Anesthetic effects of dexmedetomidine-ketamine-midazolam administered intramuscularly in five-striped palm squirrels (*Funambulus pennantii*)

**David Eshar DVM**

**Hugues Beaufrère DVM, PhD**

Received April 15, 2019.
Accepted May 28, 2019.

From the Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506 (Eshar); and the Department of Clinical Studies, Ontario Veterinary College, University of Guelph, Guelph, ON N1G 2W1, Canada (Beaufrère).

Address correspondence to Dr. Eshar (deshar@vet.k-state.edu).

**OBJECTIVE**

To evaluate efficacy and safety of anesthesia with dexmedetomidine-ketamine-midazolam (DKM) in five-striped palm squirrels (*Funambulus pennantii*).

**ANIMALS**

8 male squirrels.

**PROCEDURES**

Squirrels were anesthetized with DKM (dexmedetomidine, 0.1 mg/kg; ketamine hydrochloride, 30 mg/kg; and midazolam, 0.75 mg/kg) administered IM. Atipamezole (0.15 mg/kg) and flumazenil (0.1 mg/kg) were administered IM 40 minutes after induction of anesthesia. Vital signs and responses were recorded every 5 minutes during anesthesia.

**RESULTS**

Anesthetic induction and recovery from anesthesia were rapid and without complications in all squirrels. Median anesthetic induction time was 67.5 seconds (interquartile [25th to 75th percentile] range, 5.5 seconds), and mean ± SD recovery time after drug reversal was 147 ± 79 seconds. Heart rate, respiratory rate, and rectal temperature significantly decreased during the anesthetic period. All squirrels became hypothermic by 40 minutes after induction. The righting reflex was absent during the 40-minute anesthetic period in all squirrels, with variable responses for the palpebral reflex, jaw tone, forelimb withdrawal reflex, and hind limb withdrawal reflex. Only 2 of 8 squirrels had loss of the limb withdrawal reflex in both the forelimbs and hind limbs from anesthetic induction to 25 minutes after induction.

**CONCLUSIONS AND CLINICAL RELEVANCE**

DKM appeared to provide safe and effective anesthesia in five-striped palm squirrels, but oxygen and thermal support were indicated. At the doses administered, deep surgical anesthesia was not consistently achieved, and anesthetic depth of individual squirrels must be determined before surgical procedures are performed in palm squirrels anesthetized with this drug combination. (Am J Vet Res 2019;80:1082–1088)

---

Five-striped palm squirrels (*Funambulus pennantii*), which are also known as northern palm squirrels, are rodents in the family Sciuridae. The natural geographic distribution of this squirrel species is Southeast Asia, including India, Pakistan, Nepal, Bangladesh, and Iran. Palm squirrels are an adaptable species that inhabit tropical and subtropical dry deciduous forests, mountain forests, scrublands, plantations, grasslands, arable lands, rural gardens, and urban areas.1-3 Palm squirrels are highly adaptable, and they reportedly are an invasive species in Australia, Israel, and the United Arab Emirates.4-7 Adult palm squirrels weigh between 135 and 200 g, and body length is 20 to 30 cm, half of which consists of the tail.4 These animals have been used for research, mainly in endocrinologic studies.8-14

Because of their fractious nature, squirrel-like species often need to be chemically immobilized or anesthetized to enable clinicians and researchers to perform examinations or diagnostic procedures.15-20 Inhalation anesthesia is commonly used to immobilize rodents because of rapid induction and recovery properties.22,24,27,28 Inhalation anesthesia is typically delivered to palm squirrels in a chamber or by use of a face mask, given that endotracheal intubation is technically challenging and therefore not routinely performed. However, use of a face mask for anesthesia increases the risk of exposure of veterinary personnel to waste gases, which is a major occupational health concern.29-31 Therefore, alternative anesthetic regimens (eg, injectable anesthetic drugs for induction and maintenance of anesthesia) are desired.
when inhalation anesthesia cannot be performed or to reduce exposure to waste gas.\textsuperscript{16,17,19,32}

