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Anesthetic efficacy of dexmedetomidine-ketamine in eastern box turtles (*Terrapene carolina carolina*) is enhanced with the addition of midazolam and when administered in the forelimb versus the hindlimb

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OBJECTIVE
To compare dexmedetomidine-ketamine (DK; 0.1 and 10 mg/kg, respectively) with midazolam (M; 1.0 mg/kg) or 0.9% sodium chloride (S; 0.2 mL/kg) administered IM in the forelimb (F) or hindlimb (H) in eastern box turtles (*Terrapene carolina carolina*).

ANIMALS
20 clinically healthy, captive adult eastern box turtles.

METHODS
In a randomized, blinded, complete crossover study with 1-week washout periods, turtles were administered each of 3 treatments: F-DKS, F-DKM, or H-DKM. Palpebral reflex, muscle tone, and withdrawal responses were serially assessed and used to calculate cumulative sedation scores at each 5-minute time point. The ability to intubate was evaluated. At 60 minutes, atipamezole (1.0 mg/kg) and either flumazenil (F-DKM, H-DKM; 0.05 mg/kg) or 0.9% sodium chloride (F-DKS; 0.5 mL/kg) were administered IM.

RESULTS
All treatments resulted in clinically relevant anesthetic effects. F-DKM produced significantly higher sedation scores than H-DKM or F-DKS at all time points between 10 and 60 minutes ($P < .05$). Sedation score variability was observed with all treatments with significantly higher variability for H-DKM ($P < .05$). Intubation was successful in 32, 89, and 11% of turtles in F-DKS, F-DKM, and H-DKM, respectively. Median (range) recovery time was 10 (5–22), 16 (7–45), and 12 (4–28) minutes for F-DKS, F-DKM, and H-DKM, respectively.

CLINICAL RELEVANCE
In eastern box turtles, forelimb dexmedetomidine-ketamine resulted in clinically relevant anesthetic effects that were heightened with the addition of midazolam. Hindlimb administration of midazolam-dexmedetomidine-ketamine resulted in reduced and more variable anesthetic effects compared to forelimb administration, supporting a hepatic first-pass effect.

Keywords: eastern box turtle, sedation, dexmedetomidine, ketamine, midazolam, anesthesia

Eastern box turtles (*Terrapene carolina carolina*) are frequently presented to veterinarians as client-owned or wildlife patients. Chemical restraint may be required to facilitate thorough physical examinations, diagnostics, and treatments in this species, owing largely to their ability to fully retract into their shells. Despite this, evidence-based studies evaluating sedative and anesthetic protocols in eastern box turtles are lacking.

Considering the immense diversity of body sizes, diets, activity levels, natural habitats, and metabolic rates amongst chelonian species, there is a need to develop and apply species-specific anesthetic protocols.[1] While no evidence-based studies have evaluated anesthetic protocols in eastern box turtles specifically, a single prospective study has investigated one protocol in a related species with good success. In ornate box

Received October 9, 2023
Accepted November 16, 2023

doi.org/10.2460/ajvr.23.10.0226

turtles (*Terrapene ornata ornata*), forelimb IM administration of dexmedetomidine-ketamine-midazolam (0.1, 10, and 1.0 mg/kg, respectively) rapidly and consistently produced a light plane of anesthesia for approximately 40 minutes.[2] Subsequent administration of atipamezole and flumazenil (0.5 and 0.05 mg/kg, respectively) 60 minutes after initial injections resulted in smooth recoveries without any adverse effects. The individual drugs used in the above combination—dexmedetomidine, ketamine, and midazolam—are widely used in reptile chemical restraint either alone or in combination. In addition to providing reliable and dose-dependent sedation in reptiles, dexmedetomidine, an alpha-2 adrenergic agonist, and midazolam, a benzodiazepine, can be rapidly and reliably antagonized with atipamezole and flumazenil, respectively.[3–5] This can mitigate prolonged recovery times, a current anesthetic challenge in reptiles, and is often a major consideration for drug selection in this taxon.[3–5] Though no specific antagonist is available, ketamine, an NMDA receptor agonist and dissociative anesthetic, is also frequently used in reptile chemical restraint due to its efficacy, short half-life, and high safety profile.[3–5] In light of the apparent success of this drug combination, objective evaluation in other box turtle species is warranted.

