The Effect of the Canine \textit{ABCB1}-1Δ Mutation on Sedation after Intravenous Administration of Acepromazine

D. Deshpande, K.E. Hill, K.L. Mealey, J.P. Chambers, and M.A. Gieseg

\textbf{Background:} Dog breeds with the \textit{ABCB1}-1Δ mutation have substantially truncated nonfunctional P-glycoprotein. Dogs homozygous for this mutation (mut/mut) are susceptible to the toxic adverse effects of ivermectin, loperamide, and vincristine. Anecdotal reports suggested \textit{ABCB1} mut/mut dogs showed increased depth and duration of acepromazine sedation. Hypothesis/Objectives: That \textit{ABCB1} mut/mut dogs have increased depth and duration of sedation after acepromazine IV compared to normal dogs (nor/nor).

\textbf{Animals:} Twenty-nine rough-coated collies were divided into 3 groups of dogs based on their \textit{ABCB1} genotype: 10 mut/mut, 10 mut/nor, and 9 nor/nor.

\textbf{Methods:} Dogs were given 0.04 mg/kg of acepromazine IV. Level of sedation, heart rate, respiratory rate, and blood pressure were recorded for 6 hours after acepromazine administration. Area under the curves (AUCs) of the normalized sedation score results were calculated and compared.

\textbf{Results:} The median sedation scores for \textit{ABCB1} mut/mut dogs were higher than nor/nor dogs at all time points and were higher in mut/nor dogs for the first 2 hours. These differences were not found to be significant for any individual time point (P > .05). The median sedation score AUC for mut/mut dogs was significantly higher than nor/nor dogs (P = .028), but the AUC for mut/nor dogs was not (P = .45). There were no significant differences between groups for heart rate, respiratory rate, and blood pressure (P > .05).

\textbf{Conclusions and Clinical Importance:} In \textit{ABCB1} mut/mut dogs acepromazine dose rates should be reduced and careful monitoring performed during sedation.

\textbf{Key words:} Collie; MDR1; P-glycoprotein.

The \textit{ABCB1} gene (previously known as the \textit{MDR1} gene) codes for P-glycoprotein (P-gp), a transmembrane ATPase that transports small molecules from inside to outside of the cell membrane in an energy dependent process.\textsuperscript{1} P-gp is also a major component of the blood barrier influencing the exposure of the central nervous system to certain drugs.\textsuperscript{2} A 4-bp mutation (\textit{ABCB1}-1Δ) has been identified in dogs that results in premature stop codons and a nonfunctional P-gp.\textsuperscript{3,4} Dogs that are homozygous for this mutation (\textit{ABCB1} mut/mut) have no functional P-gp resulting in an increased sensitivity to a variety of xenobiotics including ivermectin, vincristine, doxorubicin, loperamide, and others.\textsuperscript{5–10} Dogs that are heterozygotes have shown increased sensitivity to some of these drugs (vincristine, ivermectin) compared to \textit{ABCB1} nor/nor dogs. In particular, the increased sensitivity to ivermectin in \textit{ABCB1} mut/mut dogs is thought to result from a build-up of the drug in the central nervous system. This increased concentration causes an ivermectin toxicosis that can lead to coma and death at doses that would be safe in an \textit{ABCB1} nor/nor dog.\textsuperscript{11}

The \textit{ABCB1}-1Δ mutation is thought to have originated in an ancestral herding dog during the 19th century in England before breed standards were introduced.\textsuperscript{12} Collies show the highest frequency of the mutation with approximately 75% of dogs from this breed carrying at least 1 copy. Other breeds that also commonly carry the mutation include the Australian shepherd, longhaired whippet, McNab, silken windhound, Shetland sheepdog, English sheepdog, and old English sheepdog.\textsuperscript{12}

Many drugs are transported by P-gp and the exact structure–function relationship between substrates has been hard to fully identify.\textsuperscript{13} Several drugs that are commonly used in veterinary sedation and analgesia of dogs are thought to be transported by P-gp including acepromazine, morphine, butorphanol, and fentanyl.\textsuperscript{1} Routinely used sedatives and analgesics have not been investigated to determine if their actions are potentiated in dogs with the \textit{ABCB1}-1Δ mutation. However, there are anecdotal reports in the literature that dogs that are...
homzygous for the \( ABCB1-1\Delta \) mutation show increased sensitivity to these drugs.\(^{11}\)

The aim of the current study was to determine if dogs that were homozygous for the \( ABCB1-1\Delta \) mutation (\( ABCB1 \) mut/mut) would show increased and prolonged sedation after administration of acepromazine compared to heterozygous mutants (\( ABCB1 \) nor/mut) and homozygous normal (\( ABCB1 \) nor/nor) dogs. Our null hypothesis was the dogs that were \( ABCB1 \) mut/mut would not show greater sedation than dogs with nor/nor or nor/mut genotypes.

