Evaluation of the effects of a dexmedetomidine-midazolam-ketamine combination administered intramuscularly to captive red-footed tortoises (Chelonoidis carbonaria)

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OBJECTIVE
To evaluate the effects of a dexmedetomidine-midazolam-ketamine (DMK) combination administered IM to captive red-footed tortoises (Chelonoidis carbonaria).

ANIMALS
12 healthy adult red-footed tortoises.

PROCEDURES
In a prospective experimental study, DMK (0.1, 1.0, and 10 mg/kg, respectively) was administered IM as separate injections into the right antebrachium. Atipamezole (0.5 mg/kg, IM) and flumazenil (0.05 mg/kg, SC) were administered into the left antebrachium 60 minutes later. Times to the first treatment response and maximal treatment effect after DMK administration and time to recovery after reversal agent administration were recorded. Vital signs and reflexes or responses to stimuli were assessed and recorded at predetermined intervals.

RESULTS
DMK treatment produced deep sedation or light anesthesia for \( \geq 20 \) minutes in all tortoises. Induction and recovery were rapid, with no complications noted. Median times to first response, maximum effect, and recovery were 4.5, 35, and 14.5 minutes, respectively. Two tortoises required additional reversal agent administration but recovered < 20 minutes after the repeated injections. Mean heart and respiratory rates decreased significantly over time. All animals lost muscle tone in the neck and limbs from 35 to 55 minutes after DMK injection, but other variables including palpebral reflexes, responses to mild noxious stimuli (eg, toe pinching, tail pinching, and saline ([0.9 NaCl] solution injection), and ability to intubate were inconsistent.

CONCLUSIONS AND CLINICAL RELEVANCE
DMK administration produced deep sedation or light anesthesia with no adverse effects in healthy adult red-footed tortoises. At the doses administered, deep surgical anesthesia was not consistently achieved. Anesthetic depth must be carefully evaluated before performing painful procedures in red-footed tortoises with this DMK protocol.

Suitable combinations of injectable immobilization drugs should ideally involve short-acting anesthetics with a wide safety margin that are also reversible.\(^7,8,10,11\) Regimens that involve SC or IM injection of combinations of ketamine and \(\alpha_2\)-adrenoceptor agonists are routinely used as a practical method for anesthetizing chelonians, especially in settings where inhalation anesthesia equipment is not available or when the animal retracts its head and cannot be readily intubated.\(^8,12,13\) Injections are traditionally administered in a forelimb owing to the presence of renal-portal and hepatic-portal circulation systems.\(^8\)

There are some previous reports of injectable anesthesia in red-footed tortoises. In 1 study,\(^14\) a combination of ketamine hydrochloride (0.5 mg/kg) and propofol (7.0 mg/kg) was injected into the dorsal cer-
tical sinus to facilitate radiography and CT of these animals, and maintenance with smaller doses of ketamine (0.2 mg/kg) and propofol (4.0 mg/kg) were required. In other investigations, red-footed tortoises were anesthetized with ketamine (40 mg/kg, IM) and midazolam (2.0 mg/kg, IM) for ultrasonography or with midazolam (2.0 mg/kg, IM), ketamine (40 mg/kg, IM), and propofol (15 mg/kg, IV) for exsanguination. A combination of ketamine (3.16 mg/kg) and xylazine (0.13 mg/kg) administered IM in a forelimb produced sedation sufficient for collection of a jugular venous blood sample within approximately 15 minutes in another study, and a combination of butorphanol (1.0 mg/kg), ketamine (40 mg/kg), and midazolam (2.0 mg/kg) administered IM in a forelimb resulted in sufficient relaxation to allow intubation for inhalation anesthesia of a red-footed tortoise undergoing surgical treatment for cutaneous melanoma. In 1 prospective, crossover-design study to evaluate drug combinations for pharmacological restraint of red-footed tortoises, the animals received each of 3 IM treatments (ketamine [30 mg/kg], ketamine [50 mg/kg] plus midazolam [1.0 mg/kg], and ketamine [50 mg/kg] plus butorphanol [1.0 mg/kg]). In that study, there were no significant differences among treatments for the time to onset of anesthesia (median, 6 to 16 minutes), and none of the treatments resulted in a surgical plane of anesthesia, although all treatments produced a degree of sedation that allowed jugular venous blood sample collection, oral swabbing, and biometric testing.

