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Review

Implications of ABC transporters on the disposition of typical veterinary medicinal products

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ABSTRACT

The ATP-Binding Cassette (ABC) transporters ABCB1, ABCC2 and ABCG2 are efflux transporters that facilitate the excretion of drugs, contribute to the function of biological barriers and maintain low cytoplasmic substrate concentrations in cells. ABC transporters modulate drug absorption, distribution and elimination according to the level of expression in the intestine, liver, kidney, and at biological barriers such as the blood-brain barrier. Moreover individual transporters are known to convey multi-drug resistance to tumour cells. While these diverse functions have been described in laboratory animal studies and in humans, the available information is very limited in animal species that are typical veterinary patients. This brief review summarizes the available data on organ distribution and expression levels in animals, genetic defects in dogs resulting in a non-functional P-gp expression, and describes examples of kinetic investigations directed to assess the clinical relevance of species differences in ABC-transporter expression.

Contents

1. Introduction

The ATP-Binding Cassette (ABC) comprise a superfamily of transporting ATP-ases that are integrated in cellular membranes. These are ubiquitous in biological systems, and are expressed in virtually all living organisms from bacteria to humans, and fulfil numerous important cell functions, facilitating transport of metabolic products, lipids and sterols across cell membranes (for a review see Borst and Elferink, 2002). Various inherited or acquired human diseases are associated with a dysfunction of ABC transporters, such as the Dubin–Johnson syndrome, cystic fibrosis, Tangier disease, immune deficiency, Pseudo-Xanthoma Elasticum and several forms of cholestasis. To date, 49 transporters have been identified in humans, phylogenetically allocated to 7 subfamilies, denoted ABCA to ABCG (http://nutrigene.4t.com/humanabc.htm). Several ABC transporters are highly substrate specific, recognizing a limited number of physiological substrates,

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whereas others accept diverse compounds as substrates, including drugs, toxins and plant metabolites that are present in the daily diet. A limited number of ABC transporters play a role in drug and metabolite transport. In particular the following transporters are involved in drug disposition: ABCB1, ABCC1, ABCC2, ABCC4 and ABCG2, although this number is likely to grow (Szakacs et al., 2004). These transporters are localized in the plasma membranes in the intestine, liver, kidney, blood-brain barrier and other vital biological barriers explaining their effect on the disposition of drugs.

The relevance of ABC transporters in veterinary medicine is evident. Many widely used drugs licensed for use in veterinary medicine are substrates for one or more transporters, e.g. digoxin, verapamil, loperamide, cimetidine, ivermectin, macrolides and fluoroquinoles. Also, chemotherapeutic agents in cancer therapy are increasingly applied on veterinary relevant animal species due to the increased willingness of pet owners for advanced therapies. The latter applies also to diverse human drugs, which are applied in companion animal practice.

To date there is only a limited amount of information available for clinicians to include drug–drug interactions related to ABC transporters into their decision paradigms. This review aims to provide an overview of the major ABC transporters involved in drug disposition, the current data in veterinary relevant animal species and their impact.

2. Multi-drug resistance in cancer therapy

Permeability glycoprotein, P-gp, encoded by the ABCB1 gene, was the first mammalian member of ABC proteins discovered by Juliano and Ling (1976). Initially, a decreased drug permeability was noticed in Chinese hamster ovary cells, displaying resistance to colchicines and a wide range of amphiphilic, but otherwise unrelated chemotherapeutic agents. The decreased sensitivity was associated with the expression of a 170 kDa protein, and hence named Permeability glycoprotein (P-gp). P-gp was subsequently referred to as Multi-Drug Resistance protein 1 (MDR1) in the field of cancer therapy, as it confers resistance of cancer cells to various structurally unrelated chemotherapeutic agents.

Forthcoming studies with the aim to explain multi-drug resistance in cancer cells and the advances in genomic analyses lead to the discovery of other efflux transporters, different from P-gp. Closely related transporters were cloned from hamster cell lines, termed mdr2 or abcb1b, and from the human liver, initially designated Multi-Drug Resistance protein 3, MDR3, and later as ABCB4 (Gros et al., 1986; Jongsma et al., 1987; Van der Bliek et al., 1986). Thereafter, MRP1 or Multi-drug Resistance associated Protein 1 (ABCC1) (Cole et al., 1992) and related transporters, including MRP2 (ABCC2) (Buchler et al., 1996; Mayer et al., 1995) and MRP4 (ABCC4) (Boguski et al., 1993; Kool et al., 1997) were described. Breast Cancer Resistance Protein, BCRP or ABCG2, is one of the most recent discovered members of the ABC-transporter superfamily (Allikmets et al., 1998; Doyle et al., 1998).

