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A ROLE OF P-GLYCOPROTEIN IN MODULATION OF ANTIBIOTIC PHARMACOKINETICS

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ABSTRACT

P-glycoprotein (P-gp) belongs to superfamily of ABC (ATP-binding cassette) drug transporters. It is expressed in intestines, brain, kidneys, testes, liver, adrenal gland, lungs heart and eyes. This protein functions as a biological barrier by extruding toxic substances and xenobiotics out of cells. P-gp could modulate pharmacokinetics of antibacterial drugs through limitation of their oral absorption and penetration into target organs. Drug-drug interactions with antibacterials could be mediated by inhibition or induction of P-gp. Among the antibiotics recognized as substrates and modulators of P-gp are structurally unrelated compounds as fluoroquinolones, macrolides, ansamycines, tetracyclines and antracyclines. These findings provide a basis for the understanding of the pharmacokinetics, pharmacodynamics and toxicodynamics of the antibiotics in healthy and diseased individuals.

Key words: P-glycoprotein, Antibiotics, Pharmacokinetics

INTRODUCTION

P-glycoprotein (P-gp) belongs to superfamily of ABC (ATP-binding cassette) drug transporters and it was initially identified because of its over expression in cultured tumor cells associated with an acquired cross-resistance to multiple cytotoxic anticancer agents (12, 14). It was also recognized to be expressed in many normal tissues, suggestive of a physiological function. P-gp is located in the apical membrane of the enterocyte of the gastrointestinal tract, suggesting that the transporter functions to facilitate excretion of substrates from the systemic circulation into the gut lumen. Similarly, localization of P-gp in the canalicular domain of the hepatocyte and the brush border of the proximal renal tubule is consistent with a role for the transporter in the biliary and urinary excretion of xenobiotics and endogenous substrates. In addition, it has a protective function at the site of important physiological barriers such as blood-brain and blood-testes. It is also expressed in tissues as adrenal gland, lungs, heart and eyes (43).

P-gp shows broad substrate specificity, recognizing a large number of compounds with unrelated pharmacological properties. It acts as one of general means for cells to protect themselves from undesirable invasion by compounds, including antibiotics, which freely diffuse across membranes. Function of this protein affects drug pharmacokinetics through limitation of their oral absorption and penetration into target organs such as the brain and testes (18, 26). In individuals with increased P-gp expression or function, reduced oral bioavailability, decreased maximal plasma concentrations, increased renal clearance and reduced area under the curve (AUC) would be expected (15).

This work is aimed to discuss the role of P-gp in the absorption and disposition of antibiotics and participation of these drugs in drug-drug interactions by modulation of the activity of P-gp.

Antibacterial drugs

The role of P-gp in the modulation of antibiotic pharmacokinetics has been mainly studied for fluoroquinolones and macrolides. Nowadays the number of evidences about the interaction of P-gp with other antibacterial

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drugs is significantly increasing.

β-lactams

Transporters, including P-gp, identified at the blood–brain and blood–CSF barriers are important for the disposition of β-lactams because active efflux may be detrimental for treatment of meningitis and other infections of the CNS (27, 34). For example, failures in treatment of inflammation of CNS with cefalothin have been attributed to the active efflux of this molecule (35). Dicloxacillin is recognized as a substrate for P-gp and inter-individual differences in its pharmacokinetics were explained by variations in MDR1 genotype (22). These findings are supported by the fact that the induction of this protein by rifampin administration provokes decreasing of differences in the dicloxacillin pkarmacokinetics (clearance, AUC and C_{max}) between various MDR1 genotypes (22). Isoxazolyl-penicillin flucloxacillin has the potential to induce expression of both CYP3A4 as well as P-gp, through activation of the nuclear hormone receptor pregnane-X-receptor (PXR) which would offer an explanation for the observed clinical drug-drug interactions between the antibiotic and cyclosporine (11).

Fluoroquinolones

Fluoroquinolones have been reported as a class of drugs able to undergo efflux, which can explain the low bioavailability of some of them after oral administration. It has been shown in *in situ* and *in vitro* studies, that absorption of grepafloxacin and sprafloxacin is increased by concomitant administration of P-gp inhibitors (23). It has to be taken into account that involvement of other transporters in addition to P-gp can also play a role because there are not highly specific compounds with inhibitory activity. For example, in ciprofloxacin transport are involved more than one efflux and even influx proteins (23). Fluoroquinolones are not only substrates but also inhibitors for ABC transporters. Grepafloxacin, levofloxacin and sparfloxacin inhibit P-gp activity in MDCKII-MDRI and Caco2 cell lines which is a prerequisite for modulated oral absorption and disposition of other substrates for P-gp as macrolide drugs when administered concomitantly (33).