In addition to drug selection, the injection site is another pertinent consideration for reptile chemical restraint. Unlike mammals, reptiles possess both renal and hepatic portal systems that receive blood flow directly from the caudal half of the body. As such, there is concern that caudal parenteral administration of drugs could result in direct delivery to the kidneys or liver. Depending on the drug and dose, this could result in target organ damage (ie, nephrotoxicity, hepatotoxicity) and/or hastened drug metabolism.[4,6,7] In several studies, the pharmacokinetics of antibiotics metabolized primarily by the kidneys did not significantly differ between cranial and caudal injection sites in reptiles;[6–9] however, the same may not be true for other drugs. Multiple pharmacodynamic studies in reptile species documented reduced clinical efficacy of hepatically metabolized anesthetic and analgesic drugs when administered in the caudal half of the body.[10–16] These findings are suggestive of a clinically relevant hepatic first-pass effect and are supported by the current understanding of reptilian caudal vascular anatomy. Studies in the red-eared slider (*Trachemys scripta elegans*) and green iguana (*Iguana iguana*) demonstrated that the majority of venous blood from the hind limbs drains into the ventral abdominal vasculature and passes directly through the liver before entering the systemic circulation.[4,17,18] The clinical relevance of this information is not fully understood and, as prospective evidence remains limited and pharmacokinetic data are scarce, parenteral administration of drugs into the caudal half of the body in reptiles remains controversial.[1,4,5,19] The effect of injection location on dexmedetomidine, ketamine, and midazolam specifically in reptiles has been minimally investigated and has not been studied in box turtles. As all 3 drugs are primarily metabolized by the liver in the studied species,[20] they may be subject to a hepatic first-pass effect after hindlimb administration in chelonians, which could result in alterations in the onset, degree, and duration of effects.

The objective of this study was to evaluate the anesthetic effects of forelimb vs hindlimb administration and the addition of midazolam on intramuscular (IM) dexmedetomidine-ketamine in eastern box turtles (*Terrapene carolina carolina*). We hypothesized that hindlimb rather than forelimb administration of dexmedetomidine and ketamine would result in reduced anesthetic efficacy, while the addition of midazolam would result in enhanced anesthetic efficacy in eastern box turtles.

Methods

Animals

This study was approved by the North Carolina State University Institutional Animal Care and Use Committee (22-272-O). Twenty, clinically healthy, client-owned eastern box turtles (*Terrapene carolina carolina*) (9 males, 11 females) living in central North Carolina, were enrolled. Turtles were group-housed as 2 separate populations of 9 (3 males, 6 females) and 11 individuals (6 males, 5 females). Both groups were housed in similar outdoor enclosures and fed balanced omnivorous diets. Approximately 1 month before the study, packed cell volume (PCV), lactate (Lactate Plus; Nova Biomedical), and a biochemistry panel (VetScan VS2, Avian/Reptilian Profile Plus; Zoetis; specific analytes presented in **Table 1**) were obtained for each turtle. The study was conducted in August–September 2022, during which time daily temperatures in central North Carolina ranged from approximately 19 °C to 35 °C.

Study design

Client-supplied food was withheld from turtles for a minimum of 24 and 72 hours before the first

Table 1—Baseline packed cell volume (PCV), lactate, and biochemistry (VetScan VS2, Avian/Reptilian Profile Plus, Zoetis; Lactate Plus, Nova Biomedical) results for 20 clinically healthy, captive adult eastern box turtles (*Terrapene carolina carolina*; 9 males, 11 females).

Parameter	Median	Range
PCV (%)	28	21–44
Lactate (mmol/L)	3	0.8–15.5
AST (U/L)	136	49–301
Bile Acids (umol/L)	0	0–4
Creatine Kinase (U/L)	626	0–1185
Uric Acid (mg/dL)	0.1	0–0.7
Glucose (mg/dL)	86	44–154
Calcium (mg/dL)	15.5	11.0–>20.0
Phosphorus (mg/dL)	4.1	2.0–12.1
Total Protein (g/dL)	5.6	3.9–8.3
Albumin (g/dL)	2	1.4–3.7
Globulin (g/dL)	3.8	2.5–4.9
Potassium (mmol/L)	4.2	3.2–5.1
Sodium (mmol/L)	137	131–143

Blood was collected approximately 1 month before the study.

and subsequent study days, respectively; the latter was performed to minimize the risk of regurgitation based on results from the first study day (described below). Environmental food sources were not controlled but were assumed to be a minimal part of the diet. On each study day, turtles were acclimated to the climate-controlled testing room (21–22 °C) for 1 hour. During that time, each turtle was weighed and received a physical examination. On the second and third study days, baseline heart rates were also collected using a Doppler ultrasonic flow device (Model 811-B; Parks Medical Electronics Inc) placed in the caudal cervical region. Due to oversight, baseline heart rates were not obtained on the first study day. Turtles were then left undisturbed for at least 15 minutes before treatment.