Although other ABC transporters are involved in multi-drug resistance of cancer cells (Gillet et al., 2007; Szakacs et al., 2004), ABCB1, ABCC1 and ABCG2 are the major transporters with clinical relevance in cancer therapy, various studies in humans suggested a prognostic value of transporter expression and therapy outcome (Dhooge et al., 1999; Styczynski et al., 2007; Tsunoda et al., 2006; van der Pol et al., 2003). In veterinary therapy, multi-drug resistance in the therapy of tumour patients has received little attention, despite the increasing use of cytostatic agents in dogs and the positive detection of P-gp in tumours of various tissue origin (Culmsee et al., 2004; Ginn, 1996; Petterino et al., 2004; Steingold et al., 1998)). For example, in dogs diagnosed for canine lymphoma, closely related to human non-Hodgkin's lymphoma, P-gp immunoreactivity in biopsies was detected in a low number of the patients at the moment of diagnosis. After relapse P-gp expression was higher and more frequent compared with the pre-treatment samples, suggesting that it has a role in cell survival (Lee et al., 1996). Chemotherapeutics that have been identified as substrates for one or more ABC transporters in other animal species and in humans are widely used in veterinary therapy including anthracyclines (daunorubicin, doxorubicin), vinca-alkaloids (vincristine and vinblastine) and antifolates (methotrexate). Although, interspecies differences in substrate affinity seem to be limited, functional studies indicated an important difference between species: in contrast to human MRP1, in dogs, mice, rats and cattle, MRP1 failed to confer the resistance to doxorubicin, despite an otherwise high functional similarity (Ma et al., 2002; Nunoya et al., 2003; Stride et al., 1997; Taguchi et al., 2002). The role of P-gp, MRP1, BCRP and other transporters in clinically relevant multi-drug resistance in animal species is of high interest as a prognostic factor and would indicate the possible need for drug resistance circumvention, such as the co-administration of transporter-inhibitors (Morschhauser et al., 2007), liposomal formulations (Tulpule, 2005) or small interfering RNA's (Li et al., 2006).

The use of chemotherapeutics in cancer therapy, particularly because of the narrow safety margin, in combination therapies with other substrates or inhibitors is at risk of clinically relevant drug–drug interactions and the clinician should thus consider his choice carefully (Aszalos, 2007). This is particularly relevant in veterinary medicine as it is largely unknown whether veterinary licensed drugs are modulators of ABC transporters in animals. Combination therapies thus require careful observation of the patient for drug–drug interactions.

3. ABCB1 and protection of the central nervous system

Early studies on the expression of P-gp in normal tissues indicated substantial expression levels in the adrenal gland, intestines, liver and kidney of rodents as well as humans (Cordon-Cardo et al., 1990; Fojo et al., 1987; Thiebaut et al., 1987). However, despite its localization and the suggestion that P-gp plays a role in drug elimination (Thiebaut et al., 1987), its importance for pharmacokinetics remained unrecognized for many years. It was only in 1994 that its role as an important factor in drug disposition became evident by a well-known incident: a colony of *mdr1a* (−/−) knock-out mice was treated for a mite infestation with the endectocidal drug ivermectin. Most of the knock-out mice died, while the wild-type animals remained unaffected by the given therapy. The observed ivermectin toxicity resulted from an increased permeability of the blood-brain barrier in the knock-out mice. This incident clearly demonstrated that not only cytostatic agents, but also other therapeutic agents are substrates for P-gp and that it prevents the penetration of its substrates into barrier-protected tissues (Schinkel et al., 1994).

The finding that ivermectin is a substrate for P-gp has implications in veterinary medicine. Collie-dogs and other herding breeds are at risk for carrying a mutant P-gp. This mutant has a 4 base pair deletion in the ABCB1 gene resulting in the absence of a functional form of P-gp. From clinical experience it had been known for a long time that collies are highly sensitive for intoxications by ivermectin, which is explainable now by the mutation in the ABCB1 gene as described above (Mealey et al., 2001). This defect has thereafter been detected in related herding breeds of the collie lineage (Shetland Sheepdog, Australian Shepherd, Old English Sheepdog, English Shepherd, Border Collie) and two breeds of the sighthound class (Longhaired Whippet and Silken Windhound) (Geyer et al., 2005a; Neff et al., 2004)) and the White Swiss Shepherd (Geyer et al., 2007).

Homozygous mutant dogs are highly sensitive to ivermectin showing adverse neurologic effects after a single dose of 120 μg/kg, whereas dogs homozygous for the normal P-gp can receive 2000 μg/kg in a single dose without signs of toxicity. Heterozygous dogs may experience adverse effects at doses greater than 300 μg/kg (Mealey, 2006).

Comparable intoxications have not been noticed for the related avermectines such as selamectin, that is licensed as a transdermal formulation. Selamectin is a substrate for human P-gp (Brayden and

Griffin, 2008), but did not cause side effects in ivermectin-sensitive collies (Bishop et al., 2000; Novotny et al., 2000). Moxidectin, a semi-synthetic macrocyclic lactone of the milbemycin family, is also licensed for the use in dogs as a transdermal formulation and has been shown to be extensively absorbed into the systemic circulation (http://www.emea.europa.eu/vetdocs/PDFs/EPAR/advocate/029703en6.pdf). Moxidectin safety was assessed in ivermectin-sensitive collies and did not result in adverse clinical signs (Paul et al., 2004). Although it could not be demonstrated that moxidectin is a substrate for human P-gp (Griffin et al., 2005), it has been suggested that moxidectin is transported by rat P-gp and MRP's in primary rat hepatocytes (Dupuy et al., 2006). Intoxication has been reported for one dog (Australian shepherd) that had received repeated oral doses (Geyer et al., 2005b). Although, this dog was homozygous for the mutant P-gp, the intoxication could have resulted from accumulation of the drug in the body since moxidectin has a long elimination half-life (Lallemand et al., 2007). The selective toxicity of moxidectin is apparently not solely related to P-gp but also to other transporters in the blood-brain barrier. BCRP is a good candidate since high milk to plasma ratios of moxidectin in the milk of sheep were detected (Imperiale et al., 2004) and BCRP facilitates the excretion of xenobiotics in the milk of mice and sheep (Jonker et al., 2005; Pulido et al., 2006).

