

The Effect of the Canine *ABCBI-1Δ* Mutation on Sedation after Intravenous Administration of Acepromazine

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Background: Dog breeds with the *ABCBI-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 *ABCBI* mut/mut dogs showed increased depth and duration of acepromazine sedation.

Hypothesis/Objectives: That *ABCBI* mut/mut dogs have increased depth and duration of sedation after acepromazine IV compared to normal dogs (nor/nor).

Animals: Twenty-nine rough-coated collies were divided into 3 groups of dogs based on their *ABCBI* genotype: 10 mut/mut, 10 mut/nor, and 9 nor/nor.

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.

Results: The median sedation scores for *ABCBI* 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 ($P > .05$).

Conclusions and Clinical Importance: In *ABCBI* mut/mut dogs acepromazine dose rates should be reduced and careful monitoring performed during sedation.

Key words: Collie; MDR1; P-glycoprotein.

The *ABCBI* gene (previously known as the *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.¹ P-gp is also a major component of the blood barrier influencing the exposure of the central nervous system to certain drugs.² A 4-bp mutation (*ABCBI-1Δ*) has been identified in dogs that results in premature stop codons and a nonfunctional P-gp.^{3,4} Dogs that are homozygous for this mutation (*ABCBI* mut/mut) have no functional P-gp resulting in an increased sensitivity to a variety of xenobiotics includ-

Abbreviations:

AUC	area under the curve
P-gp	p-glycoprotein

ing ivermectin, vincristine, doxorubicin, loperamide, and others.⁵⁻¹⁰ Dogs that are heterozygotes have shown increased sensitivity to some of these drugs (vincristine, ivermectin) compared to *ABCBI* nor/nor dogs. In particular, the increased sensitivity to ivermectin in *ABCBI* 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 *ABCBI* nor/nor dog.¹¹

The *ABCBI-1Δ* mutation is thought to have originated in an ancestral herding dog during the 19th century in England before breed standards were introduced.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 shepherd, and old English sheepdog.¹²

Many drugs are transported by P-gp and the exact structure-function relationship between substrates has been hard to fully identify.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.¹ Routinely used sedatives and analgesics have not been investigated to determine if their actions are potentiated in dogs with the *ABCBI-1Δ* mutation. However, there are anecdotal reports in the literature that dogs that are

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homozygous for the *ABCB1-1Δ* mutation show increased sensitivity to these drugs.¹¹

The aim of the current study was to determine if dogs that were homozygous for the *ABCB1-1Δ* 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 this 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 for 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 before 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 scale 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

Table 1. Sedation scoring system.

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
3. Interactive behavior	Pupils dilated; abnormal facial expression	-1
	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
4. Response to restraint	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
	Lies on floor, no restraint required	2
5. Response to noise	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
6. Gait	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
7. Posture	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
7. Posture	Mild aberrations in gait, wobbly	1
	Walks normally	0
	Lateral recumbence	3
	Sternal recumbence	2
7. Posture	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 sedation score at 2 hours (just before acepromazine dosing) was used as the dogs baseline sedation score. For the statistical analysis this baseline sedation score was subtracted from all the sedation scores post-acepromazine for each dog, creating a normalized sedation score. Normalized sedation 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) post-acepromazine over the 6 hours was calculated from the normalized sedation scores using R and a linear interpolation (version 3.1.2, R Core Team 2014).¹⁵ The AUCs for each genotype were compared using the Wilcoxon rank sum test. For heart rate, respiration rate, and mean arterial blood pressure a one-way ANOVA was performed for each time point if the data were found to be normally distributed using Levene’s test. If the data for an individual time point were found not to be normally distributed then it was analyzed with Kruskal–Wallis rank sum test. The *P*-value was adjusted for multiple comparisons using the Holm–Bonferroni method. All statistical tests were performed in R (version 3.1.2, R Core Team 2014).

Results

Genotype Results

A total of 31 rough-coated collies were enrolled in this study and had their *ABCBI* 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 sedation score increased for all 3 *ABCBI* genotype groups during the 2-hour acclimation period

Table 2. Age, sex, weight, and genotype distribution of the 29 rough-coated collies used for sedation scoring.