In a complete crossover design with a 1-week washout period between treatments, turtles received each of 3 treatments: forelimb dexmedetomidine (Dexmedesed; Dechra), ketamine (Ketaset; Zoetis), and 0.9% sodium chloride (0.9% NaCl, Hospira) (F-DKS, 0.1, 10 mg/kg and 0.2 mL/kg, respectively), forelimb dexmedetomidine, ketamine, and midazolam (Athenex) (F-DKM, 0.1, 10, and 1.0 mg/kg, respectively), and hindlimb dexmedetomidine, ketamine, and midazolam (H-DKM, 0.1, 10, and 1.0 mg/kg, respectively). The treatment order was randomized for each turtle.

To standardize the total injection volume and number of injections, 0.9% NaCl was added to F-DKS at an equivalent volume of midazolam. All drugs were administered as separate IM injections in the cranial brachium (F-DKS, F-DKM) or caudal thigh (H-DKM) using 0.5 mL insulin syringes with 28G 1/2-inch needles. Forelimb injections were alternated right or left side for successive weeks. Due to a missing left hindlimb in 1 turtle, only right hindlimb injections were performed. Doses were chosen based on the results of pilot data collected from naïve, clinically healthy, wild-caught eastern box turtles (North Carolina State University Turtle Rescue Team) and the results of a prior study in ornate box turtles.[2]

Monitoring and scoring

Immediately after treatment administration, each turtle was placed in an individual open-top container (approximately 34 cm X 34 cm X 12 cm) and continuously observed for time to first effect, defined as slowing or cessation of ambulation, and time to cessation of gross, purposeful movement. Five minutes after treatment administration, each turtle was manually moved to a table and maintained there for the remainder of the test period. If the turtle attempted to ambulate, it was returned to the container for an additional 5 minutes and this process repeated.

From the time of treatment administration, each turtle was serially monitored every 5 minutes for spontaneous ventilation via visual assessment, heart rate, and degree of sedation (described below). The cloacal temperature was measured at 20, 40, and 60 minutes (Mindray Passport 12; Mindray North America). To avoid excessive stimulation that could affect the level and progression of sedation, heart rate or temperature collection was aborted if turtles subjectively demonstrated resistance (eg, retracting into the shell, kicking, and/or struggling). A modified sedation scoring rubric was developed based on prior chelonian sedation studies.[2,21] Each of the 10 parameters was categorized as present (0), reduced (1), or absent (2) at each time point and totaled to calculate a cumulative sedation score ranging from 0–20, with 0 correlating to no sedation and 20 correlating to maximum sedation **(Table 2)**. Palpebral reflex was assessed using a cotton tip applicator gently tapped on the medial canthus. Muscle tone was evaluated via manual manipulation of the neck, limbs, plastron, and jaw. For the latter, a disposable plastic fingernail pick was used to aid in manipulating the mandible. Limb and tail withdrawal responses were assessed via a single clamp of a 14 cm Kelly hemostat. All sedation scoring was performed by 1 of 2 investigators (ACH, RGC) blinded to treatment groups and randomly assigned to turtles on each study day. To support consistency in scoring technique and clearly define scoring criteria, both blinded investigators jointly assessed 2 aforementioned pilot turtles. They then independently assessed 4 subsequent pilot turtles and compared scores to ensure similarity in scoring before the primary study.

Intubation was attempted using a lightly lubricated 2-mm uncuffed endotracheal tube in any turtle with an absent jaw tone. If successful, a mainstream capnograph (TidalGuard; Newtech, Inc) was attached and used to assess spontaneous ventilation for the remainder of the intubation period. Apnea was defined as the absence of an end-tidal carbon dioxide measurement for >1 minute. If a turtle was apneic, assisted ventilation of approximately 1 breath per minute was provided via a manual

Table 2—Sedation scoring criteria for adult eastern box turtles (*Terrapene carolina carolina*) after IM administration of dexmedetomidine-ketamine-0.9% sodium chloride (0.1, 10 mg/kg, and 0.2 mL/kg, respectively) in the forelimb or dexmedetomidine-ketamine-midazolam (0.1, 10, and 1.0 mg/kg, respectively) in the forelimb or hindlimb.

Variables	Score
Lower eyelids open[a]	0–2
Palpebral reflex[a]	0–2
Neck muscle tone	0–2
Forelimb muscle tone[a]	0–2
Hindlimb muscle tone[a]	0–2
Forelimb withdrawal response[a,b]	0–2
Hindlimb withdrawal response[a,b]	0–2
Tail withdrawal response[b]	0–2
Plastron tone	0–2
Jaw tone	0–2
Total	0–20

Variables were assessed every 5 minutes for 60 minutes in the order listed and scored as present (0), reduced (1), or absent (2) by 1 of 2 investigators blinded to treatment.

[a]Left and right sides were scored independently and averaged. [b]Limb and tail withdrawal responses were only tested if limb muscle tone was absent.

resuscitator bag (FiO_2 0.21). Three-lead electrocardiography (Mindray Passport 12; Mindray North America) was attempted during pilot data collection but was unsuccessful and not attempted during the primary study.