Serious adverse effects of P-gp mutant dogs have also been reported for other drugs that are substrates for human P-gp: loperamide with central nervous system (CNS) side effects (Sartor et al., 2004), doxorubicin, vincristine (Mealey et al., 2003) and digoxin (Henik et al., 2006) with adverse effects in organs different from the CNS. For example, a collie with lymphoma was treated with vincristine and doxorubicin and developed myelosuppression and gastro-intestinal toxicity even at lowered doses, but tolerated cyclophosphamide. The patient appeared to be heterozygous for the mutant P-gp that likely has resulted in lower cytoprotection and delayed elimination of the drugs (Mealey et al., 2003).

The former cases indicate that clinicians have to be cautious with the use of drugs that are P-gp substrates in particular in the canine populations that are at risk for carrying the mutant P-gp. As a tool for the clinician, a first step in individualized veterinary medicine was made by the commercially available genetic test for the analysis of the P-gp genotype of the patient (Mealey, 2004).

4. ABCC2 mutants

MRP2 was formerly called canalicular Multi-specific Organic Anion Transporter (cMOAT), but is now classified as a product of the ABCC2 gene (Buchler et al., 1996; Mayer et al., 1995; Paulusma et al., 1996). cMOAT was originally identified in mutant rat strains deficient for the excretion of bilirubin-glucuronides and other organic anions (Oude Elferink et al., 1990).

While its role in multi-drug resistant cancer cells is only modest, MRP2, localized in the hepatocyte canalicular membrane and at the luminal membrane of renal proximal tubule cells, contributes significantly to the elimination of endogenous conjugated metabolites, and of xenobiotics and their conjugates with glutathione, glucuronate or sulphate.

An inherited disease, the human Dubin–Johnson syndrome, characterized by persistent hyperbilirubinemia, was found to be associated with the absence of functional active cMOAT/MRP2 (for a review see Konig et al., 1999). A similar hereditary deficiency in the biliary secretion of conjugates has been observed in Corriedale sheep (Alpert et al., 1969) and rats (TR− and EHBR mutant rats). The human and ovine mutants result in a dark discolorization of the liver, while this is not observed in the mutant rats. The discolorization is due to the disposition of pigments that are formed by amino-acid catabolism (Kitamura et al., 1992; Zimniak, 1993). To our knowledge it is not known whether this mutation is wide-spread in this race of sheep and whether the MRP2 deficiency has had clinical consequences for drug-therapy.

5. Physiological functions of BCRP

BCRP, the ABCG2 gene product, is one of the latest discovered members of the ABC-transporter family (Allikmets et al., 1998; Doyle et al., 1998; Miyake et al., 1999) and its expression was first associated with resistance to chemotherapeutics used in breast cancer patients, resulting in the name breast cancer resistance protein (Allen et al., 1999). At the same time the gene was characterized in the human placenta (Allikmets et al., 1998) where it has an important role in the protection of the fetus, preventing the trans-placental passage of drugs and toxins. BCRP is a so-called half transporter with six membrane-spanning domains and requires homodimerization for its function in contrast to P-gp that consists of twelve membrane-spanning domains. Its expression has been demonstrated in nearly all organs in humans (Doyle et al., 1998). Its function is similar to that of P-gp as part of tissue-barriers, limiting the penetration into the brains and the fetus, and the absorption of toxins from the intestinal lumen.

With the aim to study the function of BCRP *in vivo*, a *bcrp* knock-out mouse was developed by The National Cancer Institute (NKI) in the Netherlands, and again accidentally, an unintended modification of the diet with an increase in dietary chlorophyll (from alfalfa (*Medicago sativa*) leaf concentrate) leading to an unexpected phototoxicity in the knock-out mice, apparently associated with an increased absorption and cellular accumulation of a chlorophyll degradation product, pheophorbide A (Jonker et al., 2002). Other known substrates for BCRP are endogenous metabolites including porphyrins and estrogen-conjugates, riboflavin (vitamin B12), phyto-estrogens, drugs, toxins and their conjugates (Adachi et al., 2003; Suzuki et al., 2003; Zamek-Gliszczynski et al., 2005).