A suitable injectable anesthetic combination should involve short-acting anesthetic drugs with a wide safety margin that preferably are reversible.\textsuperscript{16,17,19,32} Regimens involving SC or IM injection of ketamine and \(\alpha_2\)-adrenoceptor agonist combinations are routinely used as a practical method of anesthesia for rodents, especially when inhalation anesthesia equipment is not readily available (eg, field settings) or when an animal cannot readily be intubated (eg, palm squirrels).\textsuperscript{16,17,19,27,32} Dexmedetomidine is an \(\alpha_2\)-adrenoceptor agonist that is reversible and provides analgesia, sedation, and muscle relaxation.\textsuperscript{28} Ketamine is a dissociative, centrally acting antagonist of the N-methyl-d-aspartate receptor that also provides analgesia.\textsuperscript{28} Midazolam, a benzodiazepine, is reversible and commonly used with minimal adverse effects to sedate rodents.\textsuperscript{28} Coadministration of dexmedetomidine and midazolam to rats results in a dose-related analgesic effect and rapid induction of sedation.\textsuperscript{39} In black-tailed prairie dogs (\textit{Cynomys ludovicianus}), another member of the Sciuridae, anesthesia with DKM appears to be an effective alternative to isoflurane for brief procedures that require rapid induction and analgesia.\textsuperscript{17} For this reason, the DKM combination was chosen for use in the study reported here.

Reports on the use of injectable anesthetics in palm squirrels are sparse in the literature. Five-striped palm squirrels were immobilized for intracardiac collection of blood samples by anesthetic induction with isoflurane followed by IM administration of a high dose of ketamine (100 to 150 mg/kg).\textsuperscript{4} To the authors’ knowledge, data on clinically appropriate injectable anesthetic regimens for use in five-striped palm squirrels have not been published. The objective of the study reported here was to determine the physiologic effects, including assessment of anesthetic variables and vital signs, after administration of a DKM combination in five-striped palm squirrels during a period suitable for most testing and therapeutic procedures.\textsuperscript{16,19,32} Our hypothesis was that the DKM combination would provide safe and effective anesthesia in palm squirrels.

**Materials and Methods**

**Animals**

Eight adult sexually intact male five-striped palm squirrels were included in the study. Body weight ranged between 100 and 145 g. The squirrels were confiscated from local illegally owned private collections and were newly arrived at the Jerusalem Zoo, where they were group housed in a climate-controlled (room temperature, approx 24°C) quarantine room with a light-dark cycle of 14 hours of light and 10 hours of darkness. The wire enclosure within the room contained wooden hiding boxes with wood shavings, branches, and canvas ropes for environmental enrichment. Routine husbandry care included daily observation to monitor health. Squirrels had free access to water and were fed a diet consisting of seeds, nuts, and fruit. Food was not withheld from the squirrels before they were anesthetized during the study. The squirrels were evaluated as part of their quarantine health examination; they had an acclimation period of at least 1 week before the start of the study. The study protocol was reviewed and approved by the Jerusalem Zoo Ethics Committee and the Institutional Animal Care and Use Committee at Kansas State University (No. 4088).

**Experimental procedures**

Squirrels were placed in individual 31 X 19 X 20-cm clear plastic animal containers and moved from the quarantine housing location to the designated examination room (room temperature, approx 25°C). Each squirrel was anesthetized once. The DKM doses used in the study reported here were chosen on the basis of results from preliminary experiments. In the preliminary experiments, which were conducted with other palm squirrels, lower dosages than those used in the final study provided only partial or brief immobilization.

The drug combination used in the study reported here was considered effective if squirrels were stably immobilized in a deep sedative state (most responses were absent) or a full surgical anesthetic state (complete loss of all responses, including deep pain) for at least 40 minutes. Squirrels were anesthetized with a combination of dexmedetomidine\textsuperscript{a} (0.1 mg/kg), ketamine hydrochloride\textsuperscript{b} (30 mg/kg), and midazolam\textsuperscript{c} (0.75 mg/kg), which were administered into the thigh musculature as 3 separate injections. Drugs were injected by use of a 31-gauge, 5/16-inch needle attached to a 0.5-mL insulin syringe.\textsuperscript{d}

After the drugs were injected, the squirrels were placed back into the clear plastic containers and closely monitored during the induction period. Anesthetic induction time was defined as the interval from drug injection to loss of the righting reflex. Once loss of the righting reflex was observed (anesthetic induction; baseline), squirrels were positioned in sternal recumbency, and eye lubricant was topically applied bilaterally.

Atipamezole\textsuperscript{e} (0.15 mg/kg) and flumazenil\textsuperscript{f} (0.1 mg/kg) were administered IM at 40 minutes after anesthetic induction. To determine the full anesthetic effects of DKM in this study, the squirrels were allowed to spontaneously breathe room air without supplemental oxygen or active thermal support. During recovery from anesthesia, the squirrels were placed in a heated clear plastic container for monitoring of vital signs. Once a squirrel was observed to be fully responsive, it was removed to the group holding facility.