Antagonist administration and recovery

Sixty minutes after treatment administration, turtles were manually restrained for antagonist administration. Due to the weight of the endotracheal tube connector and concern for accidental and/or traumatic extubation, intubated turtles were extubated before handling. Each turtle received atipamezole (Antisedan; Zoetis; 1.0 mg/kg) with either 0.9% NaCl (0.5 mL/kg) (F-DKS) or flumazenil (Hikma Pharmaceuticals; 0.05 mg/kg) (F-DKM, H-DKM) administered as separate IM injections using 0.5 mL insulin syringes with 28G 1/2-inch needles. To standardize the total injection volume and number of injections, 0.9% NaCl was given with atipamezole at an equivalent volume of flumazenil in F-DKS turtles. Injections were administered contralateral to the initial injections in the forelimb (F-DKS; F-DKM) or in the left hindlimb (H-DKM). For the single turtle missing its left hindlimb, antagonists were administered in the right hindlimb (H-DKM). Reintubation was attempted for any previously intubated turtles, and if successful, the endotracheal tube was maintained in place until swallowing was observed. Respiratory rate, heart rate, and level of sedation were assessed every 5 minutes until recovery, defined as the turtle lifting its head and moving its eyes in response to movement of a standardized object (ink pen) in its field of vision (ie, visual tracking).

Statistical analysis

Statistical analyses were performed using commercially available software (Prism Version 9.2; GraphPad Software, Inc; R Version 4.1.2 with *lme4* and *lmer*Test packages; The R Foundation) with significance set at $P < .05$. Data were tested for normality using a Shapiro-Wilk test. A Friedman's test with Dunn's test for multiple comparisons was used to compare paired cumulative sedation scores of each turtle among treatments at each 5-minute time point, paired time to first effect among treatments, paired time to cessation of purposeful, spontaneous movement among treatments, and paired recovery time among treatments. As heart rates were not collected when turtles were resistant to Doppler assessment, paired heart rate comparisons were not consistently possible; thus, a Kruskal-Wallis test with Dunn's test for multiple comparisons was used to compare unpaired heart rates among groups at each 5-minute time point. Variances for each treatment at each time point were calculated, and variance through time was compared among treatments using a paired Mann-Whitney test with Bonferroni correction for multiple comparisons. A McNemar test was used to compare paired intubation success rates among treatments. Heart rates collected on the second and third study days were compared using a paired t test. Lastly, inter-investigator variability in scores for each treatment at each time point was evaluated using a Mann-Whitney test.

Results

All 20 turtles were deemed clinically healthy and PCV, lactate, and biochemistry results were within clinically acceptable limits (Table 1).[22,23] Overall, the median (range) body weight was 422 (321–494) g for males and 452 (367–562) g for females.

F-DKM resulted in significantly higher sedation scores than F-DKS and H-DKM at each 5-minute time point from 10 to 60 minutes ($P < .05$) **(Figure 1)**. F-DKS resulted in higher median sedation scores than H-DKM at most time points, but no significant differences were identified. Across all time points, H-DKM resulted in significantly more variable sedation scores than F-DKS ($P = .0073$) or F-DKM ($P = .0278$). The sedation score interquartile range and range were wider for H-DKM compared to F-DKS and F-DKM at all time points from 25 to 60 minutes **(Table 3)**. The number of turtles for which each of the 10 scored parameters was recorded as absent for at least 3 consecutive time points during each treatment period is presented **(Table 4)**.

Median (range) time to first effect (slowing or cessation of ambulation) was 2.4 (1.0–4.1), 1.9 (0.8–4.0), and 2.8 (0.8–10.0) minutes for F-DKS, F-DKM, and H-DKM, respectively. While these times were clinically similar, the time to first effect was significantly faster for F-DKM compared to H-DKM ($P = .0170$). Median (range) time to the cessation of purposeful, spontaneous movement was 5.0 (1.0–10.0), 4.8 (1.0–10.0), and 10.0 (2.5–25.0) minutes for F-DKS, F-DKM, and H-DKM, respectively; this was significantly faster for F-DKM compared to H-DKM ($P = 0.0273$). Additionally, this period was subjectively smooth for all turtles with no adverse responses observed.