	<i>ABCBI</i> nor/nor	<i>ABCBI</i> nor/mut	<i>ABCBI</i> mut/mut
Number of dogs	9	10	10
Males	5	5	4
Females	4	5	6
Age (mean ± SD)*	3.9 ± 3.8	4.3 ± 2.4	3.4 ± 3.1
Weight (mean ± SD)*	18.9 ± 3.4	21.5 ± 3.2	22.3 ± 4.9

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

Table 3. The median dog sedation scores (range) of the 3 *ABCBI-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)

in the consult room before IV dosing of acepromazine (Table 3). After IV dosing of acepromazine at 0.04 mg/kg sedation scores of all dogs were increased by the 30-minute time point (Fig 1). Sedation scores then declined over the following 6 hours until the majority of dogs had sedation scores similar to before treatment values. Median normalized sedation scores were highest in the *ABCBI* mut/mut dogs and these higher scores persisted longer compared to the *ABCBI* nor/mut dogs and *ABCBI* nor/nor groups (Fig 1). The *ABCBI* nor/mut dogs had increased sedation scores at 1 hour, 90 minutes and 2 hours compared to the *ABCBI* nor/nor dogs. However, none of the sedation scores for the 3 genotypes were found to be significantly different at any of the individual time points.

To compare the sedation scores for each genotype over the total 6 hours after acepromazine sedation the AUC for each dogs normalized sedation score was calculated and the median, ranges and interquartile ranges compared (Fig 2). Compared to the nor/nor dogs, the AUCs of the *ABCBI* mut/mut dogs were found to be significantly higher (*P* = .028) while the AUCs of the *ABCBI* nor/mut dogs were not (*P* = .447).

Analysis of the heart rate, respiratory rate and mean arterial blood pressure over 6 hours after IV dosing of acepromazine showed no statistical correlation with any of the 3 *ABCBI* genotypes and remained within clinical acceptable limits.

Discussion

The results from this study suggest that dogs that are homozygous for the *ABCBI-1Δ* mutation are likely to show increased and prolonged sedation after IV acepromazine compared to *ABCBI* heterozygous or normal dogs. We selected a dose rate of 0.04 mg/kg of acepromazine because we considered that this dose would be sufficient to see moderate levels of sedation in all dogs but should not result in life threatening adverse effects even if sedation was dramatically pronounced and extended in *ABCBI* mut/mut dogs. It is unknown what level of sedation would result with higher or lower doses rate of acepromazine.

We have not determined what effect giving acepromazine intramuscularly would have on sedation in *ABCBI* mut/mut dogs. However, the *ABCBI-1Δ* mutation results in increased drug levels in the central nervous system because of a non-functional P-gp.¹⁶ This is

