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P-gp substrate-induced neurotoxicity in an Abcb1a knock-in/Abcb1b knock-out mouse model with a mutated canine ABCB1 targeted insertion

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ABSTRACT

Certain dog breeds, especially Collies, are observed to exhibit neurotoxicity to avermectin drugs, which are P-glycoprotein (P-gp) substrates. This neurotoxicity is due to an ABCB1 gene mutation (ABCB1-1Δ) that results in non-functional P-gp expression. A developed Abcb1a knock-in/Abcb1b knock-out mouse model expressing the ABCB1-1Δ canine gene was previously reported and mice exhibited sensitivity upon ivermectin administration. Here, model and wild-type mice were administered P-gp substrates doramectin, moxidectin, and digoxin. While knock-in/knock-out mice exhibited ataxia, lethargy and tremor, wild-type mice remained unaffected. In addition, no neurotoxic clinical signs were observed in either mouse type administered domperidone, a P-gp substrate with no reported neurotoxicity in ABCB1-1Δ Collies. Overall, neurotoxic signs displayed by model mice closely paralleled those observed in ivermectin-sensitive Collies. This model can be used to identify toxic P-gp substrates with altered safety in dog populations and may reduce dog use in safety studies that are part of the drug approval process.

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1. Introduction

P-glycoprotein (P-gp) is a large (170 kDa) membrane-associated protein and member of the ATP-binding cassette (ABC) transporter family. It is comprised of a single polypeptide chain that is conformationally arranged into two similar domains of transmembrane helices and has the ability to bind a wide variety of structurally diverse compounds that range in size and charge (Aller et al., 2009; Endicott and Ling, 1989; Ford et al., 2003; Gottesman and Pastan, 1993; Schinkel, 1997). Physiologically, this protein serves to protect cells from potentially toxic xenobiotics and endogenous metabolites by actively expelling these compounds from the cell in an ATP-dependent manner (Schinkel, 1997). ABCB1, the gene that encodes for P-gp, was first identified as the multidrug resistance (MDR1) gene due to its ability to bestow multidrug resistance on mammalian tumor cells upon treatment with cytotoxic drugs (Ueda et al., 1987). The protective role of the ABCB1 gene product was further substantiated by its tissue distribution, as P-gp is chiefly expressed in hepatic, brain, intestinal and renal tissues. Within the brain, P-gp is prominent in capillary endothelial cells that contribute to blood–brain barrier function. In other tissues it is typically found near the luminal membrane domain of polarized epithelial cell layers (Ambudkar et al., 2008; Cordoncardo et al., 1989; Fojo et al., 1987; Schinkel, 1997).

Several dog breeds within the herding group, particularly Collies, harbor a mutation in the ABCB1 gene that has been associated with severe adverse effects upon administration of ivermectin, an antiparasitic veterinary drug and known P-gp substrate (Fecht and Distl, 2008; Pulliam et al., 1985; Seward, 1983). Sequence analysis of cDNA from dogs that displayed the ivermectin-sensitive phenotype revealed a 4-bp exonic deletion near the 5′ end of the open reading frame (Mealey et al., 2001). The identified mutation, commonly referred to as ABCB1-1Δ or MDR1-1Δ, results in a frameshift that introduces a premature stop codon and yields a truncated, nonfunctional P-gp that lacks over 90% of the amino acid sequence found in the wild-type protein (Baars et al., 2008; Kawabata et al., 2005; Mealey et al., 2001; Roulet et al., 2003). The lack of functional P-gp in dogs with the ABCB1-1Δ mutation leads to increased blood concentrations of P-gp substrates as well as an accumulation of these substrates at the blood–brain barrier and subsequent diffusion into the central nervous system.

P-gp has the ability to bind a diverse set of molecules, as a result this protein has the potential to affect the tissue distribution and pharmacokinetics of many drugs, especially those within the avermectin family. The United States Food and Drug Administration

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Center for Veterinary Medicine (US FDA CVM) requires that all drugs be evaluated for safety as part of the drug approval process. Since avermectins are known to cause toxicity in ABCB1-1Δ dogs, studies which employ ivermectin-sensitive ABCB1-1Δ Collies as animal subjects are typically conducted to evaluate the safety of these drug candidates. These "Ivermectin Sensitive Collie Safety Studies" are becoming increasingly difficult to conduct as the availability of these Collie colonies for research purposes is decreasing. In addition, P-gp's broad substrate specificity may contribute to drug sensitivity induced by the administration of non-avermectin P-gp substrates to dogs with compromised P-gp activity. Therefore, alternative screening methods need to be developed to identify potentially toxic P-gp substrates and assess their safety for use in ABCB1-1Δ dogs.

