Comparison of isoflurane, sevoflurane, and desflurane as inhalant anesthetics in prairie rattlesnakes (*Crotalus viridis*)

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OBJECTIVE
To characterize induction and recovery characteristics of 3 commonly used inhalant anesthetics in prairie rattlesnakes (*Crotalus viridis*): isoflurane, sevoflurane, and desflurane.

ANIMALS
12 healthy adult prairie rattlesnakes.

PROCEDURES
In a randomized crossover design, snakes underwent anesthetic induction with 5% isoflurane, 8% sevoflurane, or 18% desflurane, with a washout period of ≥7 days between anesthetic events. Anesthetic depth parameters were recorded throughout induction and recovery, including time to loss and return of righting reflex, muscle tone, ability to intubate, response to pressure, and time to return to spontaneous respiration. Every 5 minutes throughout the anesthetic procedures, heart rate, respiratory rate, and percentage expired anesthetic gas were recorded.

RESULTS
No snakes died during the study. Sevoflurane anesthesia resulted in anesthetic gas avoidance behavior in snakes during induction and had the significantly longest recovery time to extubation and time to return of pressure response, compared with the other inhalant anesthetics. Anesthesia with isoflurane resulted in a significantly longer time to return of righting reflex, compared with sevoflurane or desflurane. No significant difference was noted in time to loss of pressure response among the 3 anesthetic gases. Desflurane anesthesia resulted in the significantly quickest loss of righting reflex among the anesthetic protocols; despite this, 4 of 12 desflurane anesthetized snakes did not achieve an anesthetic plane deep enough for intubation.

CONCLUSIONS AND CLINICAL RELEVANCE
Isoflurane and sevoflurane, but not desflurane, inhalation anesthesia resulted in consistent and predictable loss of righting reflex and induction of anesthesia deep enough to allow intubation in snakes. (J Am Vet Med Assoc 2020;257:945–949)

Inhalant anesthetics are commonly used to anesthetize snakes for handling, examination, sample collection, and other veterinary procedures. Because of the difficulty of venous access in snakes, large injection volumes, inconsistent anesthetic depth, and potentially prolonged recovery following IM administration of anesthetics, inhalant drugs are often preferred for anesthetic induction and maintenance. Administration of anesthetics through inhalation for venomous species provides safer drug administration, compared with injection. The induction can be performed through a chamber, thus eliminating direct handling for drug administration. Despite being used commonly, there are few studies that have evaluated the effectiveness of inhalant anesthetics on snakes, no studies on venomous species, and only a few studies that have evaluated the effectiveness of these anesthetics in reptiles.

In a survey provided to the Association of Reptile and Amphibian Veterinarians, the most commonly used anesthetic is isoflurane. Sevoflurane has increased in popularity for reptiles in recent years owing to its lower blood-to-gas solubility, resulting in even more rapid induction and recovery. Desflurane was not documented to be used by any veterinarians surveyed.

Anesthetic depth in reptiles is frequently evaluated by loss or return of righting reflex, muscle relaxation, presence or absence of corneal reflex, or pressure response with forceps or a needle applied to the tail or foot. Semmes-Weinstein monofilaments are the current standard foresthesiometry, with pressure applied for assessment of nerve function in humans with neuropathies. These monofilaments have been used for assessment of analgesic effectiveness in rats and anesthetic depth in garter snakes.
The purpose of the study reported here was to evaluate the effects of 3 inhalant anesthetics (ie, isoflurane, sevoflurane, and desflurane) on the time to specific anesthetic end points in prairie rattlesnakes (Crotalus viridis). In addition, we determined whether any differences exist in speed of induction and speed of recovery between the different inhalant anesthetic agents. We hypothesized that inhalant anesthetics would provide safe, short-acting anesthesia sufficient for handling and that differences will be found in time to induction and time to recovery, with desflurane associated with the shortest times.

Materials and Methods

Snakes

Four male and 8 nongravid female healthy adult prairie rattlesnakes (mean body weight, 410 g [0.9 lb]) were individually housed in enclosuresa (36 X 19 X 18 inches) for the duration of the study. Room temperature was maintained at 23.9°C to 26.7°C (75°F to 80°F), and relative humidity ranged from 24% to 26%. All snakes were bedded on newspaper and provided water ad libitum. Snakes were offered killed mice every 2 weeks and were never handled or used in experiments during their feeding week. The experimental protocol was approved by the University of Illinois Institutional Animal Care and Use Committee (protocol No. 18177).