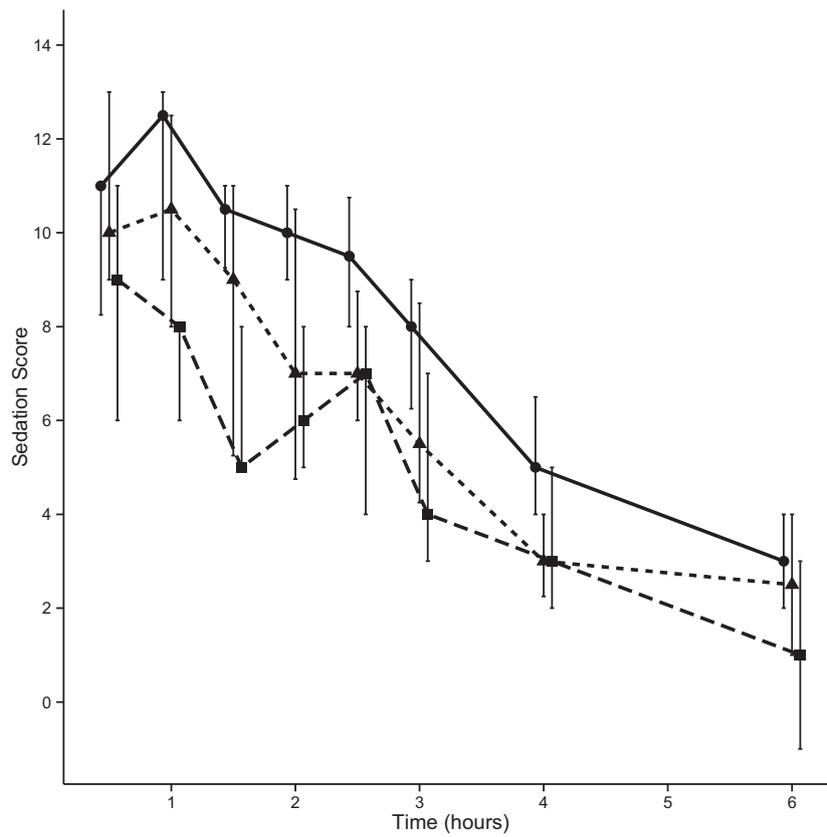


Fig 1. Median dog sedation scores normalized to baseline (\pm the interquartile ranges), for the *ABCBI-1 Δ* genotypes mut/mut (solid line), mut/nor (short dashes), and nor/nor (long dashes) measured over 6 hours after IV administration of acepromazine.

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 *ABCBI* 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 *ABCBI* mut/nor dogs did not show statistically significant increased sedation compared to the *ABCBI* nor/nor animals. This is in agreement with sensitivity to macrocyclic lactones such as ivermectin where heterozygous carriers of the *ABCBI* mutation do not show an increased sensitivity compared to homozygous normal dogs at normal doses.³ However, it is known that *ABCBI* mut/nor dogs do show some increased toxicity to ivermectin for doses greater than 120 $\mu\text{g}/\text{kg}$ especially if they are administered over multiple days.¹⁷ Furthermore the *ABCBI-1 Δ* mutation can predict the hematologic toxicity of vincristine in both mut/mut and mut/nor dogs.¹⁸ It is unknown if higher doses of acepromazine would have resulted in increased sedation in the *ABCBI* 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 *ABCBI* mutation.

Acepromazine is considered a relatively “safe” sedative, whereas the level of sedation seen in an individual *ABCBI* 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 *ABCBI* 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.¹⁹ It would be advisable to perform a CBC, serum chemistry, and urinary analysis on dogs that carry *ABCBI-1 Δ* 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.^{20–22} These opiates are known to cause sedation in dogs and are frequently used in combinations with acepromazine.²³ These drugs have not been

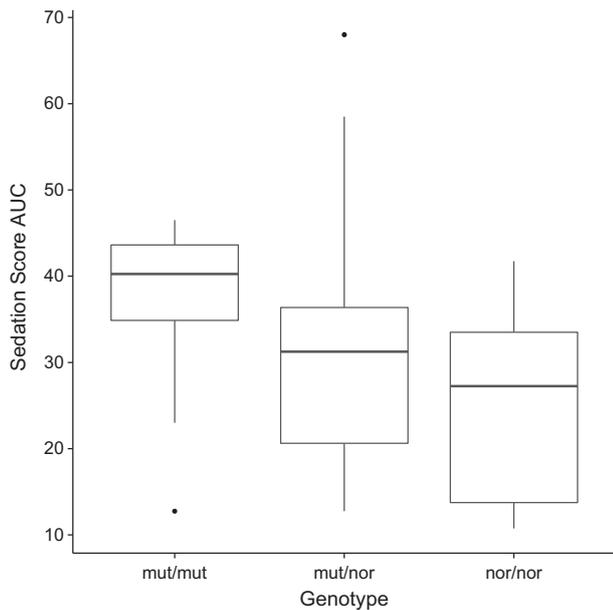


Fig 2. Box and whisker plot of the sedation score AUCs for each genotype. The box shows the 1st and 3rd quartiles with the median plotted as the band inside the box. Whiskers show the upper and lower ranges with outliers plotted as circles.

tested for the effects on sedation in *ABCB1* mut/mut dogs but there is anecdotal evidence that butorphanol may result in increased sedation.¹¹ Combinations of these drugs may result in an increased depth and duration of sedation that exceeds what was seen in this study. 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 *ABCB1* 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 *ABCB1-1Δ* 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.^{24,25} It is also clear that the sedation score of a dog will increase with saline treatment as a dog adapts and relaxes to a new environment.^{26,27} To mini-

mize 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 *ABCB1* nor/nor acted as suitable control to the *ABCB1* 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 *ABCB1* 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 *ABCB1-1Δ* 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.

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

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