To this end, we have developed a knock-in/knock-out mouse model expressing the mutated ABCB1-1Δ canine gene in order to phenotypically mirror the neurotoxic signs observed in ivermectin-sensitive Collies. The *in vivo* murine model was generated by targeting both murine Abcb1a and Abcb1b genes, which are the two homologous isoforms of ABCB1 present in mice (Ambudkar et al., 2008). This strategy involved the replacement of murine Abcb1a with mutated ABCB1-1Δ cDNA and the invalidation of murine Abcb1b in order to prevent expression of murine Abcb1b and Abcb1a and promote canine ABCB1-1Δ expression. The benefit of using a knock-in/knock out mouse model for P-gp substrate toxicity as opposed to Abc1a/b knock-out or P-gp deficient CF-1 mice is that the developed mouse model more accurately genotypically reflects dogs with the ABCB1-1Δ mutation. Furthermore, similar genetic engineering techniques were used to develop a knock-in/knock-out mouse line that contains wild-type canine ABCB1. In future studies, when they are available for use, these mice that express wild-type canine P-gp will be used as controls. In a previous study, we detailed the development of the ABCB1-1Δ mouse model and established correlations between the adverse clinical signs observed in canines and the signs observed in the Abcb1a knock-in/Abcb1b knock-out mice upon administration of the P-gp substrate ivermectin. Results from that study showed that a 10 mg/kg b.w. dose of ivermectin administered via subcutaneous injection was sufficient to cause marked ataxia, lethargy and tremors in the knock-in/knock-out mouse model between 1 and 7 h post administration. Throughout the study, the clinical signs of neurotoxicity observed in these mice increased in severity and their condition continued to deteriorate. In contrast, no persisting signs of neurotoxicity were observed 7 h after ivermectin administration in wild-type C57BL/6J mice (Orzechowski et al., 2012). Furthermore, the clinical signs of neurotoxicity observed in the mouse model closely paralleled those signs observed in ABCB1-1Δ Collies upon exposure to ivermectin.

In the research described here, the use of the Abcb1a knock-in/Abcb1b knock-out mouse model is evaluated further as a method for screening P-gp substrates that potentially could prove to be toxic to ABCB1-1Δ dogs. Here, we have challenged Abcb1a knock-in/Abcb1b knock-out mice with doramectin, moxidectin, digoxin and domperidone, four known P-gp substrates used in veterinary medicine for various indications. The antiparasitic agents doramectin and moxidectin, as well as the cardiac drug digoxin, have been previously documented to induce neurotoxicity in dogs with the ABCB1-1Δ mutation (Fecht and Distl, 2008). The antiemetic domperidone has not been observed to elicit clinical signs of neurotoxicity in ABCB1-1Δ dogs and was administered as a control to further substantiate that the clinical signs observed with P-gp substrate administration in the mouse model reflect clinical observations in ABCB1-1Δ dogs (Neff et al., 2004). Establishing correlations between the Abcb1a knock-in/Abcb1b knock-out mouse response and canine response to P-gp substrates will support the potential use of the mouse model in place of ivermectin-sensitive Collies in assessing the safety of avermectins. Furthermore, the knock-in/knock-out mouse model could be used to screen molecules or new drug candidates that are non-avermectin P-gp substrates but may have altered pharmacokinetic and toxicity profiles in canines carrying the ABCB1-1Δ mutation. Overall, the availability of this model has the potential to serve as a tool used by the FDA to assess the safety and improve labeling of drugs intended for canine use.

2. Materials and methods

2.1. Chemicals

All drugs were commercially available preparations. Injectable digoxin (Baxter International) and an oral formulation of doramectin (Pfizer) were used as supplied. An injectable formulation of moxidectin (Fort Dodge Animal Health) and an oral formulation of domperidone (Dechra Veterinary Products) were both diluted with propylene glycol (Sigma Aldrich) prior to administration.

2.2. Abcb1a knock-in/Abcb1b knock-out mice

The generation and characterization of Abcb1a knock-in/Abcb1b knock-out mice with a targeted ABCB1-1Δ insertion was detailed previously (Orzechowski et al., 2012). Maintenance and breeding of the mouse colony was handled by the U.S. FDA's Center for Food Safety and Applied Nutrition (CFSAN), a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC), in accordance with all relevant institutional guidelines. All mice, including wild-type C57BL/6J control mice, were housed individually in standard polycarbonate micro-isolator filtered cages supplied with TEK-Fresh cellulose bedding. Deionized water and standard certified 18% pelleted rodent diet (Harlan Laboratories) were provided for mice *ad libitum*. Animals were kept on a 12 h light/dark cycle with environmental temperatures ranging from 18 °C to 25 °C with 40–70% humidity. As part of the CFSAN animal program, sentinel mice were used to ascertain the health of study animals. Serum and fecal samples collected from sentinel mice housed in rooms with colony mice tested negative for at least 18 common infectious disease agents. Forty (40) Abcb1a knock-in/Abcb1b knock-out mice and twenty-four (24) C57BL/6J wild-type mice were used in this study. These mice ranged in age from 17 to 30 weeks old, had weights between 21.4 and 53 g, and included both genders. Previous studies had shown no correlation between time to onset or severity of clinical signs and adult mouse age or gender. All animal protocols were approved and monitored by CFSAN's Institutional Animal Care and Use Committee (IACUC).

2.3. P-gp substrate administration

Each P-gp substrate was administered individually to ten (10) Abcb1a knock-in/Abcb1b knock-out mice and six (6) C57BL/6J wild-type mice via subcutaneous injection or oral gavage. The administered dose of each P-gp substrates was based on the median lethal dose (LD_{50}) provided on the Material Safety Data Sheets (MSDSs) and was at least five times less than the LD_{50} reported for normal, non-genetically engineered mice. Body weights for mice ($n = 64$) used in this study were obtained the day prior to P-gp substrate administration. Doramectin ($n = 16$) and digoxin ($n = 16$) were administered subcutaneously. For subcutaneous injections, doses of P-gp substrate were prepared and injected into the scruff region of the neck using a 1 cc syringe fitted with a 26 gauge, 3/8″ needle. Formulations for subcutaneous injections were prepared as follows: doramectin (10 mg/mL) was diluted in propylene glycol