Measurements obtained immediately after induction were used for subsequent comparisons. Measurements of vital signs were obtained at baseline and at 5-minute intervals in the following order: heart rate,
respiratory rate, rectal temperature, and \( \text{SpO}_2 \). Heart rate was determined by use of Doppler ultrasonography with the probe placed over the thorax. Respiratory rate was measured by direct visual observation of chest movements. Heart rate and respiratory rate were monitored between the 5-minute time points by use of a stethoscope. Rectal temperature was measured with a digital thermometer. The \( \text{SpO}_2 \) was measured by use of handheld pulse oximeters, which were alternately placed on the paw of a forelimb and hind limb.

After vital signs were recorded at each 5-minute time point during the anesthetic period, responses were evaluated in the following order: loss of the righting reflex, loss of the palpebral reflex, assessment of jaw tone, forelimb withdrawal reflex, and hind limb withdrawal reflex. Responses were scored on a scale of 0 to 2 (0 = response was present, 1 = response was reduced, and 2 = response was absent). Loss of the righting reflex was assessed by gently rolling each squirrel into lateral recumbency and evaluating attempts to right itself into sternal recumbency. Loss of the palpebral reflex was evaluated by gently touching the rostral canthus of the eye 2 times with a cotton-tipped applicator. Jaw tone was assessed by gently opening the mouth (pulling down on the mandible with a cotton-tipped applicator). Both of the withdrawal reflexes were assessed by use of a toe pinch (a plastic forceps was used to pinch the metacarpal and metatarsal digits with increasing amounts of applied pressure [each pressure was applied 2 times at each location] until a response was determined). A surgical plane of anesthesia was defined as the loss of all monitored responses. Recovery was defined as the return of all responses.

**Statistical analysis**

Outcome variables were assessed over time by use of linear mixed models, with time as a fixed effect and squirrel as a random effect. Results at 5-minute time points were compared with baseline values. Residual plots were used to assess linearity, homogeneity of variances, normality, and outliers. Quantile plots were also used on the residuals to assess normality. Ordinal categorical variables (all responses) were only reported as descriptive statistics. All analyses were performed with a statistical program. Values were considered significant at \( P < 0.05 \).

**Results**

All squirrels were stable throughout the peri-anesthetic period and had no adverse effects related to anesthesia during or after conclusion of the study. Median anesthetic induction time was 67.5 seconds (interquartile [25th to 75th percentile] range, 5.5 seconds). Mean ± SD recovery time was 147 ± 79 seconds after administration of reversal agents.

Heart rate from 15 minutes after anesthetic induction through the end of the study was significantly \( P = 0.026 \) lower than the respiratory rate at baseline (Figure 1). Rectal temperature from 10 minutes after anesthetic induction through the end of the study was significantly \( P < 0.001 \) lower than the rectal temperature at baseline (Figure 3). We were unable to consistently measure \( \text{SpO}_2 \) throughout the 40-minute anesthetic period in all squirrels regardless of the limb on which the oximeter was placed. Responses were measured in all 8 squirrels over the 40-minute anesthetic period (Table 1).

**Discussion**

In the study reported here, 8 five-striped palm squirrels were anesthetized with DKM administered induction through the end of the study was significantly \( P = 0.026 \) lower than the respiratory rate at baseline (Figure 1). Rectal temperature from 10 minutes after anesthetic induction through the end of the study was significantly \( P < 0.001 \) lower than the rectal temperature at baseline (Figure 3). We were unable to consistently measure \( \text{SpO}_2 \) throughout the 40-minute anesthetic period in all squirrels regardless of the limb on which the oximeter was placed. Responses were measured in all 8 squirrels over the 40-minute anesthetic period (Table 1).
IM, and use of this combination appeared to provide safe and effective anesthesia in this species. To the authors’ knowledge, no similar regimens have been used in palm squirrels, and the combination of agents tested in the present study was designed to provide balanced and partially reversible anesthesia that can be used for prolonged or pain-inducing procedures that might require more than brief or deep sedation. On the basis of results of this study, DKM appeared to be a viable option for the induction and maintenance of anesthesia in palm squirrels, particularly when inhalation anesthesia is not desired or not an option (eg, field settings).

The IM route of administration was chosen for the present study because it generally allows for uniform and rapid drug absorption. Determining the doses for the various anesthetic drugs in this study was challenging because of the low body weight of the squirrels and the relatively small volumes for injection. To ensure accuracy of drug delivery, each of the 3 drugs was administered separately. A combination of the 3 drugs in 1 syringe could have been administered IM into a single site, but it could also have led to greater tissue trauma from the larger volume of injectate and associated pain in these small animals. Drug dilutions were considered, but they were not used because of concerns about increased amounts of soft tissue trauma from a larger volume of injectate. The SC administration of DKM could have helped address these concerns and can be considered in future studies. Regardless, none of the squirrels in the present study had evidence of injection site trauma during postprocedural follow-up evaluations.

