen *et al*. 2000). In turn, the P-gp inhibitors erythromycin and ketoconazole reduced the biliary clearance of for example fexofenadine (Ayrton & Morgan, 2001).

As discussed already above in the chapter on drug absorption, efflux transporters facilitate also the excretion of drug from the systemic circulation into the gut lumen. This was first experimentally demonstrated in mice after i.v administration of paclitaxel, which appeared in the intestinal lumen also in animals in which the bile flow into the intestinal lumen was interrupted by gallbladder cannulation (Sparreboom *et al*., 1997). Similar mechanisms of intestinal secretion of talinolol were described after intravenous infusion (Gramatte & Oertel, 1999) and approximately 10% of the intravenous dose of digoxin (1 mg) was effluxed into small intestine in healthy human volunteers (Drescher *et al*., 2003).

Another prominent example is ivermectin, which is also actively secreted from blood into the intestinal lumen. This is of clinical significance, since many gastrointestinal parasites do not feed on plasma and hence could otherwise not be reached by the anthelmintic following its parenteral application (Laffont, 2002). It could be shown that all segments of the rat small intestine excreted the parent drug after systemic administration, and the clearance of ivermectin via secretion into the small intestines was approximately 5-fold higher than the biliary clearance (Laffont, 2002).

The role of drug transporters in the modulation of antibiotic pharmacokinetics has been mainly studied for β-lactams, fluoroquinolones and, to a lesser extent, macrolides and aminoglycosides (Van Bambeke *et al*., 2003). P-gp and MRP2 for example, were shown to mediate the blood-to-lumen secretion of the fluoroquinolone grepafloxacin in the rat intestines (Naruhashi *et al*., 2001, 2002, 2003), as well as by human intestinal Caco-2 cell monolayers (Yamaguchi *et al*., 2000; Lowes & Simmons, 2002). Both, MRP2 and P-gp also contribute to grepafloxacin excretion by the liver, kidneys or CNS (Cormet-Boyaka *et al*., 1998; Sasabe *et al*., 1998; Takano *et al*., 1998; Wakasugi *et al*., 1998; Murata *et al*., 1999). In wild type Wistar rats, the biliary clearance of grepafloxacin was markedly reduced by the P-gp-inhibitor cyclosporin A (Yamaguchi *et al*., 2002). According to other investigations, the decrease in biliary and overall intestinal clearances of ciprofloxacin observed in the presence of azlocillin, cephalexin and cyclosporine can be explained by competition at the level of organic anion and/or cation transport systems, including MRP2 (Barrieáre *et al*., 1990; Dautrey *et al*., 1999).

P-gp and MRP2 may contribute at least in part also to the renal tubular secretion (Ito *et al*., 1997b; Sasabe *et al*., 2004). The well known effect of probenicide on penicilins clearance resulted in prolongation of $t_{1/2\beta}$ of the antibiotic and could be explained with inhibition of ABC transporters (Ayrton & Morgan, 2001). Investigations with pazufloxacin suggest the excretion into the urine by more active drug transporters than P-gp and MRP2 (Shimizua *et al*., 2004). Administration of aminoglycosides leads to reduction in MRP2- and P-gp-mediated efflux in renal proximal tubules, which could be an important factor in the clinical nephrotoxicity that is associated with use of this class of antibiotics (Terlouw *et al*., 2001; Miller, 2002) as well as in drug-drug interactions.

DISEASES AND ABC TRANSPORTERS

Investigation on ABC transporters during diseased conditions started with recognition of their role in multidrug resistance in tumour cells. Increased levels of expression and changed function of ABC transporter proteins in tumour cells play an important role in anticancer therapy via development of resistance against anticancer drugs (Leonard *et al*., 2003). Felix & Barrand (2002) found that oxidative stress, by changing P-gp expression, might also affect movement of P-gp substrates in and out of the brain.

Endotoxin of Gram-negative microorganisms has been shown to down-regulate simultaneously hepatic P-glycoprotein, MRP2 and CYP3A and to impair the transport and biotransformation of several of their substrates (Zhao *et al*., 2002). Decreased MDR1 expression was found in liver under inflammatory conditions

(Hartmann *et al*., 2001). Endotoxin-induced inflammation (using LPS) caused also a reduction in the intestinal expression and activity of P-gp, MRP2, and CYP3A in rats, which elicits corresponding changes in the intestinal transport (reduction of the basolateral to apical efflux of digoxin, amiodarone and 7-benzyloxyquinoline; significant increase in the apical to basolateral absorption of these compounds) and metabolism of their substrates (decreased by approximately 50 to 70%) (Kalitsky-Szirtes *et al*., 2004). Inflammation provoked by infection with *Staphylococcus spp*., *E. coli*, or by LPS or cytokines (IL-1b, IL-2, IL-6, TNFα and IFNγ) decreased the level of expression of MDR1 mRNA (Fernandez *et al*., 2004). Moreover, IL10 deficient enterocolitis mice exhibit an impaired function and expression of P-gp in the epithelial cells along the intestines (Buyse *et al*., 2005). Taken together, these findings suggest changes in the pattern and level of expression in ABC transporters during inflammation that could result in changes in drug kinetics and hence in the outcome of drug therapy (Yamada *et al*., 1996; Kawaguchi *et al*., 2000).

