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CASE REPORT

Detection of heterozygous MDR1 nt230(del4) mutation in a mixed-breed dog: case report of possible doxorubicin toxicosis

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Abstract: P-glycoprotein (ABCB1), the product of the Multidrug Resistance Gene (MDR1) (ABCB1) gene, is the major multidrug transporter contributing to the barrier function of several tissues and organs, including the brain. A four base pair deletion mutation in MDR1 results in the absence of a functional form of ABCB1 and loss of its protective function. Severe intoxication with the ABCB1 substrate, such as with anticancer drugs, has been attributed to genetic lack of functional ABCB1. This mutation has been detected in more than 10 dog breeds as well as in mixed-breed dogs living in different countries. In Brazil, evaluation for this mutation is not as widely available and is rarely used by veterinarians, so drug intoxication may be underdiagnosed. This is the first report from Brazil of doxorubicin neurotoxicity in a mixed-breed dog with the MDR1 nt230(del4) mutation.

Keywords: canine, toxicology, cancer, P-glycoprotein

Introduction

The multidrug resistance (MDR) transporter P-glycoprotein (ABCB1) is the product of the MDR1 (ABCB1) gene and belongs to the family of membrane-bound ATP-binding cassette (ABC) transporters.[1] It is expressed in many tissues with secretory or excretory functions, including the liver (canalicular membrane of hepatocytes), kidney (luminal membrane of the proximal tubules), and intestine (brush border membrane of enterocytes), where it limits drug absorption from the gut and promotes drug excretion into the bile and urine. ABCB1 is also expressed at physiological barriers, where it acts as an important impediment to the distribution of substrate drugs to selected tissues and restricts passage of xenobiotics through the blood–brain barriers, the blood-testes barrier, and the placenta.[2–4]

In 2001, a mutation in the MDR1 gene was described in ivermectin-sensitive collies and identified as MDR1 nt230(del4).[5] The mutation involves a frameshift deletion mutation that generates multiple premature stop codons, resulting in a severely truncated P-glycoprotein composed of <10% of the wild-type amino acid sequence.[6] This allele probably results in a complete loss of ABCB1 function.

Due to the nature of ABCB1 as an efflux pump to protect the cell against a wide variety of substances, ABCB1 substrates vary greatly in size, structure, and function. Substrates range from small molecules, such as organic cations, amino acids, and some antibiotics, to macromolecules, such as polysaccharides and proteins.[7] These substrates include multiple hydrophobic and amphipathic drugs, many of which are commonly used in veterinary medicine.[8–14] (Table 1). Because ABCB1 has an important role in drug pharmacokinetics, related mutations that result in reduced expression lead to

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Table 1 Substrates for P-glycoprotein

Anticancer drugs
Doxorubicin
Vincristine
Vinblastine
Immunosuppressants
Ciclosporin
Antiparasitic drugs
Ivermectin
Moxidectin
Steroid hormones
Aldosterone
Cortisol
Dexamethasone
Antimicrobial agents
Tetracycline
Doxycycline
Levofloxacin
Ketoconazole
Itraconazole
Analgesics
Morphine
Methadone
Antidiarrheals
Loperamide
Anticonvulsants
Phenothiazine
Cardiac drugs
Digoxin
Diltiazem
Verapamil
Talinolol

increased oral bioavailability and reduced elimination via the liver, kidney, and intestines.[15,16] However, the most dramatic clinical consequences of impaired ABCB1 function are the neurotoxic effects described since 1983 in cases of ivermectin toxicosis in collies.[17,18] Clinical signs of neurotoxicity include mydriasis, stupor, lethargy, vomiting, ataxia, tremors, ptyalism, coma, blindness, convulsion, and death.[19–21] Little is known about the effects of these substances in dogs that are heterozygous for the mutation, but the recommendation has been made that heterozygous dogs should be treated with caution when using drugs that are known ABCB1 substrates (http://www.vetmed.wsu.edu/depts-vcpl/drugs.aspx).