6. ABC transporters in drug absorption and disposition

The relevance of the individual transporters in drug disposition dependents on its tissue distribution and substrate recognition. The organ-specific and subcellular distribution of ABC transporters has been evaluated by immunohistochemistry mainly in human tissues (Cordon-Cardo et al., 1990; Konig et al., 1999; Maliepaard et al., 2001; Thiebaut et al., 1987).These studies indicated that P-gp and BCRP have a wide and overlapping tissue distribution affecting drug absorption, distribution and elimination, whereas MRP2 was found to be most important in the liver and kidneys as a transporter of hydrophilic conjugates and anions into bile and urine. Mechanistic pharmacokinetic studies were mostly done in laboratory animals, comprising also experiments in knock-out mice, whereas substrate identification studies used *in vitro* systems with defined cell cultures, such as Caco-2 cell monolayers and genetically modified cell-lines overexpressing human or rodent transporters (Marathe and Rodrigues, 2006).

The ABC transporters P-gp, BCRP and MRP2 are situated in the apical membrane of intestinal epithelial cells mediating the efflux of their substrates and counteract the intestinal absorption. Although the extent of intestinal absorption of drugs and xenobiotics is dependent on a wide range of factors, an impaired absorption in human and rodents was often observed for P-gp substrates (Adachi et al., 2003; Greiner et al., 1999; Kim et al., 1998; Kruijtzer et al., 2002; Sparreboom et al., 1997; Yamaguchi et al., 2002) as well as BCRP substrates (Jonker et al., 2000; Sesink et al., 2005; van Herwaarden et al., 2003; Zaher et al., 2006).

The unidirectional transport to the intestinal lumen (exsorption or desorption) has now been recognized as an additional route for drug elimination, competing with the excretion into the bile and urine, and depending on the expression and activity of ABC-efflux transporters (Mayer et al., 1996).

The role for the intestines with a high expression of P-gp as an excretory organ has previously been demonstrated in *mdr1a/1b* knock-out mice. A pronounced decrease in intestinal secretion of various P-gp substrates, including digoxin, was observed in these animals as compared to the corresponding wild-type strain (Schinkel et al., 1997). In humans, intestinal perfusion experiments demonstrated that the

enteral elimination of digoxin, administered IV, was decreased in the presence of the P-gp inhibitor quinidine (Drescher et al., 2003). The role of P-gp in intestinal elimination of ivermectin was demonstrated in an experiment with rats. The intestinal elimination of systemically applied ivermectin appeared to be five fold higher than the elimination via the bile, and co-administration of verapamil, an inhibitor of P-gp, markedly decreased the intestinal secretion (Laffont et al., 2002). Similar to P-gp, MRP2 and BCRP contribute to intestinal elimination, though mainly of drug-conjugates (Adachi et al., 2005; Dietrich et al., 2001; Sesink et al., 2005; van Herwaarden et al., 2003).

The kidneys are well equipped for the excretion of compounds into the urine (for a review see Chandra and Brouwer, 2004). Various transporters belonging to the SLC-superfamily are present in the basolateral and apical membrane of the tubule cells along the nephron and contribute to cellular uptake and efflux (van Montfoort et al., 2003). With regard to ABC transporters, it appears that MRP2 is highly expressed in the proximal tubule cells of most animal species. Its functional characterization at this specific location, however, is rather limited (van de Water et al., 2005). The role of P-gp in renal excretion is yet not well established and contradictory (Hedman et al., 1991; Kageyama et al., 2006; Nishihara et al., 1999; Shimizu et al., 2004; Smit et al., 1998). BCRP is highly expressed in the kidneys of rodents and is functionally involved in the urinary excretion of its substrates (van Herwaarden et al., 2006), and particular in the excretion of sulphated conjugates in mice (Mizuno et al., 2004). In contrast, the expression of BCRP in the human kidney is apparently low (Huls et al., 2008), but no data are available about its functional role in mechanisms of renal excretion.

The liver is still considered to be the major detoxifying organ, despite the increasing recognition of the (pre-systemic) elimination of drugs by the intestines co-expressing transporters and biotransformation enzymes in the intestinal wall (Adachi et al., 2005; Enokizono et al., 2007; Wacher et al., 1995) The liver parenchymal cells (the hepatocytes) are specialized in the uptake, metabolism and excretion of endo- and xenobiotics. Phase I and phase II metabolizing enzymes are highly expressed in the liver and form sequential elements in the detoxification process prior to active excretion of the drug and metabolites. The vectorial transport from the blood into the bile is dependent on transporters located in the sinusoidal (basolateral) membrane and in the apical (canalicular) membrane. Drug transporters in the basolateral membrane are mainly members of the SLC family (SLC, solute carrier such as OATP, OAT and OCT), while members of the ABC-transporter family, including ABCB1, ABCB4, ABCC2 and ABCG2, facilitate the canalicular drug transport. Several members of the ABCC-family in the basolateral membrane transport also substrates out of the hepatocytes back into the sinusoidal blood stream. P-gp is the major canalicular transporter for neutral and cationic substances (Hiroyuki Kusuhara and Yuichi, 1998; Smit et al., 1998). Human MDR3 (ABCB4), related to P-gp (ABCB1), has a certain degree of overlap in substrates. Its overall transport affinity, however, is much lower than that of P-gp (Smith et al., 2000). MRP2 is the major transporter of organic anions (such as for example ampicillin and ceftriazone), and hydrophilic phase II conjugates (Dietrich et al., 2001; Hiroyuki Kusuhara and Yuichi, 1998; Konig et al., 1999; Oude Elferink et al., 1995) into the bile. The role of BCRP in hepatic xenobiotic and conjugate excretion has been demonstrated in mice (Hirano et al., 2005; Merino et al., 2005; van Herwaarden et al., 2003), however in the interpretation of these data it needs to be considered that mice highly express BCRP in the liver and these findings may not be representative for other animal species: BCRP and MRP2 have a different importance between rats and mice in the biliary excretion of conjugates (Zamek-Gliszczynski et al., 2005).

