Frequency of the mutant MDR1 allele associated with ivermectin sensitivity in a sample population of Collies from the northwestern United States

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Objective—To determine the frequency of the MDR1 gene mutation (polymorphism) associated with ivermectin sensitivity in a sample population of Collies in Washington and Idaho.

Animals—40 healthy client-owned Collies.

Procedure—A blood sample (8 ml) was collected from each dog and used for RNA extraction. Reverse transcriptase was used to generate MDR1 cDNA. Polymerase chain reaction (PCR) primers were designed to amplify a 1,061-base pair region of the MDR1 gene. The PCR products were sequenced to determine whether the Collies had 0, 1, or 2 mutant alleles. Pedigrees of some dogs were available for analysis to determine relatedness of affected dogs.

Results—Of the 40 Collies, 9 (22%) were homozygous for the normal allele (normal), 17 (42%) were heterozygous (carrier), and 14 (35%) were homozygous for the mutant allele (affected). Pedigree analysis revealed that some, but not all, affected dogs were related to each other within the 4 most recent generations.

Conclusions and Clinical Relevance—A high percentage of a sample population of Collies in Washington and Idaho are affected or carriers of the mutant MDR1 allele associated with ivermectin sensitivity. A similar frequency of this mutation may be detected in dogs from other geographic areas. Pharmacologic treatment with ivermectin, loperamide, vincristine, and other drugs that are substrates of P-glycoprotein include loperamide, digoxin, the antiemetic ondansetron, many chemotherapeutic drugs including vincristine, vinblastine, and doxorubicin, and a number of other drugs.15 Collies with 2 mutant alleles have the ivermectin-sensitive phenotype and are believed to be susceptible to neurotoxic effects caused by other P-glycoprotein substrates. The objective of the study reported here was to determine the frequency of MDR1 polymorphism in a sample population of Collies in Washington and Idaho.

Materials and Methods

Animals—Forty healthy client-owned Collies were included in the study. Owner consent was obtained, and the study was approved by our institutional animal care and use committee. Rough- and smooth-coated Collies were represented. Owners who were interested in determining the MDR1 genotype of their Collies allowed their dogs to be included in the sample population. Owners were solicited to enroll their Collies in the study primarily through announcements made at meetings of the Inland Empire Collie Club and word-of-mouth advertising. A pedigree (representing the 4 most recent generations) was available for 8 dogs.

Collection and extraction of RNA—A venous blood sample (8 ml) was collected from each dog and used for extraction of RNA. Leukocytes were prepared by use of density-gradient centrifugation. Total RNA was extracted from the leukocytes, using a commercially available reagent.

Reverse-transcriptase polymerase chain reaction (PCR) and genomic sequencing—For reverse-transcriptase reactions, a PCR amplification kit was used with oligo(dT) primers. The cDNA was then amplified by use of primers described elsewhere in separate PCR reactions, using DNA polymerase with 2.5 mM MgCl₂. The PCR consisted of 36 cycles with denaturing, annealing, and extension conditions.

Ivermectin-induced toxicosis in Collies was first described in 1983.1,2 Ivermectin causes neurologic toxicosis in some, but not all, Collies at doses that are 0.005 times the dose required to cause toxicosis in other dogs.3,4 Despite numerous investigations,3-6 the cause of this toxicosis in dogs. In that study, many Collies had signs of neurologic toxicosis after administration of routinely recommended doses of the anti-diarrheal agent loperamide. It was postulated that the cause of ivermectin sensitivity in Collies was related to the apparent breed susceptibility to loperamide-induced neurotoxicosis.

A mutation in the MDR1 gene has been described in ivermectin-sensitive Collies.7 The mutation involves a frameshift deletion mutation that generates a premature stop codon, which prevents synthesis of the complete protein product. The product of the MDR1 gene, P-glycoprotein, is an important component of the blood-brain barrier8-11 and is an ATP-dependent drug transporter that is expressed in the luminal membrane of capillary endothelial cells in the brain. In that location, it functions in a protective capacity to transport a number of substrates, including ivermectin, from brain tissue back into the capillary lumen. Other substrates for P-glycoprotein include loperamide, digoxin, the antiemetic ondansetron, many chemotherapeutic drugs including vincristine, vinblastine, and doxorubicin, and a number of other drugs.13 Collies with 2 mutant alleles have the ivermectin-sensitive phenotype and are believed to be susceptible to neurotoxic effects caused by other P-glycoprotein substrates. The objective of the study reported here was to determine the frequency of MDR1 polymorphism in a sample population of Collies in Washington and Idaho.
of 95°C (10 seconds), 64°C (15 seconds), and 72°C (60 seconds) in a thermocycler. The PCR products were resolved by electrophoresis in 1% agarose gels that contained ethidium bromide. Expected size of the MDR1 band was 1,061 base pairs. The PCR products were purified, using a commercially available kit, and sequenced by personnel at a commercial laboratory using dye-terminator chemistry and an automated DNA sequencer. Sequences from dogs in this study were compared with the canine MDR1 sequence (GenBank AF 045016).