**Materials and Methods**

**Animals**

All dogs enrolled in the study were client-owned animals that were returned to their owners at the completion of the study. Owners gave signed, informed consent for study enrollment and animal ethics approval for the study was obtained from the Massey University Animal Ethics Committee (MUAEC No. 12/63).

**Genotyping**

The \( ABCB1 \) genotyping was performed by obtaining buccal samples from 31 rough-coated collies from DNA testing. Genotyping was performed by the Veterinary Clinical Pharmacology Laboratory at Washington State University, USA (www.vcpl.vetmed.wsu.edu).

**Physical Exam and Blood Testing**

One week before sedation scoring, each dog was brought into the clinic and a physical examination performed that included heart and lung auscultation, temperature, pulse rate, abdominal palpation and mucus membranes evaluation. Blood and urine samples were also collected at this time. Complete blood count (CBC) and serum chemistry panels were performed on the blood samples (New Zealand Veterinary Pathology Limited). A urinary test strip (Combur-Test Strips; Roche, Auckland, New Zealand) and refractometer (Leica 10436 Veterinary Refractometer) were used to perform an in-house urinary analysis. Dogs were included in the study if they were within 1–10 years of age and the physical examination was considered normal and the CBC, serum chemistry and urinalysis results were within established reference ranges.

**Sedation Testing**

Dogs were sedation scored in groups of no more than 4 dogs per day. All dogs were brought into the Massey University Veterinary Teaching Hospital early in the morning and moved to a quiet consultation room for the duration of the study. Dogs were fasted overnight but were given free access to water throughout the study and were monitored constantly. The level of sedation was scored at 0, 0.5, 1, and 2 hours after IV administration of acepromazine. After IV dosing dog sedation was evaluated at 30, 60, and 90 minutes and 2, 2.5, 3, 4, and 6 hours. Heart rate and respiratory rate were also measured by palpation and by visual observation. Blood pressure (systolic, mean, and diastolic) was measured with a noninvasive oscillometric blood pressure monitor (SurgiVet V9000). One minute after the 2-hour sedation score was performed all dogs received 0.04 mg/kg acepromazine via slow intravenous injection into the cephalic vein. The 2-hour sedation score was considered the baseline.

Sedation scoring was carried out by the same person (DD) who was blinded to the genotype results of the dogs. The sedation score was based upon a previously published sedation scoring system with small modifications.\(^{14}\) This provided a detailed scoring system that assessed several behavioral variables. Using this scoring system, normal alert dogs would score approximately 0, excited dogs would have negative scores, and dogs showing increasing levels of sedation would have positive scores. The sedation score recorded 7 variables (Table 1). For variable 4—response to restraint, dogs were assessed while having heart rate, respiratory rate and blood pressure taken. For variable 5—response to noise, a handclap was used to generate the noise. For variable 6—gait, this was assessed.

| Observation | Description | Score |
|-------------|-------------|-------|
| 1. Vocalization | Quiet | 0 |
| | Whining softly but quiets with soothing touch | -1 |
| | Whining continuously | -2 |
| | Barking continuously | -3 |
| 2. Appearance | Eyes sunken, glazed, or unfocused; ventromedial rotation | 3 |
| | Eyes glazed but follow movement | 2 |
| | Protrusion of nictitating membrane; normal visual responses | 1 |
| | Normal appearance | 0 |
| | Pupils dilated; abnormal facial expression | -1 |
| 3. Interactive behavior | Recumbent; no response to voice or touch | 3 |
| | Recumbent; lifts head in response to voice or touch | 2 |
| | Recumbent but stands in response to voice or touch | 1 |
| | Moves toward/away from voice or touch; appears anxious | 0 |
| | Wags tail/excited/growls or hisses when approached or touched | -1 |
| | Very excited/jumps/bites or swats when approached | -2 |
| 4. Response to restraint | Lies on floor, no restraint required | 2 |
| | Lies on floor with light restraint of head or neck | 1 |
| | Sits up on floor; with light restraint | 0 |
| | Requires regular correction | -1 |
| | Struggles continuously against restraint | -2 |
| 5. Response to noise | No response to a hand clap | 3 |
| | Minimal response to a hand clap | 2 |
| | Slow or moderate response to a hand clap | 1 |
| | Brisk response to a hand clap; raises head with eyes open | 0 |
| 6. Gait | Unable to get up | 5 |
| | Sits up but unable to stand, falls when attempting to stand | 4 |
| | Gait very unsteady, standing but ataxic | 3 |
| | Gait clearly unsteady | 2 |
| | Mild aberrations in gait, wobbly | 1 |
| | Walks normally | 0 |
| 7. Posture | Lateral recumbence | 3 |
| | Sternal recumbence | 2 |
| | Sitting or ataxic while standing | 1 |
| | Standing | 0 |
| | Moving continuously | -1 |
by walking the dog on a leash in the same room. Variable 7—posture, was assessed 1 minute after assessing the gait.