7. ABC transporters in drug absorption and disposition of common veterinary drugs

In veterinary medicine, the preferred route of drug administration is the oral application, particularly when multiple doses are required. Oxytetracycline, a congener of the tetracycline antimicrobials, has a very low oral bioavailability in pigs of 3–9% and subsequently high doses are applied to achieve effective plasma concentrations. The low bioavailability not only results in large inter-individual differences in plasma concentrations, but also in a considerable fraction of the dose that is excreted unchanged with the faeces and subsequently reaches the environment. We could demonstrate that oxytetracycline is a substrate for human P-gp, but only a small fraction of the total permeability was related to active transport and at clinically relevant doses oxytetracycline is likely to saturate intestinal P-gp. These data indicated that it is not possible to improve the oral bioavailability of oxytetracycline by the modulation of P-gp, but suggest a risk for drug–drug interactions as it inhibited the transport of other P-gp substrates, Rhodamine123 and ivermectin (Schrickx and Fink-Gremmels, 2007a).

The cardiac drug digoxin has a limited oral bioavailability in both human and dogs that is related to P-gp expression. P-gp is subsequently a factor in the variation of plasma levels. For example, pretreatment of dogs with phenobarbital, an inducer of P-gp, decreased plasma levels in dogs (Ravis et al., 1987).

The function of ABC transporters to allow drug secretion from the central compartment into the intestinal lumen, is favourable in a number of typical veterinary indications. For example, due to a high secretion of ivermectin into the gastro-intestinal tract, parenteral application is effective in treating gastro-intestinal parasite infestations. Fluoroquinolones can be applied orally or by parenteral injection. Despite a low rate of biliary elimination, drug concentrations are high in the gut lumen, as first demonstrated for ciprofloxacin, the first widely marketed fluoroquinolone, (Sorgel et al., 1989). Fluoroquinolones are substrates for multiple human ABC transporters (Alvarez et al., in press), as demonstrated for enrofloxacin, ciprofloxacin and danofloxacin-mesylate (Merino et al., 2006; Pulido et al., 2006; Schrickx and Fink-Gremmels, 2007b). Danofloxacin-mesylate is licensed for the use in calves suffering intestinal tract infections, but needs to be administered by the parenteral route. Danofloxacin-mesylate highly distributes into the target tissues, and concentrations exceeding those in plasma were detected in the gut lumen (Friis and Nielsen, 1997; Lindecrona et al., 2000; McKellar et al., 1998; Shem-Tov et al., 1998a). The luminal compartment is the major site of gram negative infections and hence luminal drug concentrations are even more relevant for the efficacy than tissue concentrations. We demonstrated that danofloxacin-mesylate is a substrate for multiple human ABC transporters, suggesting that intestinal elimination is the underlying mechanism for danofloxacin-mesylate secretion into the luminal compartment of the intestines (Schrickx and Fink-Gremmels, 2007b).

8. Drug–drug interactions

The use of drugs that are substrates for one or more ABC transporter in combination therapies with other substrates or inhibitors comprise the risk of clinically relevant drug–drug interactions, not only in cancer therapy. That drug–drug interactions leading to inhibition of efflux transporters can result in an increased rate of exposure and drug toxicity is exemplified by the interaction of the P-gp substrate digoxin with other cardiac drugs, such as verapamil and quinidine, or cyclosporine, resulting in marked increases in digoxin plasma concentrations and the appearance of clinical signs of intoxication in man and rats (Fromm et al., 1999; Sachiyo Funakoshi et al., 2003; Verschraagen et al., 1999). The interaction between digoxin and cyclosporine has also resulted in cases of severe digitalis toxicity in dogs (Robieux et al., 1992). Although cyclosporine decreased renal elimination of digoxin in *in situ* perfused kidney in the dog (De Lannoy et al., 1992), the interaction is likely to occur in the intestines increasing the intestinal absorption of digoxin (Igel et al., 2007).

Many compounds that are substrates for P-gp are also substrates for (human) CYP3A4 and these act synergistically in the small intestines to reduce the oral bioavailability. The overlapping substrate

specificities result in complex pharmacokinetic profiles of multi-drug regimens and drug–drug interactions.

Cyclosporine A is widely used in veterinary medicine in immune-mediated disorders. It has a limited oral bioavailability and extensive metabolism. Ketoconazole has been shown to increase cyclosporine A blood levels when co-administered with cyclosporine A and is used in veterinary medicine to reduce the dosage and cost of cyclosporine A (Daigle, 2002). It is likely that the underlying mechanism is inhibition of both P-gp and cytochrome *P450 3A* as in human (Robson, 2003).