Results

The deletion mutation associated with ivermectin sensitivity in Collies was detected in a large number of dogs in the study. Of the 40 dogs, 9 (22%) were homozygous for the normal (wild-type) MDR1 sequence and were classified as normal, 14 (35%) were homozygous for the mutant allele and classified as affected, and 17 (42%) were heterozygous and classified as carriers (Fig 1).

Analysis of the 8 pedigrees was performed and revealed that 4 of the dogs in the study were closely related. For 1 pair of siblings, test results indicated that 1 of the dogs was affected (homozygous mutant), whereas the other was heterozygous (1 normal allele and 1 mutant allele). For another closely related pair of dogs (a dam and her female offspring), test results indicated that the dam was heterozygous, but her female offspring was affected (ie, homozygous for the mutant allele). There were 2 affected dogs that were not related to other dogs in the study within the 4 most recent generations. Furthermore, the 8 dogs reported here were unrelated (within the 4 most recent generations) to a sample population of Collies from Michigan that were included in another study. In that study, all of the dogs from Michigan were heterozygous or homozygous for the described MDR1 mutation.

Discussion

Ivermectin sensitivity in Collies has been associated with homozygous expression of a deletion mutation of the MDR1 gene. The product of the MDR1 gene, P-glycoprotein, is an integral component of the blood-brain barrier. At the blood-brain barrier, P-glycoprotein actively extrudes drugs from brain tissue back into capillaries, resulting in lower concentrations of drugs in brain tissues that are substrates for P-glycoprotein. In MDR1 knockout mice, lack of P-glycoprotein leads to abnormally increased accumulation of certain drugs in the brain with resultant undesired adverse neurologic effects. In ivermectin-sensitive Collies, this mutation consists of a 4-base pair deletion that generates a premature stop codon, resulting in a severely truncated and nonfunctional protein product.

Other investigators have estimated that approximately 30 to 40% of Collies are sensitive to ivermectin. Our study yielded similar results. In our study population, the frequency of the homozygous mutant genotype was 14 of 40 (35%). Interestingly, in a separate sample population of Collies from Michigan, all of those dogs had at least 1 mutant allele. Analysis of the pedigrees that were available (8/40 dogs of this study and 13/15 dogs from Michigan) indicated that none of the dogs from Washington or Idaho in our study were related to the dogs from Michigan within the 4 most recent generations. Collectively, these results suggest that the MDR1 mutation associated with ivermectin sensitivity is widely dispersed in Collies.

Sporadic descriptions of ivermectin sensitivity have been reported in a few other breeds including Shetland Sheepdogs, Australian Shepherds, and Old English Sheepdogs. Whether these breeds have the same MDR1 mutation that has been reported in Collies is unknown. In people, several MDR1 mutations have been described; therefore, it is reasonable to assume that other breeds of dogs may not have the same MDR1 genotype as Collies.
Determination of the MDR1 genotype of Collies is clinically important for several reasons. First, ivermectin is only 1 of several clinically relevant substrates for P-glycoprotein that can cause neurotoxicosis. Loperamide, available as an over-the-counter antidiarrheal agent, causes neurotoxicosis in Collies at doses routinely used in other breeds. Similar to ivermectin, loperamide is generally excluded from entering brain tissue in high concentrations as a result of the actions of P-glycoprotein. In affected Collies, loperamide achieves high concentrations in brain tissue, causing neurologic toxicosis. In support of this fact, 1 of the Collies in our study was treated with a recommended dose of loperamide as a puppy and developed severe (nearly fatal) neurologic toxicosis. That dog was homozygous for the mutant allele. Other drugs that are substrates for P-glycoprotein and that can cause neurotoxicosis in affected Collies include vincristine, vinblastine, ondansetron, and potentially, moxidectin. These drugs should not be used in Collies homozygous for the mutant MDR1 allele.

Other nonneurologic implications exist for Collies with the MDR1 mutation described. For example, P-glycoprotein also is normally expressed at the luminal border of the intestinal tract, where it functions as an antiabsorption mechanism for a number of drugs, including digoxin, cyclosporin A, dexamethasone, and antiviral drugs. In affected Collies, oral bioavailability of these drugs is likely to be greater than in unaffected dogs. This would result in higher plasma concentrations and a higher likelihood of adverse drug reactions in affected Collies.

It is likely that a high percentage of Collies brought to veterinarians for treatment are affected by the MDR1 mutation described here. It is important that veterinarians consider this factor when selecting pharmacologic treatments for Collies. Furthermore, an adverse drug reaction involving neurologic toxicosis should be considered for Collies that have abnormal CNS signs.

References