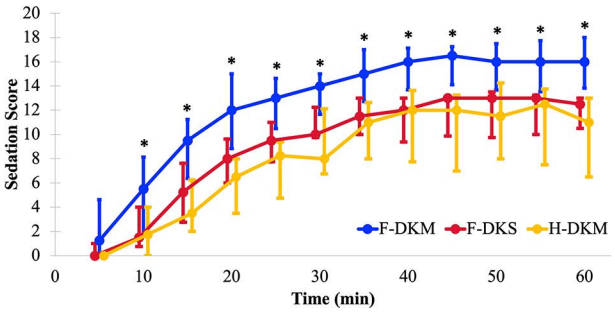

Figure 1—Median and 25–75th percentile cumulative sedation scores for 20 clinically healthy, captive adult eastern box turtles (*Terrapene carolina carolina*; 9 males, 11 females) administered IM dexmedetomidine-ketamine-0.9% sodium chloride (0.1, 10 mg/kg, and 0.2 mL/kg, respectively) in the forelimb (F-DKS) or IM dexmedetomidine-ketamine-midazolam (0.1, 10, and 1.0 mg/kg, respectively) in the forelimb (F-DKM) or hindlimb (H-DKM). A score of 0 represents no sedation and a score of 20 represents maximal sedation. *Significantly higher ($P < .05$) for F-DKM compared to F-DKS and H-DKM at all time points from 10 to 60 minutes.

Table 3—Interquartile range (difference between 75th and 25th percentiles) and range (difference between maximum and minimum) cumulative sedation scores for 20 clinically healthy, captive adult eastern box turtles (*Terrapene carolina carolina*; 9 males, 11 females) after administration of IM dexmedetomidine-ketamine-0.9% sodium chloride (0.1, 10 mg/kg, and 0.2 mL/kg, respectively) in the forelimb (F-DKS) or IM dexmedetomidine-ketamine-midazolam (0.1, 10, and 1.0 mg/kg, respectively) in the forelimb (F-DKM) or hindlimb (H-DKM).

	Interquartile range			Range		
	Treatment			Treatment		
Time (min)	F-DKS	F-DKM	H-DKM	F-DKS	F-DKM	H-DKM
5	1.0	4.6	0.3	4.0	9.0	3.0
10	3.0	5.6	4.0	8.0	14.0	6.0
15	4.9	5.3	4.3	9.0	11.0	9.0
20	3.6	5.0	4.5	10.0	11.0	15.0
25	3.3	2.6	4.6	9.5	10.0	15.0
30	2.5	2.0	5.4	12.0	11.0	16.0
35	3.0	3.3	4.6	10.0	9.5	16.0
40	3.6	3.1	5.9	10.5	9.0	17.0
45	3.3	3.3	6.3	10.0	9.0	14.5
50	3.8	2.8	6.3	10.5	10.0	13.0
55	3.3	3.8	6.3	10.5	9.0	16.0
60	2.5	3.8	6.5	9.0	7.0	18.0

A score of 0 represents no sedation and a score of 20 represents maximal sedation.

Table 4—Number of clinically healthy, captive adult male and female eastern box turtles (*Terrapene carolina carolina*) out of 20 turtles total for which each scored parameter was recorded as absent for at least 3 consecutive time points from 5 to 60 minutes after each of 3 treatments (IM dexmedetomidine-ketamine-0.9% sodium chloride [0.1, 10 mg/kg, and 0.2 mL/kg, respectively] in the forelimb [F-DKS], IM dexmedetomidine-ketamine-midazolam [0.1, 10, and 1.0 mg/kg, respectively] in the forelimb [F-DKM] or hindlimb [H-DKM]).

Parameter	F-DKS	F-DKM	H-DKM	No. of turtles
Lower eyelid open[a]	20	20	20	20
Palpebral reflex[a]	0	3	0	20
Neck muscle tone	18	20	15	20
Forelimb muscle tone[a]	13	20	13	20
Hindlimb muscle tone[a]	3	16	9	20
Forelimb withdrawal response[a,b]	4	15	5	20
Hindlimb withdrawal response[a,b]	0	6	0	20
Tail withdrawal response[b]	1	3	0	20
Plastron tone	20	20	15	20
Jaw tone	13	20	10	20

[a]Left and right sides were scored independently and are listed as absent only if absent bilaterally. [b]Limb and tail withdrawal responses were only tested if limb muscle tone was absent.

A small, pale, well-demarcated, raised lesion was identified at the left lateral border of the glottis of a single turtle upon opening the mouth for intubation during the first study day. The etiology of the lesion was unknown, and intubation was not attempted for this turtle on any study day. As such, this turtle was not included in the intubation analysis. Of the remaining turtles, 13/19, 19/19, and 10/19 turtles in F-DKS, F-DKM, and H-DKM, respectively, had absent jaw tone for at least 3 consecutive time points, with a median (range) time to initial loss of jaw tone of 20 (10–30), 20 (5–35), and 28 (15–45) minutes, respectively. Overall, intubation was successfully achieved in 6/19, 17/19, and 2/19 turtles F-DKS, F-DKM, and H-DKM, respectively. This outcome was statistically significant for F-DKM compared to F-DKS (P = .0026) and H-DKM (P = .0003). The median (range) time to successful intubation was 28 (15–40), 30 (15–45), and 50 (50) minutes for F-DKS, F-DKM, and H-DKM, respectively. Intubation nonsuccess was attributable to glottal tone or the development of jaw tone with stimulation.