**Statistical Analysis**

The sedation score at each time point was calculated from the summed total of the 7 variables that make up the sedation scale (Table 1). Sedation scores for each time point during the 2-hour acclimation period were compared for significant differences with the Kruskal–Wallis rank sum test. For each dog the sediment score at 2 hours (just before acepromazine dosing) was used as the dog’s baseline sediment score. For the statistical analysis this baseline sediment score was subtracted from all the sediment scores post-acepromazine for each dog, creating a normalized sediment score. Normalized sediment scores for each time point post-acepromazine were compared for significant differences with the Kruskal–Wallis rank sum test. For each dog the area under the curve (AUC) over the 6 hours was calculated from the normalized rank sum test. For each dog the sediment score was subtracted from all the sediment scores post-baseline sediment score. For the statistical analysis this baseline adjustment was done for each genotype group during the 2-hour acclimation period.

**Genotype Results**

A total of 31 rough-coated collies were enrolled in this study and had their ABCB1 genotypes determined. From these 31 dogs, 2 dogs were excluded from sedation. One dog had increased serum BUN, and creatinine concentrations, and lower urine specific gravity suggestive of renal disease. A second dog was excluded as it was greater than 10 years of age when it was available for sedation scoring. Of the remaining 29 dogs there was an almost even mix of the 3 genotypes, with age, weight, and sex distributions similar across the 3 genotypes (Table 2). Several of the dogs were closely related to each other as either siblings or parents and offspring.

**Sedation Trial Results**

The median dog sediment scores (range) of the 3 ABCB1-1Δ genotypes over the acclimation period 2 hours before IV acepromazine dosing. No significant differences were found at any time point between groups ($P > .05$).

| Time (hours) | nor/nor | mut/nor | mut/mut |
|-------------|---------|---------|---------|
| 0           | 0 (–5, 5) | 0 (–3, 0) | 0 (–4, 2) |
| 0.5         | 0.5 (–1.1) | 0 (–1, 4) | 0 (–1, 3) |
| 1           | 1 (1.3) | 1 (–2, 4) | 0.5 (–1, 4) |
| 2           | 2 (0.5) | 1.5 (–2, 4) | 1 (–1, 4) |

*No statistical significant differences were identified between groups ($P > .05$).*

Table 3. The median dog sediment scores (range) of the 3 ABCB1-1Δ genotypes over the acclimation period 2 hours before IV acepromazine dosing. No significant differences were found at any time point between groups ($P > .05$).
likely to be the mechanism responsible for increased acepromazine sedation seen in this study. It is reasonable to assume that this will still be the case in dogs dosed intramuscularly. This suggests that for *ABCB1* mut/mut dogs the doses of acepromazine should be reduced and dogs should be monitored carefully, regardless of the route of administration.

We found that the *ABCB1* mut/nor dogs did not show statistically significant increased sedation compared to the *ABCB1* nor/nor animals. This is in agreement with sensitivity to macrocyclic lactones such as ivermectin where heterozygous carriers of the *ABCB1* mutation do not show an increased sensitivity compared to homozygous normal dogs at normal doses. However, it is known that *ABCB1* mut/nor dogs do show some increased toxicity to ivermectin for doses greater than 120 μg/kg especially if they are administered over multiple days. Furthermore the *ABCB1*-1Δ mutation can predict the hematologic toxicity of vincristine in both mut/mut and mut/nor dogs. It is unknown if higher doses of acepromazine would have resulted in increased sedation in the *ABCB1* mut/nor dogs and our study does not rule this out. Increased sedation may have been found to be statistically significant in the mut/nor groups if more dogs were included in the study.