Midazolam has been frequently used in anesthetic protocols for reptiles because of its sedative, anxiolytic, and muscle relaxation properties. Dexmedetomidine can be added to protocols for chemical restraint in reptiles, as it can produce sedation and muscle relaxation and provide analgesia. The addition of ketamine, a dissociative, centrally acting antagonist of the N-methyl-D-aspartate receptor, can increase the level of sedation and provide some analgesia, which can facilitate the performance of more invasive procedures or result in a longer duration of immobilization. Intranasally administered midazolam (0.5 or 1.5 mg/kg) or dexmedetomidine (0.05 or 0.15 mg/kg) was not found to produce adequate sedation for diagnostic and handling procedures in red-footed or Indian star tortoises (Geochelone platynota). However, a leopard tortoise (Stigmochelys pardalis) with an ectopic egg in its urinary bladder underwent SC administration of medetomidine (0.15 mg/kg), midazolam (1.0 mg/kg), and ketamine (5 mg/kg), which produced deep sedation within approximately 30 minutes and allowed endoscopy-guided egg removal. Ketamine-dexmedetomidine-hydromorphone (or morphine) combinations administered IM provide anesthesia sufficient to facilitate endoscopic evaluation of gonads, although aquatic and semiaquatic species seem more sensitive to the treatment (requiring 10 to 20 mg of ketamine/kg and 0.05 mg of dexmedetomidine/kg) than terrestrial tortoises (requiring 20 to 40 mg of ketamine/kg and 0.1 mg of dexmedetomidine/kg).

The clinical application of injectable combinations of dexmedetomidine-midazolam-ketamine (DMK) was previously reported for several chelonian species. In African spurred tortoises (Centrochelys sulcata), SC administration of DMK (0.07 to 0.1, 1.0, and 2.5 to 5.0 mg/kg, respectively) produced moderate to deep sedation, which was suitable for cloacal endoscopy, and recovery was rapid after reversal treatment with atipamezole. In red-eared slider turtles (Trachemys scripta elegans), SC administration of DMK (0.1, 1.0, and 2.0 mg/kg, respectively) produced moderate to deep sedation, which was suitable for cloacal endoscopy and intrathecal injection, with turtles recovering rapidly after reversal treatment.

The objective of the study reported here was to determine the physiologic effects and anesthetic properties of a DMK combination (0.1, 1.0, and 10 mg/kg, respectively) after IM administration to healthy red-footed tortoises. We hypothesized that this protocol would produce deep sedation (with immobilization) or anesthesia in these animals without adverse effects.

Materials and Methods

Animals

Twelve adult (6 males and 6 females) zoo-kept red-footed tortoises were included in the study as part of their annual health examination performed in the month of September. The tortoises were housed in 5 zoological collections and were observed daily by the caretakers to monitor general appearance and behavior. The animals were deemed healthy on the basis of daily observations by their keepers and the results of physical examination by a veterinarian. The study protocol was approved by each zoo’s ethics committee and the Institutional Animal Care and Use Committee at Kansas State University (No. 4366.1).

Experimental procedures

Prior to experimental procedures, animals were brought indoors to allow time for acclimation to the study room and temperature (mean, 25 °C). At this time, a physical examination was performed for each tortoise and body weight was determined with the scales available at each institution. Exclusion criteria included any history or visible signs of systemic illness (eg, signs of respiratory or cardiovascular abnormalities, infection, inflammation, trauma, or neoplasia) or discomfort. Baseline (pretreatment) vital signs were often not determined owing to the shy nature of the tortoises and the need to minimize handling...

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stress that could potentially affect the measured anesthetic variables.

For study purposes, treatment with the DMK combination was considered successful when a stable plane of deep sedation and immobilization (complete muscle relaxation and most reflexes or responses absent) or a surgical plane of anesthesia (complete loss of all reflexes and responses, including responses to deep pain) was produced.

All injections were performed with 3-mL syringes and 22-gauge, 1.5-inch needles (Luer-Lok syringes with PrecisionGlide needles; Becton, Dickinson, and Co). Dexametomidine hydrochloride (Dexdomitor; 0.1 mg/kg), midazolam hydrochloride (1.0 mg/kg), and ketamine hydrochloride (Ketaset; 10 mg/kg) were administered as 3 separate IM injections in the right antebrachium.