Case report

In 2011, a blood sample from an eight-year-old female mixed-breed dog was sent to our laboratory for MDR1 mutation testing. The animal had been diagnosed with lymphoma two months previously and was started on a chemotherapeutic protocol containing cyclophosphamide, doxorubicin, vincristine, and prednisolone (Table 2). During the first administration of doxorubicin (30 mg/m^3 by slow intravenous push) the patient showed unusual clinical signs, including salivation, tachypnea (60 breaths per minute), tachycardia (160 beats per minute), depression, and ataxia, but these signs resolved within 24 hours. The attending veterinarian believed that these clinical signs were related to advanced disease and elected to proceed with the planned protocol. However, the clinical signs recurred with increased intensity four weeks later following the next dose of doxorubicin. This time, the dog suffered from loss of consciousness, tachycardia, tremors, and convulsions. Under these circumstances, the veterinarian administered anticonvulsive doses of rectal diazepam, but the dog failed to respond to it but did respond when he administered phenobarbital (6 mg/kg IM). Following administration of phenobarbital, the convulsions ceased and the dog regained consciousness. On physical examination, the dog was febrile (39.8°C) and weak. Hematological analysis (complete blood count, white cell count), urinalysis, serum biochemical analysis, and ultrasonography were performed and the only abnormality present was thrombocytopenia

Table 2 Chemotherapeutic treatments and clinical responses in an eight-year old, female, mixed-breed dog with lymphoma and the MDR1 deletion mutation (heterozygous genotype) associated with ivermectin sensitivity

Treatment week	Drugs administered	Toxic effects
1	Vincristine (0.5 mg/m^2 IV) L-asparaginase (400 UI/kg SC) Prednisone (2 mg/kg PO, q 24 h)	Thrombocytopenia (140,000 platelets/μL)
2	Cyclophosphamide (200 mg/m^2 IV) Prednisone (1.5 mg/kg PO, q 24 h)	Polyuria, polydipsia, loss of weight
3	Vincristine (0.5 mg/m^2 IV) Prednisone (1 mg/kg PO, q 24 h)	None
4	Doxorubicin (30 mg/m^2 IV) Prednisone (0.5 mg/kg PO, q 24 h)	Salivation, tachypnea (60 breaths/min), tachycardia (160 bpm), depression and ataxia
5	None	None
6	Vincristine (0.5 mg/m^2 IV)	Vomiting, loss of appetite
7	Cyclophosphamide (200 mg/m^2 IV)	Neutropenia (2,500 cells/μL)
8	Vincristine (0.5 mg/m^2 IV)	Vomiting
9	Doxorubicin (30 mg/m^2 IV) Phenobarbital (6 mg/kg IM) Diazepam (0.5 mg/kg)	Loss of consciousness, tachycardia (180 bpm), tremors and convulsions, fever (39.8°C), thrombocytopenia (78,000 platelets/μL)
10	Stopped treatment	None

Abbreviations: MDR, multidrug resistance gene; PO, per oral; SC, subcutaneous; IM, intramuscular; IV, intraavenous.

(78,000 platelets/μL). The veterinarian discontinued chemotherapy and although the dog did not have the collie phenotype, he suspected efflux pump dysfunction when the owner reported similar neurological clinical signs following administration of moxidectin.

To perform the diagnostic test, we used an allele-specific-based screening method. Briefly, DNA was extracted directly from a 330 μL blood sample using the Illustra Blood Genomic Prep Mini Spin Kit (GE Healthcare, Chalfont, UK) and following the manufacturer's recommended protocol. Approximately 100 ng of DNA was used for PCR amplification. Allele-specific PCR was performed as described elsewhere, using positive and negative controls[22] and with primers ABCB1A, ABCB1B, ABCB1C, and ABCB1D. The primers were designed to allow detection of the wild-type MDR1 allele by PCR1 (ABCB1A, ABCB1B, and ABCB1D) and detection of the mutant MDR1 allele by PCR2 (ABCB1A, ABCB1C, and ABCB1D). An amplicon with 326 base pairs is generated only if ABCB1B matches the wild-type allele in PCR1 or if ABCB1C matches the mutant allele in PCR2. Independent of the presence or absence of the four base pair deletion, an additional control amplicon with approximately 480 base pairs was obtained with the ABCB1A and ABCB1D primer pair in both reactions (Figure 1). Sequencing analysis was used to confirm heterozygosity in this case.