Therefore, caution with acepromazine doses would be prudent, even in dogs that are heterozygous for the *ABCB1* mutation. Acepromazine is considered a relatively “safe” sedative, whereas the level of sedation seen in an individual *ABCB1* mut/mut dog is likely to be unpredictable because of differences in drug disposition between individuals. We were careful to ensure that the dogs included in this study were apparently healthy with no detected abnormalities in liver or kidney function. Any metabolic disturbance of these organs in an *ABCB1* mut/mut dog could result in prolonged metabolism and delayed excretion with the potential for an even greater sedation level. There is no published literature on acepromazine metabolism in dogs, but in horses acepromazine is metabolized by the liver and is excreted as conjugated and unconjugated metabolites in the urine. It would be advisable to perform a CBC, serum chemistry, and urinary analysis on dogs that carry *ABCB1*-1Δ mutation before sedation or anesthesia. Dogs that are very old or very young may also require particular care caused by geriatric-associated diseases and organ immaturity, respectively.

Acepromazine is not the only drug used for sedation in dogs that could potentially be transported by P-gp. Various opioids such as morphine, methadone, butorphanol, pethidine, and fentanyl are thought to be substrates for P-gp as shown by a variety of *in vitro* and *in vivo* assays. These opiates are known to cause sedation in dogs and are frequently used in combinations with acepromazine. These drugs have not been
and relaxes to a new environment. To mini-
incorporation of these combinations is very likely to pose an increased safety risk in dogs that are AAAABCBI mut/mut. These drugs may result in an increased risk. Without thoughtful dose rate selection and careful monitoring during all stages of sedation or anesthesia, these combinations are very likely to pose an increased safety risk in dogs that are ABCBI mut/mut.

There are several limitations to this study. Firstly, the number of dogs enrolled in the study was small, although each group was similar in their age and sex distributions. Obtaining client-owned dogs for studies such as these is challenging, as owners are naturally cautious to commit their animals to unnecessary sedation. However, we did find owners and breeders were interested in working with us to increase the knowledge and improve the safety of sedation and analgesia in their dogs. This work is important to improve the safety of drug treatment in dogs carrying the ABCBI-1A mutation, whereas in the long term it would be possible for breeders to selectively breed out, or at least reduce the frequency, of the mutation by careful selection of sires for mating.

The second limitation of this study is the lack of a saline control group along with the acepromazine treatment. A control group was not included for practical reasons as owners were reluctant to commit their dogs to 2 treatments on 2 separate days. It is clear that a saline control is important for sedation studies to help avoid a type II error, but is frequently not included in many studies. To minimize the effects of the absence of a saline control we acclimated the dogs to the room used for sedation scoring for 2 hours before administration of acepromazine. The sedation score at 2 hours was then used as a baseline to measure any subsequent changes in sedation after acepromazine injection. Also, dogs that were ABCBI nor/nor acted as suitable control to the ABCBI mut/mut group.

We are also limited in drawing conclusions from these studies to rough-coated collies only. Although it is reasonable to assume that other breeds that are ABCBI mut/mut would sedate to a greater extent than homozygous normal dogs, it is not certain. Other genotypes specific to other breeds may play a role attributable to unknown interactions. More studies in dogs carrying the ABCBI-1A mutation are required to evaluate commonly used sedatives and analgesics, both individually and in combination. However, these studies are challenging to perform, require large numbers of dogs, and rely on the goodwill of owners to contribute their animals. Until more studies are completed care with drug doses and limiting sedative–analgesic combinations when possible are sensible steps to reduce the risk complications in these dogs during procedures requiring sedation or anesthesia.

Acknowledgments

This work was supported by a CAS/CAHF Project Grant, a grant from the Post-graduate Research Fund and the McGeorge Research Fund. We acknowledge the invaluable work of Raewyn Mullen for helping us find collie owners and encouraging them to participate in our study.

Conflict of Interest Declaration: Dr. Kate Hill is the Associate Editor of the Journal of Veterinary Internal Medicine.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