Anesthetic induction and recovery times in the study reported here were brief, which is generally a desired effect when working with small animals in field settings. Black-tailed prairie dogs anesthetized with a similar combination of dexmedetomidine (0.25 mg/kg), ketamine (40 mg/kg), and midazolam (1.5 mg/kg) had a similar time to induction but a much longer recovery time (mean ± SD, 54.62 ± 30.49 minutes) after administration of the reversal agents. For chinchillas (*Chinchilla lanigera*) anesthetized by IM administration of a combination of dexmedetomidine (0.015 mg/kg) and ketamine (4 mg/kg), the median time to loss of the righting reflex was 2 minutes, (interquartile [25th to 75th percentile] range, 2 to 3 minutes), and the recovery time ranged from 4 to 15 minutes. Differences in the anesthetic responses among rodent species administered different doses of the anesthetic drugs emphasize the need for species-specific anesthetic regimens, as was determined for palm squirrels in the study reported here.

Lateral recumbency (or loss of the righting reflex) of laboratory rodents after drug administration is a marker of anesthesia onset. In the study reported here, all 8 squirrels had loss of the righting reflex between baseline and 40 minutes after anesthetic induction, which suggested that DKM can provide immobilization for this range of time in this species. Because drug antagonists were administered 40 minutes after anesthetic induction, it was not possible to determine the potential duration of anesthesia that DKM can provide. Future studies with DKM in palm squirrels could allow the animals to spontaneously recover without administration of reversal agents, which would reveal the full anesthetic duration of DKM.

---

**Figure 3**—Mean ± SEM rectal temperature of 8 five-striped palm squirrels anesthetized by IM administration of DKM. See Figure 1 for key.

**Table 1**—Number of five-striped palm squirrels (*Funambulus pennantii*) anesthetized by IM administration of DKM in which the tested responses were absent.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Righting reflex</th>
<th>Palpebral reflex</th>
<th>Jaw tone</th>
<th>Forelimb withdrawal</th>
<th>Hind limb withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>20</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>25</td>
<td>8</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>30</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>35</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>40</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Eight squirrels were anesthetized and evaluated at each time point. Anesthetic induction was defined as loss of the righting reflex (baseline); it was detected a median of 67.5 seconds after injection of DKM. Reversal agents were administered immediately after measurements were obtained at 40 minutes after anesthetic induction.
Administration of DKM did not provide a consistent surgical plane of anesthesia in the study reported here as indicated by the variable responses to a toe pinch (ie, limb withdrawal reflex), which suggested that supplemental analgesia or partial increases in the doses of DKM might be required for invasive and pain-inducing procedures. In addition to the intragroup variability, it is important to mention that there were differences in the observed responses for the limb withdrawal reflex between the forelimbs and hind limbs, which suggested that both should be evaluated to determine the level of anesthesia and before application of invasive or pain-inducing treatments. Furthermore, response to a toe pinch might be an indicator of the depth of anesthesia, and the potential antinociceptive effect of DKM in palm squirrels should be further evaluated.38

Heart rate, respiratory rate, and rectal temperature all decreased over time in the squirrels of the present study, which is similar to results described for chinchillas32,37 anesthetized with a dexmedetomidine-ketamine combination and alpine marmots (Marmota marmota)36 anesthetized with various combinations of ketamine and α2-adrenoceptor agonists. Because a combination of 3 drugs (DKM) was used in the present study, it was challenging to determine the drug that had the greatest effect on heart rate. Black-tailed prairie dogs anesthetized with DKM had similar decreases in heart rate and rectal temperature, but the respiratory rate increased over time.37 Palm squirrels of the present study had a significant decrease in the mean ± SD respiratory rate compared with the baseline rate during the 40-minute anesthetic period (163 ± 20 breaths/min and 108 ± 30 breaths/min, respectively). Bradycardia and hypopertilation observed in these squirrels were likely attributable primarily to the effect of dexmedetomidine (an α2-adrenoceptor agonist that decreases the sympathetic tone originating from the brain and also causes peripheral vasodilation as a result of its action on adrenergic receptors, which leads to increased peripheral vascular resistance and eventually a decrease in heart rate and cardiac output).37,39 Similar to effects of all other α2-adrenoceptor agonists, dexmedetomidine can lead to bradycardia, hypotension, respiratory depression, and hypercapnia.37–39 Measurements of tidal volume and end-tidal partial pressure of carbon dioxide in palm squirrels could aid in the respiratory assessment during anesthesia induced by the administration of DKM.37