Inhibition of P-gp contributes to cellular accumulation of ciprofloxacin in macrophages but mainly in the cytosolic compartment, which can partly assist in elimination of intracellular bacteria as *Listeria monocytogenes* and *Staphylococcus aureus* (32). Between fluoroquinolones licensed for use in animals, danofloxacin mesylate appears to be a substrate for P-gp and MRP2 in Caco2 cell lines. Its absorption and secretion are decreased by simultaneously applied ciprofloxacin (29). Other studies with chicken lymphocytes show that danofloxacin base and danofloxacin mesylate at high concentrations, which could be reached in poultry after administration of usual dosage regimens, have inhibitory effect mainly on function of P-gp and to a much lesser extent on breast cancer resistance protein (BCRP) (10).

Brain distribution of fluoroquinolones is regulated by P-gp and a parallel has been observed between the propensity of fluoroquinolones to induce seizures and their rate of efflux from the CNS (4). Concentrations of grepafloxacin and a new fluoroquinolone drug HSR-903 are much higher in the brain in mdr1a (–/–) knockout mice (37). Moreover, grepafloxacin, sparfloxacin, and norfloxacin, were strong inhibitors of efflux of HSR-903, although other quinolone derivatives, which are relatively hydrophilic, had less effect on HSR-903 uptake by the brain capillary endothelial cells, suggesting a competition for the efflux transporter proteins, including P-gp (19).

Clearance of fluoroquinolones can be accelerated by the action of MDR1 in the intestine, kidney, liver or CNS (24). Lower total body clearance of grepafloxacin in mdr1a (–/–) and mdr1a/1b (–/–) suggests that urinary and biliary excretion, intestinal secretion and metabolism are reduced in these mice (25).

P-gp could affect pharmacokinetics of fluoroquinolones in different way in healthy and diseased animals. In rats with hepatic fibrosis decreased bioavailability and increased elimination of orally administered ofloxacin was observed due to up-regulation of P-gp and increased activities of cytochrome P_{450} (CYP_{450}) in small intestines (40).

Macrolide antibiotics

It is well known that macrolides are antibiotics that have inhibitory activity on metabolizing enzymes. After discovery of ABC transport proteins it is clear that they also interact with P-gp. Their clearance can be accelerated by the action of MDR1 in the intestine, kidney, liver or CNS (36). Erythromycin exhibited significant efflux out of MDCK-MDR1 cells transfected with the human mdr1 gene, suggesting that erythromycin is a good substrate for P-gp.

Intestinal P-gp determines oral absorption and disposition of erythromycin leading to increased plasma and tissue levels in Mdr1a (–/–) mice (30). It was demonstrated in *in vivo* experiments with New Zealand albino (New Zealand White) rabbits that P-gp restricts topical erythromycin absorption across the cornea. Therefore, ocular bioavailability of P-gp substrates can be significantly enhanced by proper selection of P-gp inhibitors (5). Inhibition of P-gp contributes to accumulation of azitromycin in macrophages and thus to elimination of intracellular bacteria (32).

Erythromycin is known with its inhibitory activity on the enzymes from CYP_{450} family and P-gp function (31). For instance, mechanism of the pharmacokinetic interaction between oral ximelagatran (an oral direct thrombin inhibitor) and erythromycin involve inhibition of transport proteins, possibly P-gp, resulting in decreased biliary excretion and increased bioavailability of melagatran (6). Clarithromycin reduces the renal secretion of digoxin by blocking P-gp activity in the renal tubule (39).

At the same time, efflux by P-gp does not have significant influence in the kinetics of relatively new macrolides telithromycin and roxithromycin (21).

Ansamycines

Rifampicin is characterized as an inductor of both CYP_{450} and MDR expression and it can reduce the blood level of several drugs by induction of these proteins. Since cyclosporine is a substrate and rifampicin is an inductor for both CYP3A4 and P-gp, the observed increased clearance and decreased bioavailability of the immunosuppressive agent during rifampicin treatment are most probably due to a combination of CYP3A4 and P-gp induction (38). Rifampicin can also decrease the extent of absorption of other P-gp substrates as ranitidine by induction of the activity of this protein (16).