In conclusion, inter-species and inter-individual variations in expression of ABC efflux transporters seem to contribute to the variability in the pharmacokinetics of drugs and hence influence the therapeutic results (Lin & Yamazaki, 2003b; Benet *et al*., 2004; Sun *et al*., 2004). At present, numerous investigations are available that address the expression, functionality, and individual activity of these efflux transporters in laboratory animal species as well as humans. However, with exception of the prominent example of inherited P-gp mutations in certain dog breeds, the corresponding knowledge on drug efflux transporters is very limited in veterinary target animal species. In turn, only very few studies have examined the levels of P-gp expression in poultry and their influence on the pharmacokinetics and pharmacodynamics of drugs (Edelmann *et al*., 1999; Barnes, 2001).

ABC TRANSPORTERS IN POULTRY AND FLUOROQUINOLONES

As yet, only very few studies have addressed the expression of ABC transporters in poultry, as mentioned above**.** However, fluoroquinolones have been identified as substrates for various efflux transporters in rodents as well as humans and hence we conducted a number of studies with two major objectives, first to study ABC transporters in poultry and second to assess the interaction between fluoroquinolones and ABC transporters in chickens and turkeys. The results are about to be published, but the major conclusion can already be summarized as follows:

The expression of the three considered ABC transporters, P-gp, MRP2 and BCRP in poultry resembles in many organs the expression pattern as observed in humans and in laboratory anuimal species (Haritova *et al*., 2006a). This result was to a certain extent expected, as ABC transporters are linked to an old, and highly preserved gene family. For example, the antimicrobial resistance in *E.coli* strains is mediated by the same mechanisms, an up-regulation of efflux pumps transporters, corresponding to P-gp, expression (Webber & Piddock, 2003). However, also distinct dissimilarities were observed, as for examples in the expression of ABC transporters in the adrenals (Haritova *et al*., 2006a,b). These differences need further clarification, but point immediately to the fact that ABC transporters are not only related to drug transport, but that many

natural compounds, including hormones, neurotransmitters and signalling molecules are substrates for these transporters.

The usage of fluoroquinolones has become common in human and veterinary medicine because of their broad spectrum and appreciable kinetic properties (Sarkozy, 2001). In contrast to the numerous investigations in laboratory animals, humans and certain farm animal species, the available information on the pharmacokinetics in avian species is limited (Anadón *et al*., 1995, 1997, 2002). In particular, PK-PD studies, integrating pharmacokinetic and pharmacodynamic data are virtually lacking in poultry. However, in the light of the increasing number of cases of bacterial resistance, which is usually related to the erroneous applications or overuse of certain fluoroquinolones (EU, 2003; Giraud *et al*., 2001; Johnson *et al*., 2002; 2003; Webber & Piddock, 2003) more data supporting the selection of an optimal dose regime for individual fluoroquinolones are needed. Hence, we applied the principles of PK-PD modeling in the design of dosage regimen of fluoroquinolones in poultry, using two different fluoroquinolones, but also studied the interaction between these antibiotics (danofloxacin, enrofloxacin and marbofloxacin) and ABC transporters in turkeys and later in infected poultry chicks (Haritova *et al*., 2006b,c). While the classical PK-PD modelling indicated that the dose regimes for poultry should be amended with the aim to meet the paradigms set for concentration dependent antibiotics (Drusano, 2003; Toutain *et al*., 2002), the effects of fluoroquinolones on the expression of ABC transporters were only mild (Haritova *et al*., 2006d). This is of therapeutic advantage, as it indicated that in the course of the treatment, an adaptation of the given dose, which would be very difficult in practice, is not necessary. However, the fact that fluoroquinolones are substrates of efflux pumps, explains many parameters observed in tissue distribution experiments, such as the high drug concentration on the alveolar surface, as the high concentrations of fluoroquinolones found in the gut lumen after parenteral injection in calves (Sheer, 1987; Lynch *et al*., 1990; Mann & Frame, 1992). The latter are advantageous in term of therapeutic efficacy against *E.coli* infections in young calves (Sunderland *et al*., 2003).

Taken together it can be concluded that the increasing knowledge on the expression and function of drug transporters and the increasing number of therapeutic agents that have been found to be a substrate for one or more transporters, are of great influence in the understanding (and prediction) of drug absorption, distribution and elimination. Due to its links to and its comparable biological significance, the effect of ABC transporters of ADE is often denoted as Phase III, indicating its equal importance to Phase I and Phase II reactions. Phase I (mainly CYP450) mediated biotransformation processes that determine distribution (hence efficacy) as well as the rate of presystemic and systemic elimination. Phase II reactions (conjugation reactions) that are major determinants of species-specific differences in the susceptibility to toxins, of which the GST-dependent tolerance of mice against many toxins and procarcinogens, and the UDP-GT deficiency of cats (felidae), making these animals sensitive a large groups of (phenolic) compounds. Moreover, the impact of this transport on the distribution of endogenous substrates such as hormones and neutrotransmitters and other signalling molecules is increasingly acknowledged in human medicine,

and offers also in veterinary medicine a large field of research needs.

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