The median (range) recovery time was 10.1 (5.0–22.0), 16.0 (7.2–45.0), and 12.2 (3.5–28.3) minutes for F-DKS, F-DKM, and H-DKM, respectively. Though clinically similar, median recovery time was statistically longer for F-DKM compared to F-DKS (P = .0006); no significant differences in recovery time were detected between H-DKM and F-DKS, nor between H-DKM and F-DKM. The recovery period was subjectively smooth for all turtles with no adverse effects noted. On the first study day, 4 turtles (2 F-DKS, 1 F-DKM, 1 H-DKM) regurgitated 20 to 90 minutes after recovery and this prompted the lengthened fasting times on the remaining study days. No turtles regurgitated on subsequent study days. No other adverse reactions were observed during or after any treatment. All turtles resumed normal behaviors (eg, ambulation, foraging, and eating) after returning to outdoor enclosures (2–5 hours after recovery). All turtles remained clinically healthy for 1 week after the final study day.

Median (range) baseline heart rate on the second and third study days was 41 (26–50) and 34 (24–44) bpm, respectively. Heart rate decreased over time compared to baseline for turtles in all groups **(Figure 2)**. Statistical comparisons were not performed for heart rates at the 5-minute time point, as many turtles were minimally sedate and resistant to Doppler assessment at this time point (n = 13, n = 8, and n = 16 for F-DKS, F-DKM, and

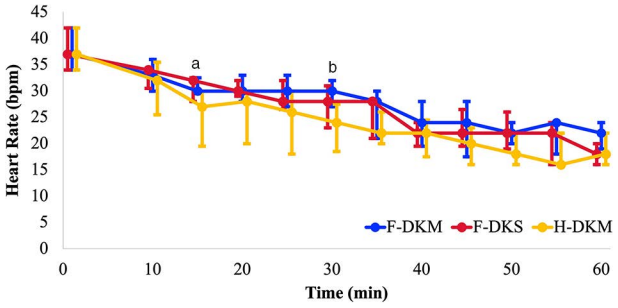

Figure 2—Median and 25–75th percentile heart rates (bpm) for 20 clinically healthy, captive adult eastern box turtles (*Terrapene carolina carolina*) administered IM dexmedetomidine-ketamine-0.9% sodium chloride (0.1, 10 mg/kg, and 0.2 mL/kg, respectively) in the forelimb (F-DKS) or IM dexmedetomidine-ketamine-midazolam (0.1, 10, and 1.0 mg/kg, respectively) in the forelimb (F-DKM) or hindlimb (H-DKM). [a]Significantly different ($P < .05$) for F-DKS compared to H-DKM at 15 minutes. [b]Significantly different ($P < .05$) for F-DKM compared to H-DKM at 30 minutes.

H-DKM, respectively). Median heart rate was consistently lower with H-DKM compared to F-DKM over the 60-minute monitoring period; this difference was only statistically significant at the 30-minute time point (Figure 2). In both intubated and nonintubated turtles, spontaneous ventilation was rarely observed (either visually or via capnograph) after cessation of spontaneous movement. As a result, all intubated turtles were manually ventilated for the duration of intubation. Median (range) end-tidal carbon dioxide concentration during intubation was 18.7 (8.4–35.4), 18.6 (7.0–35.9), and 19.7 (12.4–22.9) mmHg for turtles in F-DKS, F-DKM, and H-DKM, respectively.

Cloacal temperatures remained within 2 °C of room temperature for turtles in all groups. A single male turtle experienced marked bradycardia at the 45-minute time point on each study day. The heart rate steadily decreased from 24–26 bpm at baseline to 8–10 bpm at 45 minutes; the concurrent sedation scores at the latter time point were 11, 17, and 12, for F-DKS, F-DKM, and H-DKM, respectively. On each day, antagonists were promptly administered, and the remainder of the trial was aborted. The turtle recovered uneventfully each day. Data for this turtle were excluded from analysis for the 50-minute time point onward.

Sedation scores were not significantly different between blinded investigators at any time point for F-DKM and H-DKM. For F-DKS, sedation scores were only significantly different at a single time point (30 minutes) ($P = .0465$).