The times to first treatment response (first observed change in at least one of the assessed variables attributable to sedation or anesthesia) and maximum treatment effect (as assessed by loss of spontaneous movement and maximum decreases in variables for the anesthetic episode) were measured from the time of DMK administration. Recovery time was measured from the time of reversal agent administration. A tortoise was deemed recovered from sedation or anesthesia when all tested reflexes or responses were present; neck, jaw, and limb muscle tone scores indicated these were present (reduced or intact [vs absent]); and the animal was able to hold its head above ground and initiate spontaneous movement.

Monitoring and assessments—Immediately after DMK injection, each tortoise was placed into a large plastic container and closely monitored. Time to first response was noted by means of visual observation during this phase. The following variables were assessed immediately after DMK injection and at 5-minute intervals thereafter until the reversal agents were administered: vital signs (heart rate, respiratory rate, and cloacal temperature); spontaneous movement; skeletal muscle tone in the neck, jaw, forelimbs, and hind limbs; palpebral reflexes, forelimb, and hind limb withdrawal responses; and response to a tail pinch. After reversal agent administration, these measurements were repeated at 5-minute intervals until the tortoise had recovered from anesthesia.

Vital signs were assessed in the following order at each time point. Heart rate was measured with a Doppler ultrasonic flow detector (Model 811-B; Parks Medical Electronics Inc), which was directed toward the heart in a cervicoabrachial acoustic window. Respiratory rate was assessed by observation of skin movement in the region between the neck and shoulder region or the femoral fossa. Cloacal temperature was monitored with a laboratory-grade thermometer (MicroThera 2T; Braintree Scientific Inc).

After vital signs were recorded, the remaining variables evaluated at each time point were assessed in the following order: neck tone and jaw tone, palpebral reflexes, forelimb muscle tone, hind limb muscle tone, forelimb withdrawal, hind limb withdrawal, and response to a tail pinch. Spontaneous movement was defined as any purposeful and coordinated movement. Skeletal muscle tone in the neck, jaw, forelimbs, and hind limbs was evaluated by assessing resistance to manual manipulation (gentle head, jaw, and limb retraction). Palpebral reflexes were tested with a small cotton-tipped applicator gently touching rostral canthus of each eye twice. The forelimb and hind limb withdrawal responses were tested by pinching a digit and the skin bilaterally on each limb; the tail-pinch response was tested in the same manner. A hemostat was used to apply increasing amounts of subjectively determined pressure (each pressure applied 2 times/location) until a response was observed. The responses were subjectively assessed as present or absent.

At each time point, endotracheal intubation with a 2.0- to 3.0-mm uncuffed endotracheal tube (Sheridan; Teleflex Inc) was attempted when spontaneous movement and jaw tone were absent. Intubation was considered successful if the tube was placed uneventfully or only a minor response was elicited. Intubation was considered unsuccessful when attempted tube placement evoked a jaw contraction or gag reflex or if the animal moved spontaneously.

Every 15 minutes after DMK injection up to the time of drug reversal, each tortoise was evaluated for responsiveness to IM injection of 0.1 mL of sterile saline (0.9% NaCl) solution. The injection was administered in the left pelvic limb (femorotibialis muscle).

Reversal agents—Atipamezole (Antisedan; 0.5 mg/kg, IM) and flumazenil (0.05 mg/kg, SC) were administered in the left antebrachium 60 minutes after DMK injection. Tortoises were monitored as described until recovery from anesthesia or sedation and were later monitored closely by their keepers for any signs of resedation or other adverse effects.

Statistical analysis
Changes in heart rate, respiratory rate, and cloacal temperature were assessed over time (from the time of DMK administration [time 0 for this analysis] until the time of reversal agent administration) by use of linear mixed models, with time, sex, age, and weight as fixed effects and animal as the random effect. Residual plots were used to assess linearity, homogeneity of variances, normality, and outliers. Quantile plots were also used to assess the residuals for normality. Post hoc analysis was performed with a Tukey adjustment. All analyses were performed with a statistical program (R package version 3.1-121; R Foundation for Statistical Computing). Values of $P < 0.05$ were considered significant.

Results
The ages of tortoises in the study ranged from 11 to 52 years. The median body weight was 4.65 kg (range, 3.0 to 9.2 kg). There was no significant
effect of sex, age, or weight on any of the measured variables. The median time to first response after DMK administration was 4.5 minutes (range, 2 to 18 minutes; IQR, 3 to 8.25 minutes), and median time to maximum drug effect was 35 minutes (range, 25 to 45 minutes; IQR, 28.75 to 35 minutes). The median time to recovery after reversal agent injection was 14.5 minutes (range, 2 to 44 minutes; IQR, 9.5 to 34.75 minutes). Both induction and recovery were deemed fast and smooth (ie, no struggling, hyperactivity, signs of disorientation, or other anomalies were observed).