Paclitaxel is a chemotherapeutic agent effective in the treatment of human epithelial neoplasms. The administration of paclitaxel is problematic because it has a low oral bioavailability and it requires inorganic solvent for i.v. administration resulting in hypersensitivity reactions in both man and dog. The co-administration of the dual P-gp and CYP3A4 substrate and inhibitor cyclosporine with oral paclitaxel markedly increased the plasma concentrations in dogs with an increase in the bioavailability form approximately 20% to 100% (McEntee et al., 2003). For its veterinary use, a phase I study was completed in tumour bearing dogs and cats to determine the maximal tolerated doses (McEntee et al., 2006a,b).

Tiamulin is a semi-synthetic derivative of the antibiotic pleuromutilin and used in the control of pulmonary infections in pigs and poultry. It is well known to produce clinically important and often lethal interactions with ionophoric drugs, particularly the coccidiostats monensin and salinomycin, resulting in an increased cardiomyotoxicity as a common sign of ionophore toxicity (Ratz et al., 1997; Szucs et al., 2004). Tiamulin has later been identified as an inhibitor of P-gp in human and rodent cell lines (Baggetto et al., 1998) and is thus a potential inhibitor of P-gp in farm animal species. The recent finding that salinomycin is a P-gp substrate, suggests that the drug–drug interactions described between ionophores and pleuromutilins are not only related to inhibition of biotransformation, but also to inhibition of P-gp. Experiments with P-gp knock-out mice (*mdr1a/b*$^{-/-}$) demonstrated that oral bioavailability, brain penetration and clearance of salinomycin are markedly altered, leading to toxicity of salinomycin even at therapeutic dose levels (Lagas et al., 2008).

Combinations of the macrolide antibiotics erythromycin and oleandomycin with ionophore drugs have also been associated with toxic effects. Drug–drug interactions of antibiotics of the macrolide group have been mainly attributed to inhibition of the cytochrome *P450* system (Anadon and Reeve-johnson, 1999). However, interactions of erythromycin with digoxin have been reported repeatedly. Digoxin does not undergo biotransformation by CYP3A4 that indicates that the toxic effects are not related to inhibition of the cytochrome *P450* system, but are related to inhibition of P-gp. Recently, the inhibition of digoxin transport by various macrolides demonstrated that these can be inhibitors of P-gp, albeit with varying potency (Eberl et al., 2007). This finding also suggests that the reported interactions of several macrolides with felodipine, alfentanil and terfenadine are related to both, inhibition of cytochrome *P450 3A* and of P-gp (Anadon and Reeve-johnson, 1999).

Although all new (veterinary) pharmaceuticals undergo an extensive evaluation during premarketing approval, specific data on ABC transporters are not requested and hence a toxic syndrome due to genetic polymorphisms (as described in dogs) or drug–drug interactions are not predicted.

A recent example is the anti-emetic drug maropitant, a new drug of the class of the neurokinin-1 receptor antagonists that has recently been licensed for use in dogs. The indications of maropitant include preventing of vomiting, and treatment of vomiting induced by motion sickness and vomiting due to metabolic disorders. The dose required for the prevention of motion sickness is much higher than the dose required for the other indications. The oral bioavailability of maropitant is non-linear and ranges from 24% at the lower dose to 37% at the higher dose suggesting an extensive first-pass effect. The major biotransformation enzymes are CYP2D15 and CYP3A12. The safety margin of maropitant is limited and the concurrent use of drugs metabolized by CYP2D15 or CYP3A12 might result in adverse effects. Interactions of aprepitant (licensed for humans, a dual CYP3A4 and P-gp substrate) were observed when co-administered with corticosteroids resulting in marked increased levels of dexamethasone (a dual substrate) (Herrstedt and Dombernowsky, 2007). The overlap in CYP3A4 (the canine homologue is CYP3A12) and P-gp substrates raises the question whether maropitant is a substrate for P-gp, however no data are available about the role of transporters in maropitant disposition or about any modulatory effects.

The serotonin-reuptake inhibitor Fluoxetine was recently licensed in the United States for veterinary use as a chewable tablet for dogs to treat separation anxiety. Fluoxetine is not a substrate for murine P-gp but inhibits its function (Uhr et al., 2000) and hence may be a potential cause of unexpected drug–drug interactions in dogs.

9. Drug secretion into the milk

An organ that is particularly important in bovine medicine is the mammary gland. Bacterial infection of the mammary gland (mastitis) is frequently seen and results in significant economic losses. Antimicrobial therapy consists of intramammary application, systemic application or a combination of both. However, a systemic application (parenteral) should result in effective concentrations of antibiotics also in the mammary gland. Recently it could be shown that BCRP is highly expressed in the mammary gland epithelium during lactation, facilitating the excretion of drugs including nitrofurantoin and other xenobiotics into milk of mice (Herwaarden et al., 2006; Jonker et al., 2005; Jonker et al., 2000; Merino et al., 2005). Clinically relevant for dairy cattle is the secretion of fluoroquinolones into the milk. The licensed veterinary drug enrofloxacin and its (active) metabolite ciprofloxacin are substrates for BCRP and are excreted into the milk of sheep and mice respectively (Merino et al., 2006; Pulido et al., 2006). Danofloxacin-mesylate, another fluoroquinolone that is extensively used in veterinary medicine is a candidate substrate for BCRP (Schrickx and Fink-Gremmels, 2007b). Both drugs are often administered parenterally in cases of acute, severe inflammatory processes in the mammary gland caused by gram negative bacteria and reach therapeutic levels in the milk in cattle (Kaartinen et al., 1995; Rantala et al., 2002; Shem-Tov et al., 1998b) and other ruminant species (Escudero et al., 2007). The active transport of drugs by BCRP as the underlying mechanism of concentrating drugs in milk can be used in the development of new drugs indicated for mastitis, in particular for infections by extra-cellular pathogens. On the other hand, drugs which are BCRP substrates and indicated for the treatment of pathologies in other organs, will be secreted into the milk, resulting in undesirable contamination of consumption milk and the need to establish drug withdrawal periods for dairy cows.