Experimental design

A prospective randomized controlled study design was used. Each snake was assigned to a treatment group with an online randomizer,b such that 4 snakes received each anesthetic agent on experimental days. The 3 study days (1 day for each anesthetic) were separated with a 2 to 7-day washout period between treatments. All experiments were performed between 8:30 AM and 3 PM to reduce the impact of natural circadian rhythm on results. No other medications were administered during the study period.

Induction of anesthesia

A Mapelson D anesthetic system with an out-of-circuit, agent-specific precision vaporizer was used for induction and anesthetic maintenance. The vaporizers were calibrated prior to the start of the study, and the same agent-specific vaporizer was used throughout. The vaporizer dial was set to achieve the maximum output of 5% for isoflurane, 8% for sevoflurane, and 18% for desflurane, with a fresh gas flow rate of 1 L/min. Snakes were removed from their enclosure with a snake hook and restrained in a clear acrylic tube (length, 4 feet; diameter, 1.5 inches). The clear acrylic tube had a sealed cap on one end and a sealed cap on the other end that was fitted with an anesthetic circuit adaptor through which the anesthetic gas could enter the tube. The tube contained 2 holes (diameter, 0.5 mm each) at the cranial and caudal halves of the body to allow for gas collection and pressure-response testing. A preanesthetic respiratory rate was obtained by direct observation, and the righting reflex and response to pressure were determined by use of a 26-g force Semmes-Weinstein monofilament thread from a commercially available sensory evaluation kit.c For each snake, pressure was applied on the dorsal midline of the cranial, middle, and caudal third of the body. If snakes did not respond with gross purposeful movement when pressure was applied to all 3 locations, the test result was considered negative. Loss of righting reflex was assessed by manually positioning snakes in dorsal recumbency. Every 5 minutes following induction of anesthesia, the righting reflex and response to pressure were assessed and anesthetic gas concentration was measured via a gas analyzerd near the animal's head.

Snakes were maintained in the chamber for a minimum of 5 minutes with 2 consecutive negative righting reflex results and absent response to pressure. Once these criteria were met, snakes were removed from the chamber, intubated with a 14-gauge IV catheter,e and mechanically ventilated at 4 breaths/min by use of an electronically controlled, pressure-cycled ventilatorf set to a maximum inspired pressure of 10 cm H2O with a fresh gas flow rate of 1 L/min. Airway gas concentrations were continually evaluated through a 3.5F catheter introduced at the tip of the endotracheal tube through a T-piece at the base of the tube; this was used to ensure that inhalant anesthetic concentrations were equivalent to the maximum output of the vaporizer.

Heart rate was monitored by use of Doppler echocardiography, and respiratory rate was monitored by direct observation at the time of intubation and 5 and 10 minutes following intubation. A gas analyzer was connected between the Mapelson D circuit and the endotracheal tube with a standard gas sampling line connector. Fractional concentration of O2 in inspired gas, fractional concentration of O2 in expired gas, end-tidal partial pressure of CO2, and end-tidal anesthetic gas concentrations were measured continuously. The gas analyzer was calibrated with the manufacturer's recommended calibration gas prior to each experiment.

Inhalation anesthesia and mechanical ventilation was discontinued 10 minutes after induction, and snakes were monitored every 5 minutes for return of spontaneous respiration, righting reflex, or response to pressure. Snakes were mechanically ventilated at 4 breaths/min with room air via a manual self-inflating resuscitation bag after 20 minutes of apnea until spontaneous respiration occurred. The endotracheal tube was removed when purposeful movement was observed.

Statistical analysis

Statistical analyses were performed with available software.b The Shapiro-Wilk test was used to test normal distribution of continuous variables (ie, elapsed times). Comparisons between anesthetic agent and time to loss of righting reflex, loss of pressure response, return of righting reflex, return of pressure
response, and time to extubation were made by use of a repeated-measures ANOVA. Differences were considered significant at a value of \( P < 0.05 \).

Results

No snakes died during the study. Isoflurane and sevoflurane administration resulted in the need for positive-pressure ventilation for 20 minutes after the anesthetic gas was discontinued in 3 snakes and 1 snake, respectively, prior to returning to spontaneous respiration. The order in which snakes received the anesthetic gas was not significantly \( (P = 0.175) \) associated with the need for manual ventilation.