No animals had adverse effects detected during or after the procedure. Two tortoises had delayed recoveries (based on the authors’ experience with other animals in the study) and were administered a second dose of reversal agents 20 minutes after the first, and both recovered within approximately 20 minutes after a second dose was administered.

The mean heart rate decreased significantly (P < 0.001) over time after DMK injection, with a mean ± SEM change of –0.14 ± 0.02 beats for each 1-minute increment of time (Figure 1). The maximum decrease in heart rate, compared with the time 0 value, was 60.2% minutes after DMK administration. The mean respiratory rate also decreased significantly (P < 0.001) over time (Figure 2). However, because the values at time 0 had a much higher order of magnitude than those at all subsequent time points, the model did not fit the statistical assumptions. When the time 0 values were removed from the analysis, respiratory rate was still significantly (P = 0.014) decreased over time, but the effect was clinically negligible (mean ± SEM change, –0.02 ± 0.008 breaths for each 1-minute increment), and no differences between individual time points were found on post hoc analysis (Figure 3). The maximum decrease in respiratory rate, compared with the time 0 value, was 99.5% 40 minutes af-
ter DMK administration. There was a slight increase in mean cloacal temperature during the procedure, with the greatest change from the time 0 value (6.8%) at 60 minutes after DMK injection (Figure 4). However, the change over time was nonsignificant.

The numbers of tortoises in which muscle tone and tested reflexes or responses were absent at various time points are summarized (Table 1). All tortoises had complete muscle relaxation of the neck and limbs from 35 to 55 minutes after DMK injection; however, the results for all other tested variables were mixed and inconsistent.

### Discussion

In the study reported here, healthy adult red-footed tortoises were immobilized with a DMK combination administered IM in a forelimb. This DMK protocol resulted in a rapid onset of deep sedation or light anesthesia that lasted for ≥20 minutes in 12 of 12 tortoises and for approximately 40 minutes, long enough to perform minor clinical procedures, in more than half of the study sample. This was followed by a smooth, uneventful recovery after administration of reversal agents, and no adverse effects were detected during or after the study.

Fifty-five minutes after DMK injection, 12 of 12 tortoises had complete muscle relaxation of the neck and limbs, and 6 of 12 could be intubated at that time. The lack of glottal control and ability to intubate some of these animals suggested that this DMK protocol can also be used as a premedication or induction protocol for gas anesthesia or for some test procedures such as bronchoalveolar lavage. The ability to intubate can also be important for patient support, given the low respiratory rates observed in the tortoises in this study. Respiratory depression is considered to be a common effect of α₂-adrenoceptor agonists in reptiles.  

This lower respiratory rate was consistent with findings in other studies of chelonians that revealed bradypnea or apnea following administration of medetomidine. For example, a study in which medetomidine and ketamine were administered to gopher tortoises (Gopherus polyphemus) resulted in moderate hypercapnia, hypoxemia, and respiratory acidemia, which resolved following atipamezole administration. In ball pythons (Python regius), doxapram ameliorated the respiratory depressive effects of dexmedetomidine while preserving its antinociceptive effects.  

Thus, the addition of doxapram should be evaluated in future studies of red-footed tortoises undergoing this protocol.

The tortoises in the study reported here had a slight but nonsignificant increase in cloacal temperature during the procedure, with the greatest change from the time 0 value (6.8%) at 60 minutes after DMK injection, and this value continued to increase after reversal agent administration (data not shown). This finding contrasted with expectations, as anesthesia is generally associated with hypothermia due to vasodilation followed by a reduced response to lower temperature in the hypothalamus; the combination of these 2 physiologic changes allows cooler blood to be

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|                               | 25   | 0    | 2    | 1    | 2    | 0    | 1    | 0    | 1    | — | — | — |
redistributed throughout the body.28 No significant effects on body temperature were observed in gopher tortoises anesthetized with medetomidine and ketamine.27 One common cause of hypothermia in anesthetized patients is the inhalation of cold gases, which might have been minimized by bradypnea in tortoises of the study reported here; the lack of hypothermia could have also been secondary to ketamine administration, as it causes increased peripheral arteriolar resistance that may prevent the redistribution of cooler blood.29 The ability of tortoises to remain normothermic appeared to be a possible advantage of this DMK protocol when used for the short interval assessed in the present study.