Discussion

Administration of dexmedetomidine-ketamine-midazolam (0.1, 10, and 1.0 mg/kg, respectively) in the hindlimb of clinically healthy eastern box turtles resulted in reduced and significantly more variable anesthetic effects compared to administration in the forelimb. This was supported by significantly lower median cumulative sedation scores and wider interquartile and minimum-maximum ranges at the majority of time points after hindlimb compared to forelimb administration. Additionally, the median time to cessation of spontaneous movement for turtles in H-DKM was approximately twice as long as turtles receiving either forelimb treatment. Given the current knowledge of chelonian vascular anatomy[17] and that all 3 drugs are primarily metabolized in the liver,[20] the decreased level of sedation achieved with hindlimb administration supports a hepatic first-pass effect in the current study. Alternative explanations for the decrease in efficacy include differences in drug absorption, muscle perfusion, fat stores, and/or blood flow between forelimb and hindlimb injection sites and individual differences in response to drugs unrelated to the hepatic portal system.

The effect of injection sites on the pharmacology of anesthetic drugs has been studied in other reptile species with mixed results. In leopard geckos (*Eublepharis macularius*), a similar effect of injection site (forelimb vs hindlimb) was documented with IM administration of dexmedetomidine-ketamine.[12] In red-eared sliders (*Trachemys scripta elegans*), SC hindlimb administration of another hepatically metabolized drug, buprenorphine, resulted in significantly lower plasma concentrations compared to forelimb administration.[10] Finally, decreased anesthetic efficacy was reported in yellow-bellied sliders (*Trachemys scripta scripta*) and ball pythons (*Python regius*) after IM or IV administration of alfaxalone (also hepatically metabolized) in the caudal half of the body.[13–15] In contrast, a recent study detected no significant pharmacokinetic or pharmacodynamic differences between forelimb and hindlimb administration of IM alfaxalone in bearded dragons (*Pogona vitticeps*).[24] Similarly, no significant differences in anesthetic effects were noted with hindlimb rather than forelimb administration of a xylazine-ketamine combination in broad-snouted caimans (*Caiman latirostris*).[25] These results in combination with the present study highlight the potential for species-, drug-, and dose-based differences with regards to the injection site, but support a likely hepatic first-pass effect in chelonians and eastern box turtles, specifically.

Turtles amongst all test groups demonstrated inter-individual variability in anesthetic effects (Table 3) and this was likely influenced by alternative explanations above (eg, variable bioavailability, metabolic differences). That said, variability in sedation scores was significantly greatest amongst turtles in H-DKM, underscoring the potential clinical variability associated with a hepatic first-pass effect. Compromised hepatic or renal function could alternatively explain the individual variability; however, given that all turtles were clinically healthy with normal biochemistry results, this seems unlikely. Similarly, while possible, it is unlikely that inter-investigator variability contributed to these findings, as sedation scores generated by each blinded investigator were statistically and clinically similar save for a single time point in 1 treatment.

IM administration of dexmedetomidine (0.1 mg/kg) and ketamine (10 mg/kg) in the forelimb of clinically

healthy eastern box turtles produced significantly greater sedation when midazolam (1.0 mg/kg) was added. This was supported by significantly higher median cumulative sedation scores and a higher intubation success rate (89% vs 32%) in F-DKM compared to F-DKS. This finding is not unexpected as both published literature and clinical anecdotes support the efficacy of midazolam as a sedative in numerous reptile species[26–29] and it is routinely used in clinical practice.[3–5] In the current study, F-DKM permitted intubation and abolished muscle tone and forelimb withdrawal response in the majority of turtles; hindlimb and tail withdrawal response was largely maintained. As such, this protocol could be considered for use in clinical situations in which heavy sedation or a light plane of anesthesia are desired. While not definitively proven in eastern box turtles, ketamine and dexmedetomidine likely also provide some degree of analgesia, an added benefit if performing a painful procedure or in a turtle with a source of pain.[1,3–5,30,31]

The clinical effects of F-DKM administered to eastern box turtles in this study were largely similar to those reported with the same protocol in a related species, the ornate box turtle.[2] In both species, F-DKM resulted in observable first effects within 5 minutes, provided clinically relevant sedation lasting at least 40 minutes, and permitted smooth and clinically rapid recoveries (<30 minutes) after antagonist administration. While cumulative sedation scores were not calculated for ornate box turtles, many of the same parameters were evaluated and reported as present, reduced, or absent, permitting a qualitative comparison of anesthetic depth between species. Generally speaking, F-DKM resulted in a shorter time to peak effects and marginally deeper sedation in ornate box turtles relative to eastern box turtles in the present study. Additionally, F-DKM permitted intubation in only 89% of eastern box turtles (17/19) with a median (range) time to successful intubation of 30 (15–45) minutes, while all ornate box turtles (16/16) were intubated within 15 minutes of drug administration.[2] These variations may reflect true species differences; however, the possible contribution of environmental (eg, room temperature, time of year) or other population-based differences (eg, amenability to handling, baseline health status, age) cannot be ruled out. It is also important to note that, in contrast to the present study, assisted positive pressure ventilation was not performed for ornate box turtles in the aforementioned study despite observed hypoventilation and/or apnea.[2] Thus, consequences of hypoventilation (eg, hypoxemia, hypercapnia, acid-base imbalance, and/or tissue hypoxia) could have impacted the level of apparent anesthetic depth or response to stimulation.