References

1. Martinez M, Modric S, Sharkey M, et al. The pharmacogenomics of P-glycoprotein and its role in veterinary medicine. J Vet Pharmacol Ther 2008;31:285–300.
2. Schinkel AH, Wagenaar E, Mol CA, et al. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. J Clin Invest 1996;97:2517–2524.
3. Mealey KL, Bentjen SA, Gay JM, et al. Ivermectin sensitivity in collies is associated with a deletion mutation of the mdr1 gene. Pharmacogenetics 2001;11:727–733.
4. Roulet A, Puel O, Gesta S, et al. MDR1-deficient genotype in Collie dogs hypersensitive to the P-glycoprotein substrate ivermectin. Eur J Pharmacol 2003;460:85–91.
5. Nelson OL, Carsten E, Bentjen SA, et al. Ivermectin toxicity in an Australian Shepherd dog with the MDR1 mutation associated with ivermectin sensitivity in Collies. J Vet Intern Med 2003;17:354–356.
6. Krugman L, Bryan JN, Mealey KL, et al. Vincristine-induced central neurotoxicity in a collie homozygous for the ABCBI mutation. J Small Anim Pract 2012;53:185–187.
7. Mealey KL, Northrup NC, Bentjen SA. Increased toxicity of P-glycoprotein-substrate chemotherapeutic agents in a dog with the MDR1 deletion mutation associated with ivermectin sensitivity. J Am Vet Med Assoc 2003;223:1453–1455, 1434.

8. Sartor LL, Bentjen SA, Trepanier L, et al. Loperamide toxicity in a collie with the MDR1 mutation associated with ivermectin sensitivity. J Vet Intern Med 2004;18:117–118.

9. Mealey KL, Gay JM, Martin LG, et al. Comparison of the hypothalamic-pituitary-adrenal axis in MDR1-1delta and MDR1 wildtype dogs. J Vet Emerg Crit Care 2007;17:61–66.

10. Henik RA, Kellum HB, Bentjen SA, et al. Digoxin and mexiletine sensitivity in a collie with the MDR1 mutation. J Vet Intern Med 2006;20:415–417.

11. Mealey KL. Adverse drug reactions in herding-breed dogs: the role of P-gp. Compendium 2006;28:23–33.

12. Neff MW. Breed distribution and history of canine mdr1-1, a pharmacogenetic mutation that marks the emergence of breeds from the collie lineage. Proc Natl Acad Sci USA 2004;101:11725–11730.

13. Sarkadi B, Homolya L, Szakacs G, et al. Human multidrug resistance ABCB and ABCG transporters: participation in a chemoimmunity defense system. Physiol Rev 2006;86:1179–1236.

14. Smith LJ, Yu JK, Bjorling DE, et al. Effects of hydromorphone or oxymorphone, with or without acepromazine, on pre-anesthetic sedation, physiologic values, and histamine release in dogs. J Am Vet Med Assoc 2001;218:1101–1105.

15. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org/

16. Mealey KL, Greene S, Bagley R, et al. P-glycoprotein contributes to the blood-brain, but not blood-cerebrospinal fluid, barrier in a spontaneous canine P-glycoprotein knockout model. Drug Metab Dispos 2008;36:1073–1079.

17. Mealey KL. Canine ABCB1 and macrocyclic lactones: heartworm prevention and pharmacogenetics. Vet Parasitol 2008;158:215–222.

18. Mealey KL, Fidel J, Gay JM, et al. ABCB1-1Delta polymorphism can predict hematologic toxicity in dogs treated with vincristine. J Vet Intern Med 2008;22:996–1000.

19. Dewey EA, Maylin GA, Ebel JG, et al. The metabolism of promazine and acetylpromazine in the horse. Drug Metab Dispos 1981;9:30–36.

20. Thompson SJ, Koszdzin K, Bernards CM. Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. Anesthesiology 2000;92:1392–1399.

21. Wandel C, Kim R, Wood M, et al. Interaction of morphine, fentanyl, sufentanil, alfentanil, and loperamide with the efflux drug transporter P-glycoprotein. Anesthesiology 2002;96:913–920.

22. Callaghan R, Riordan JR. Synthetic and natural opiates interact with P-glycoprotein in multidrug-resistant cells. J Biol Chem 1993;268:16059–16064.

23. Tranquilli WJ, Thurmon JC, Grimm KA, et al. Lumb & Jones’ Veterinary Anesthesia and Analgesia, 4th ed. Ames, IA: Blackwell Publisher; 2007.xv, 1096 p.

24. Hofmeister EH, Chandler MJ, Read MR. Effects of acepromazine, hydromorphone, or an acepromazine-hydromorphone combination on the degree of sedation in clinically normal dogs. J Am Vet Med Assoc 2010;237:1155–1159.

25. Giuffrida MA. Type II error and statistical power in reports of small animal clinical trials. J Am Vet Med Assoc 2014;244:1075–1080.

26. Hofmeister EH, Egger CM. Evaluation of diphenhydramine as a sedative for dogs. J Am Vet Med Assoc 2005;226:1092–1094.

27. Hofmeister EH, King J, Read MR, et al. Sample size and statistical power in the small-animal analgesia literature. J Small Anim Pract 2007;48:76–79.