10. Species differences in the expression of ABC transporters with emphasis on veterinary target animal species

Species differences in expression of transporters should be taken into consideration in interspecies scaling as these can have implications on kinetics of substrates. Marked species differences have been observed for ABCC2 and ABCG2 that have direct consequences for the capacity of elimination of substrates including sulphate and glucuronide conjugates. A high expression of MRP2 in the liver of rats results in a high capacity of transport while other species including the dog have a lower biliary transport capacity for MRP2 substrates (Ishizuka et al., 1999; Ninomiya et al., 2005). In contrast, expression of ABCG2 is relatively low in the rat liver and high in the mouse liver (Tanaka et al., 2005) that results in mechanistic species differences in biliary excretion of sulphate and glucuronide metabolites (Zamek-Gliszczynski et al., 2006). These data indicate the need to measure the species specific expression of multiple transporters in tissues. The

expression of ABC transporters either at a transcriptional or protein level in organs of veterinary relevant animal species has, however, not been widely studied.

The current data indicate that the mRNA and protein expression of P-gp in the liver, kidneys, intestines and brains of dogs resembles that of human (Conrad et al., 2001; Ginn, 1996; Tashbaeva et al., 2007). Unfortunately, the methods used do not allow a reliable quantification and only one or two secretory transporters were included. The organ distribution of MRP2 in dogs is apparently similar to many other species, except for the rat and this could be the reason for the more limited capacity for biliary secretion of the MRP2 substrates 17 beta-estradiol glucuronide, 2,4-dinitrophenyl-*S*-glutathione and temocaprilat (ACE-inhibitor, not for use in veterinary medicine) in dogs compared to rats (Ishizuka et al., 1999). The differences in expression can also be the underlying mechanism of differences in the elimination routes of the MRP2 substrate drug enalapril and its active metabolite enalaprilat (Liu et al., 2006). Rats excreted 26% of the dose in the urine and 72% in the faeces in 72 h; dogs excreted 40% of the dose in the urine and 36% in the faeces (Tocco et al., 1982).

In pigs, the expression of P-gp and BCRP in the liver and kidneys is relatively low as it could not be detected by immunohistological methods, but only at the transcriptional level. The functional consequences remain to be assessed, but the data suggest that MRP2, which has a similar expression pattern as dogs, is most important in both the biliary and urinary eliminations of drug-conjugates (Schrickx, 2006).

In chicken and turkeys, the expression levels of ABCB1 mRNA in the intestines and liver are comparable to human, but in contrast to human, in which the renal expression exceeds that in the intestines, renal ABCB1 mRNA levels were lower than in the intestines (Edelmann et al., 1999; Haritova, 2006; Langmann et al., 2003). A remarkable finding was the relatively low expression of ABCB1 in the adrenal gland in both chicken and turkeys. The expression of P-gp in the adrenal gland in humans is highest of all organs and is apparently related to its function in steroid transport (Langmann et al., 2003). Although this difference will not relate to drug transport, it indicates species differences in physiology. The subsequent search for other closely related human ABCB1 sequences in the chicken genome, suggests that chickens have only one gene that is highly related to both human ABCB1 and ABCB4. Such a species difference was also found in the major transcription factors Pregnane X Receptor (PXR) and Constitutive activated Androstane Receptor (CAR), regulating in the expression of ABC transporters (and of the major drug metabolizing enzymes). PXR orthologues in various species show a sequence identity of more than 95% in the DNA binding domains, however the sequence identity for the ligand binding domains is as low as 75%–80% among mammalians, while in the chicken the PXR homologue, CXR, has a sequence identity with human PXR for the ligand binding domain of only 50% and with human CAR of 56%, a level that is comparable to that between mammalian PXR and CAR sequences (Handschin et al., 2000). These data suggest duplication of genes in mammals and indicate differences in the modulation of expression of transporters and biotransformation enzymes.

11. Species differences in transport of substrates

Although substrate specificities are generally similar between human, rodent and dog, species differences in the functional activity and inhibitory potency have been reported.