Rats34 anesthetized with ketamine-metomidine and mice37 anesthetized with ketamine-dexmedetomidine had decreases in SpO2 (from 85% to 65%) during the anesthesia period in previous studies. We failed to obtain SpO2 measurements in the squirrels of the present study despite the use of 2 handheld pulse oximeters and alternating placement over various parts of all 4 limbs. Use of pulse oximetry to assess oxygen saturation can result in inaccurate measurements, with decreases in tissue perfusion and lower oxygen saturation, especially in cases of vasoconstriction and hypothermia, which are an expected response when investigators use α2-adrenoceptor agonists.37,39,40 Also, because the oximeters were designed on the basis of the oxygenation curve for humans, it was possible that the palm squirrels might have had a different oxygenation pattern. Furthermore, the pulse oximeters used in the present study can be inaccurate when outside of their original calibration range.40 The study34 of rats anesthetized with ketamine-metomidine found a correlation between respiratory rate and SpO2 and yielded the following equation to relate both variables: \( \text{SpO}_2 = 63.3761 + (0.1263 \times \text{respiratory rate}) \). Application of this equation to the data for the palm squirrels of the present study yielded SpO2 values of 84% and 77% at baseline and 40 minutes after anesthetic induction, respectively, which is similar to the SpO2 reported for the rats34 and mice.37 Regardless, additional studies that involve measurement of blood gases and use of other pulse oximeters41 will be required to determine whether the observed decrease in respiratory rate was associated with hypoventilation, hypoxemia, or other blood gas abnormalities in palm squirrels anesthetized with DKM. Although squirrels of the study reported here made a full recovery from anesthesia, supplemental oxygen should be provided when DKM is used for anesthesia of palm squirrels.

Squirrels of the present study became profoundly hypothermic, with a lower rectal temperature (5.5°C lower) over the 40-minute anesthetic period. Anesthesia in general, and especially when α2-adrenoceptor agonists are used, can lead to hypothermia.32,37,38 Alpine marmots anesthetized with ketamine-xylazine or ketamine-metomidine in field settings had a decrease in body temperature to as low as 30°C, but they recovered and were released back into the wild.16 Anesthetic hypothermia can negatively affect the immune system, wound healing, blood clotting, and recovery from anesthesia.16 Although the palm squirrels of the present study made a full recovery from anesthesia, we would strongly recommend that active thermal support be provided when DKM is used for anesthesia.

The study reported here had several limitations, including a small sample size. However, studies conducted to evaluate anesthesia in other species have used similar numbers of animals, including studies that included 6 rabbits,42 7 cats,43 8 chinchillas,32 and 8 dogs.30 Only male squirrels were used in the present study, and female squirrels should be included in future studies of DKM to determine whether there are differences between sexes, as has been found for rats34 and mice.37

Fecal output and food intake were lower in chinchillas for 3 days after anesthesia with dexmedetomidine-ketamine but not after anesthesia with isoflurane in a previous study.32 In the study reported here, the squirrels could not be individually monitored to determine food intake or fecal output after anesthesia.
because they were group housed in the quarantine holding facility; however, no overt adverse effects in any of the squirrels were noted by the caretakers. Additional studies to compare the safety and efficacy of inhalation anesthetics (eg, isoflurane or sevoflurane) and the DKM regimen may also be useful.

The DKM regimen evaluated in the study reported here appeared to be a safe and effective method for anesthetizing five-striped palm squirrels. There was rapid induction and recovery from anesthesia as well as a stable plane of anesthesia for at least 40 minutes after anesthetic induction.

Acknowledgments

Supported by the Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University.

The authors declare that there were no conflicts of interest.

The authors thank Dr. Roi Lapid, Dr. Avital Paz, Dr. Nili Avni-Magen, Nufar Eshkar Carmel, and Ariella Bary for technical assistance.

Footnotes

a. Dexdomitor, Orion Corp, Espoo, Finland.

b. Ketaset, Hospira, Lake Forest, Ill.


b. BD Ultra-Fine II, Becton, Dickinson and Co, Franklin Lakes, NJ.

c. Hospira, Lake Forest, Ill.

d. BD Ultra-Fine II, Becton, Dickinson and Co, Franklin Lakes, NJ.

e. BD Ultra-Fine II, Becton, Dickinson and Co, Franklin Lakes, NJ.

References


33. Arenillas M, Gomez de Segura IA. Anaesthetic effects of alfaxalone administered intraperitoneally alone or combined with dexmedetomidine and fentanyl in the rat. Lab Anim 2018;52:588–598.