All 3 protocols in the current study produced hypoventilation and in many instances apnea, as spontaneous ventilation was rarely observed. While this was subjectively assessed in nonintubated turtles and potentially an imperfect metric, if spontaneous ventilation was present in these turtles, it was likely shallow at best. Additionally, while it is possible that the capnograph utilized in the present study was not sensitive enough in the face of a shallow, spontaneous breath, end-tidal carbon dioxide concentrations were successfully measured with every assisted breath. Recall that in F-DKS and H-DKM, turtles permitted intubation in only 6 and 2 of 38 total trials, respectively; thus, many turtles in these groups were likely hypoventilated and were not able to be provided with assisted ventilation. Although chelonians are hypoxia-tolerant in comparison to mammals and no turtles in the current study demonstrated any clinically apparent side effects of hypoventilation, any acute or chronic effects are unknown. While anecdotally not always practiced, intubation and positive pressure ventilation are the arguable standard of care for sedated or anesthetized reptiles exhibiting hypoventilation, including chelonians.[32–35] As such, F-DKS or H-DKM should be used with caution, especially in turtles with compromised cardiorespiratory function or other comorbidities that would render them less tolerant to sequelae of hypoventilation.

Turtles in all groups demonstrated a decrease in heart rate over the study period. While this was statistically significant between groups at 2 time points (Figure 2), these singular inter-group differences were not clinically relevant. The observed bradycardia is likely largely a result of the cardiovascular effects of dexmedetomidine, which causes peripheral vasoconstriction and a subsequent reflex bradycardia.[36] While this can result in decreased cardiac output, the net cardiovascular consequences of the tested drug combinations in the current study are unknown. Comparable decreases in heart rate were observed in the aforementioned study in ornate box turtles and a study using the same protocol in a larger chelonian species, the red-footed tortoise (*Chelonoidis carbonaria*).[2,21]

All 3 protocols permitted smooth and clinically rapid (< 30 minutes) recoveries, with 1 exception (45 minutes for a single turtle in F-DKM). No turtles experienced resedation and, aside from the isolated incidents of regurgitation on the first study day, the recovery period was uneventful, and turtles swiftly resumed normal behaviors. As prolonged recovery time continues to be a challenge of chelonian anesthesia, the consistent and clinically favorable recovery periods observed in the present study lends support to the suitability of the tested protocols in eastern box turtles.

Challenges associated with cardiopulmonary assessment in chelonians presented several limitations in the present study. Pulse oximetry, electrocardiography, and blood pressure monitoring are standard of care for anesthetized veterinary species; however, pulse oximetry has not been validated for reptiles[4] and electrocardiography was precluded in the present study due to detection limits of available equipment. Oscillometric blood pressure monitoring has been investigated in boids and iguanas but was not an accurate substitute for direct measurements.[37,38] Direct blood pressure monitoring has not previously been reported in eastern box turtles, and it would be challenging given the small patient size. Similarly,

while blood gas analysis would have provided additional, clinically useful information, the inherent challenges of repeated venipuncture in small reptiles discouraged its use in the current study. Mainstream capnography was utilized in this study; however, the accuracy in using end-tidal carbon dioxide ($ETCO_2$) values to estimate arterial or venous carbon dioxide concentration has not been established in reptiles, though it may be useful for monitoring trends.[4,39] Lastly, although the sedation scoring system utilized in the present study quantitatively reflected the subjective clinical differences observed between treatments and was modeled after previous chelonian sedation studies,[2,21] it has not been formally validated and, thus, may be a confounding factor. That said, to the authors' knowledge, no validated sedation scoring systems exist for chelonians.

In conclusion, forelimb dexmedetomidine-ketamine resulted in clinically relevant anesthetic effects in eastern box turtles that were heightened with the addition of midazolam. Additionally, hindlimb rather than forelimb IM administration of dexmedetomidine-ketamine-midazolam resulted in significantly reduced and more variable anesthetic effects in eastern box turtles. This finding is suggestive of a clinically relevant hepatic first pass that should be considered when administering the above combination or any hepatically metabolized anesthetic or analgesic drugs in box turtles. For all 3 evaluated protocols, recovery times were favorable after antagonist administration.

Acknowledgments

The authors thank Jeff and Eric Ginsberg, Andrea Thomson, Kent Passingham, James Robertson, Holly Amato, and the North Carolina State University Turtle Rescue Team for study assistance and support.

Disclosures

The authors declare no conflicts of interest. No AI-assisted technologies were used in the generation of this manuscript.

Funding

The authors have nothing to disclose.

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