The antiepileptic drugs phenytoin and levetiracatem, for example, are transported by mouse but not human P-gp (Baltes et al., 2007). The activity of canine transporters can be estimated from *in vitro* studies using the canine derived kidney epithelial cell line Madin–Darby canine kidney cells (MDCK). MDCK cells transfected with individual ABC transporters are widely used for functional studies in comparison to wild-type (wt) cells and the use of selective inhibitors. For example, it could be shown that the secretory transport of the P-gp substrates paclitaxel, vinblastine and digoxin in the wt-MDCK cells was sensitive for the P-gp inhibitors cyclosporine A, ketoconazole, loperamide, verapamil, nicardipine and quinidine (Taub et al., 2005), suggesting similar characteristics to human P-gp. Cell lines transfected with human, monkey, canine, rat and mouse P-gp further characterize the substrate specificity of canine P-gp. Diltiazem, cyclosporine A, dexamethasone, daunorubicin, digoxin clarithromycin, etoposide, paclitaxel, propranolol, quinidine, ritonavir, saquinavir, verapamil and vinblastine are substrates for human, monkey, canine, rat and mouse P-gp (Katoh et al., 2006; Suzuyama et al., 2007; Takeuchi et al., 2006). It is however difficult to establish the efficiency of the transport among species due to differences in the level of expression. Although the transport has been corrected for the P-gp protein level in the transfected cell lines, the specificity and affinity of the antibodies directed against human P-gp have not been measured for canine P-gp (Katoh et al., 2006).

Differences in the inhibitory potency of verapamil for digoxin and cyclosporine transport between canine and human P-gp could clearly be demonstrated, with a lower potency towards the transport by canine P-gp. However, this was not observed for the inhibition of daunorubicin by verapamil. These data indicate differences in affinity of substrates for P-gp across species but also its dependency on the substrate (Suzuyama et al., 2007).

The feline homologue of P-gp was cloned in 2000 and shown to display a high homology of 90.7% (Okai et al., 2000). This feline P-gp, however, was never functionally characterized. The rabbit homologue to MRP2 has been cloned and to a limited extent characterized (van Kuijck et al., 1997).

Data about the transporters in other species became available when certain cell types served as a model for the human blood-brain barrier, such as endothelial cells isolated from the capillaries of bovines (Bachmeier et al., 2006) or porcine species (Bauer et al., 2003; Eisenblatter and Galla, 2002). We have measured the inhibitory potencies of various known human substrates and inhibitors for porcine P-gp in an *ex vivo* lymphocyte model using Rh123 as a substrate. The potent first and second generation P-gp inhibitors as well as ivermectin and loperamide potently inhibited porcine rhodamine123 efflux with EC50 values that are comparable to data from the literature. These findings also validate the used model and suggest that peripheral lymphocytes can be used for identification of substrates and inhibitors (Schrickx, 2006).

12. Inter-individual variation in drug disposition attributable to modulation of ABC transporters

Changes in function or expression of ABC transporters, originating either from genetic variation, physiological and pathological conditions, or from exogenous factors, determine the individual variability in drug disposition and kinetics and subsequently the individual pharmacological response (Kerb, 2006; Lamba et al., 2004). The expression of transporters (and enzymes) is directly or indirectly regulated by endogenous and exogenous substances via transcription factors and co-regulators. Endogenous substances are steroids (including sex hormones), thyroid hormones and cytokines (Miyoshi et al., 2005). That physiological actions have consequences for variation in transporter expression in organs relevant for drug disposition, is also indicated by variability in expression by gender (Chandler et al., 2007; Tanaka et al., 2005), pregnancy (Wang et al., 2006), and age (Rosati et al., 2003). It may not be surprising that steroid-hormones modulate ABC-transporter expression since ABC transporters are regulated by the same transcription factors as biotransformation enzymes, for what gender differences have been long known. Exogenous factors include Herbs, such as St John's Worth, food constituents or supplements that may inhibit or induce transporters resulting in an increased and decreased exposure of drugs, respectively (Hennessy et al., 2002). This accounts in particular for flavonoids as substrates

and modulators of multiple ABC transporters (Morris and Zhang, 2006; Zhou et al., 2004). Modulation of transporters and subsequent pharmacokinetics did not receive attention yet in veterinary medicine, but are likely to affect pharmacokinetics, efficacy and toxicity in animals as well.

13. Conclusions and future objectives

In conclusion, ABC transporters are not only significant factors in multi-drug resistance of cancer cells, but also modulators of drug absorption and distribution. The knowledge on the impact of ABC transporters in veterinary medicine is confined to some clinical relevant data for MDR1 gene mutations and P-gp deficiencies in dogs. Clinically relevant drug–drug interactions can be predicted on the basis of data from human medicine, but have obtained little attention in the veterinary field, despite known serious side effects following co-medication. More recently, various investigations with fluoroquinolones have been conducted in different animal species confirming the clinical significance of multiple transporters as modulators of kinetic parameters. These investigations are often hampered by the lack of systematic data regarding the physiological levels in animal tissues and their transcriptional regulation. Initial data in pigs and poultry (Haritova, 2006; Schrickx, 2003) demonstrate significant species differences and the need to establish comparative data compilations for other animal species. A closer insight in localization and function of efflux transporters in various organs will allow to predict the clinical relevance of substrate interactions and will allow to develop new pharmaceuticals tailor made to reach pre-selected tissues, such as the mammary gland, for the treatment of common and economic important diseases in veterinary patients.

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