Although the effect of the heterozygous condition in the dog has not been evaluated, the clinical recommendation has been made to exercise caution when administering drugs like doxorubicin to dogs heterozygous for the MDR1 mutation.[8] Studies in other species have demonstrated that P-glycoprotein function is intermediate in heterozygotes. Flow cytometric rhodamine efflux studies[24] in peripheral blood mononuclear cells in vitro and tacrolimus pharmacokinetic studies in renal transplant patients[25] have shown

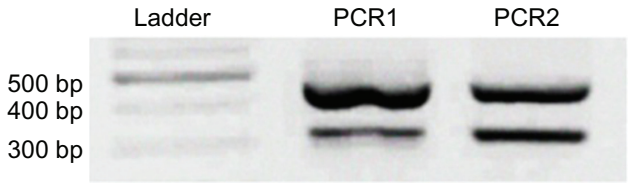

Figure 1 Results of allele-specific PCR testing in an eight-year old, female, mixed-breed dog with heterozygous MDR1 genotype. PCR1 refers to wild type allele detection and PCR2 refers to MDR1 mutant allele detection. An amplicon with 326 bp is generated only if ABCB1B matches the wild type allele in PCR1 or if ABCB1C matches the mutant allele in PCR2. Independent of the presence or absence of the 4 bp deletion, an additional control amplicon with ≈ 480 bp was obtained with primer pair ABCB1A and ABCB1D in both reactions.
Abbreviations: bp, blood pressure; PCR, polymerase chain reaction; MDR, multidrug resistance gene.

that MDR1 mutation heterozygotes have intermediately functional P-glycoprotein. The recommendation for heterozygous dogs receiving doxorubicin is a 25% reduction in dose (http://www.vetmed.wsu.edu/depts-vcpl/drugs.aspx). While it would be expected that a dog with reduced P-glycoprotein expression would have heightened susceptibility to commonly seen drug side effects, such as myelosuppression and adverse gastrointestinal reactions, it is possible that doxorubicin might cause acute neurotoxicity because it has been shown to be neurotoxic when the blood–brain barrier is breached in dogs.[26]

The MDR1 nt230(del4) mutation is an inherited trait and genetic screening is important both for gene pools and for individual patients.[27,28] Recent genetic screening studies performed in several dog breeds identified the mutation not only in collies, but also in more than 10 dog breeds as well as mixed-breed dogs.[29–32] After identification of the MDR1 nt230(del4), several molecular techniques were developed for use as diagnostic screening tools and have been successfully used in at-risk breeds prior to application of therapeutic protocols involving known ABCB1 substrates. Unfortunately, in Brazil, the screening methods have only recently been made commercially available and are underutilized, so it is likely that many historical cases of intoxication have gone unrecognized. In this case, the patient had unusual signs following two administrations of doxorubicin, leading the owner to report similar signs following administration of moxidectin. Moxidectin is a drug known to produce neurotoxicity in dogs deficient in ABCB1 activity. This case illustrates the potential for heightened susceptibility to toxicity in dogs with heterozygous MDR1 mutation and demonstrates the need for further pharmacogenetic investigation in populations and also in individual animals.

Conclusion

This is the first case report from Brazil of a dog suffering from drug intoxication, potentially as the result of a dysfunctional efflux pump. It is of particular interest that this dog was not a collie-related breed and was found to be heterozygous for the mutation, so it is quite possible that dogs displaying unusual neurotoxicity following administration of a known ABCB1 substrate drug may in fact carry the mutation. Genetic screening of dogs for this mutation prior to the administration of known substrates will allow dose reduction or alternative treatment choices to be made and will significantly reduce morbidity in affected animals.

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Disclosure

None of the authors have any financial or personal relationship that could inappropriately influence or bias the content of the paper.

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