To assess the depth of sedation or anesthesia in tortoises of our study, several variables other than muscle relaxation were evaluated. These included palpebral reflexes; responses to a tail pinch, toe pinch in all 4 limbs, and saline solution injections; and ability to intubate. The responses to most of these tests were variable and inconsistent, even when all tortoises had complete muscle relaxation in the neck, forelimbs, and hind limbs and most had complete jaw relaxation. For example, the palpebral reflex was intact when responses to noxious stimuli (toe pinch, tail pinch, and saline solution injection) were absent in some of these animals. This was similar to observations reported for red-eared sliders anesthetized with alfaxalone50 or a combination of medetomidine and ketamine.31 The tail-pinch response remained intact or reduced for 8 of 12 animals throughout the immobilization event in the present study. The tail pinch response is not often tested in chelonian anesthesia studies. Instead, limb withdrawal responses are often evaluated. In a study32 that investigated combinations of ketamine, midazolam, and xylazine in red-eared sliders, the absence of a toe-pinch response and lack of neck withdrawal upon manual extension were considered indicative of anesthesia. Some of the animals in that study32 began to purposely move while they had apparently absent withdrawal responses, a finding that was attributed to the dissociative properties of ketamine. The observations in the present study suggested that the toe-pinch withdrawal response and response to saline solution injection might be unreliable for assessment of nociception in these tortoises, either because the responses are inherently inconsistent in this species or because it is difficult to pinch through the thick skin on the limbs sufficiently to induce nociception without causing trauma to deeper structures. Considering that the tail pinch response was the only one to remain present throughout the procedure in most tortoises of our study, this may be a more sensitive indicator of peripheral nociception than either the toe-pinch or saline injection responses. This might have occurred because red-footed tortoises have thinner skin on the tail than on the limbs. Future studies may also determine the antinociceptive effects of DMK in this species by use of a thermal noxious stimulus, as described for dexmedetomidine and midazolam in other reptiles.33

The variable preservation of commonly tested reflexes and responses in the present study suggested that the described DMK protocol can produce deep sedation or light anesthesia, but not a consistent surgical plane of anesthesia,20,54 in red-footed tortoises. However, this degree of immobilization might allow veterinarians to perform several clinical procedures such as blood sample collection, imaging, oral swabbing, and some minimally invasive endoscopic examinations.12,13,22,23,35–38 The authors of previous reports on anesthesia of red-footed tortoises used much higher doses of the drugs used in the present DMK protocol. For example, ketamine (40 mg/kg, IM) and midazolam (2.0 mg/kg, IM) facilitated ultrasonographic examination,5 and much lower drug doses may be needed with this DMK protocol. In another prospective study,18 red-footed tortoises underwent 3 different treatments, including ketamine (30 mg/kg), ketamine (30 mg/kg) plus midazolam (1 mg/kg), or ketamine (30 mg/kg) plus butorphanol (1 mg/kg), and each treatment failed to produce a surgical plane of anesthesia. It is possible that tortoises of this species can tolerate much higher dosages of DMK than we evaluated to increase the degree of sedation or anesthesia; however, further studies are needed to assess the efficacy and safety of DMK with various doses of the components.

Other than the lack of reliability of the tested reflexes and responses to gauge the tortoises’ anesthetic depth, limitations of the present study included a lack of blood pressure monitoring and assessment of the cardiorespiratory effects of the DMK protocol. Blood gas analysis and capnography or closed-chamber plethysmography can be used in future studies to determine whether the lower respiratory rates are considered physiologically appropriate with this protocol.24 Also, these tortoises were tested at a room temperature of approximately 25 °C during the month of September, and the observed responses could have been different under different ambient temperatures39 or at different times of the year.40

The DMK protocol described in this report produced deep sedation or light anesthesia in this sample of healthy red-footed tortoises for ≥ 20 minutes, with no response to mild noxious stimuli and successful intubation in some of the tested animals. No apparent adverse effects were noted during the procedure and recovery or on follow-up assessments by the tortoises’ keepers. Considering the inconsistencies in preservation of several tested responses, including deep pain responses throughout the testing period, this DMK protocol alone is not recommended for surgical procedures.

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