Species specific differences were observed in the effect of rifampicin on transcriptional level of expression of MDR1. It upregulates MDR1 mRNA expression in human hepatocytes and hepatoma cells but in rats Mdr1a and Mdr1b are not inducible by this antibiotic (20).

Tetracyclines

Oxytetracycline has been used for decennia in veterinary medicine due to its extensive spectrum of antimicrobial activity. A major limitation has been, and still remains, its low bioavailability following oral administration. Experiments with Caco-2 cells show that PSC833, a potent inhibitor of P-gp, decreased the secretion of oxytetracycline without affecting its absorption and indicate that this drug is a substrate for this protein. The affinity of oxytetracycline to this transporter seems to be rather low, as suggested by the low efflux ratio of 1:1.3. In competition experiments, oxytetracycline decreased the effluxes of other P-gp substrates such as Rhodamine-123 and ivermectin, findings of clinical relevance, as they clearly indicate potential drug-drug interactions at the level of P-gp-mediated drug transport (28).

Doxycycline, a tetracycline antimicrobial agent with structural similarity to doxorubicin, induced expression of MDR1 mRNA and P-gp. It reduced intracellular accumulation of doxorubicin, a substrate for P-gp and thus could contribute to clinical chemotherapeutic failure in cancer patients as a consequence of generating P-gp-expressing. Moreover, cells expressing P-gp demonstrated reduced intracellular accumulation of this tetracycline compared to that of cells that did not express P-gp suggesting that doxycycline is a substrate for P-gp (17).

Jonofore and other antibiotics

Dietary antibiotic as monensin, a model for dietary toxin, altered P-gp expression in poultry. Monensin increased P-gp expression in the liver and duodenum. Other antibiotic, bacitracin, reduced P-gp expression by 45% in the liver, but did not alter expression in the duodenum. This study indicates that dietary constituents regulate the expression of P-gp and these changes may represent an important physiological response to foods containing toxins (1).

Anticancer antibiotics

Nowadays, treatment of cancer in small animals became more and more important in veterinary practice. Multidrug resistance in cancer cells is discussed widely during last decade and a lot of efforts were spent to find a way to overcome this complicated problem (8). This section is pointed at the problems with multidrug resistance associated with the use of anticancer antibiotics and mediated by P-gp, only. Doxorubicin is one of the classical examples for an anticancer agent which is good substrate for P-gp. In P-gp-overexpressing cells, it becomes a difficult task to maintain a high intracellular doxorubicin level for a reasonable length of time (42). The pharmacokinetics of doxorubicin in blood and tissues of female

Balb/c mice was altered by earlier exposure to doxorubicin, as the animals that were treated once a week for 2 weeks showed an increased rate of doxorubicin elimination from blood and tissues following the second treatment because of overexpression of P-gp. These results have implications both in multiple-dosing regimens, as well as multiple-drug regimens, where doxorubicin is used in combination with other drugs that are substrates for P-gp (9). Idarubicin, an anthracycline antineoplastic agent, was characterized as a substrate for P-gp and its uptake in the cells is increased by verapamil and amiodarone, which function as inhibitors of this protein. Thus, these drug combinations increase acute negative inotropic effect of anthracyclines on the heart (41).

CONCLUSION

We have to acknowledge that P-gp expression and function at apical site of the cell membranes could have significant influence on drug disposition by restricting the penetration of antibiotics in some tissues or limit their absorption from gastrointestinal tract or enhanced their elimination. Opposite effect could be observed if this protein is down-regulated or inhibited. Interplay with drug metabolism also has to be taken into account (2). Notwithstanding, real situations became more complicated because other transporters as BCRP and MRPs were recognized as proteins which can modulate drug kinetics. Moreover, there is substantial overlap in substrate specificities for the different ABC transporters. Transport proteins which function at the basal site of cell membranes belonging to solute carrier family of transporters are also involved. Species specific differences in the expression pattern and activity of drug transporters require their investigation in different animal species and even breeds (7, 15). In dose determination strategy it has to be considered that efflux transport is energy dependent and saturable process when high concentrations of drugs are administered. In addition, bacterial cells also have ABC and drug efflux transporters which function in a coupled exchange with protons or sodium ions along a concentration gradient as symport or antiport (3, 13). Therefore, it is important to evaluate the role of drug transporters in target animal species not only from pharmacokinetic point of view but also because of pharmacodynamic and toxicological considerations.

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