Sevoflurane resulted in anesthetic gas avoidance behavior in snakes during induction and the longest recovery times to extubation \( (P = 0.016) \) and time to return of pressure response \( (P = 0.046) \), compared with the other inhalant anesthetics. Isoflurane anesthesia, compared with desflurane anesthesia, resulted in a 14-minute longer time to return of pressure response in snakes \( (P = 0.003) \).

Anesthesia with isoflurane resulted in a significantly longer time to return of righting reflex in snakes \( (\text{mean}, 28 \text{ minutes}; \text{median}, 23 \text{ minutes \{IQR, 20 to 36 minutes\}}) \), compared with sevoflurane anesthesia \( (\text{mean}, 16 \text{ minutes}; \text{median,} 15 \text{ minutes \{IQR, 12 to 14 minutes\}}; P = 0.026) \) and desflurane anesthesia \( (\text{mean,} 12 \text{ minutes; median,} 8 \text{ minutes \{IQR, 0 to 14 minutes\}}; P = 0.001) \). No significant \( (P = 0.346) \) difference was found in time to return of righting reflex in snakes between desflurane anesthesia and sevoflurane anesthesia \( \text{(Table 1)} \).

Desflurane anesthesia resulted in a significantly shorter time to intubation of snakes \( (\text{for those that were able to be intubated)} \), compared with anesthesia with isoflurane. Desflurane anesthesia resulted in the quickest loss of righting reflex in snakes among the anesthetic protocols \( (P = 0.029) \). The loss of righting reflex was 2.5 minutes faster following desflurane anesthesia, compared with sevoflurane anesthesia \( (P = 0.004) \). Despite the faster loss of righting reflex with desflurane, its use did not achieve a deep enough anesthetic plane for intubation in 4 of 12 snakes. These 4 snakes were unable to be intubated because of persistent jaw tone, preventing the mouth from being opened.

The present study was the first evaluating 3 inhaled anesthetic agents in a venomous reptilian species. Previous inhalant anesthetic comparisons in reptiles have focused primarily on MAC.\(^{11,13,14,24}\) Determination of MAC requires a prolonged equilibration period prior to restimulation when gross purposeful movements are observed.\(^{11,13,24}\) On the basis of the hypothesis for the present study, the objectives were met without the need to maintain snakes under inhalation anesthe sia for an extended period. The 3 inhaled anesthetic agents used in the present study were administered to the rattlesnakes at different MAC fractions. In Du meril monitors anesthetized with isoflurane, the MAC is similar to that in mammals but is lower than that in other reptiles, such as radiated rat snakes, green iguanas, or desert iguanas.\(^{10,11,141}\) Evaluation of MAC of sevoflurane, isoflurane, and desflurane in green iguanas revealed decreases in anesthetic gas concentrations over time, emphasizing the unique reptilian cardiorespiratory system and the necessity for species-specific inhalant anesthetic evaluation.\(^{15}\)

A comparison of isoflurane and sevoflurane in colubrid snakes produced profound respiratory and cardiac depression with both anesthetics.\(^{10}\) In contrast, heart rate did not differ under anesthesia with sevoflurane and isoflurane in green iguanas or in desert tortoises anesthetized with sevoflurane.\(^{6,25}\) Similarly in the present study, a few snakes anesthetized with isoflurane and sevoflurane required positive-pressure ventilation until spontaneous respiration returned and no heart rate differences were found between the anesthetic gases.

Several differences were detected in induction and recovery events for the inhalant anesthetics used in the present study. Most noticeably, isoflurane produced the longest time to return of righting reflex, sevoflurane had the longest time to return of pressure response, and desflurane had the shortest time to intubation and fastest time to loss of righting reflex. Interestingly, a comparison of isoflurane, sevoflurane, and desflurane in green iguanas noted no significant differences for any induction or recovery events for any of the inhalant agents.\(^{15}\) The discrepancies noted between the 2 reptilian species stress the importance of species-

Table 1—Mean ± SD interval from time of initial administration of an anesthetic agent to specific events during anesthetic induction and from time of cessation of administration of an anesthetic agent to specific events during recovery in 12 prairie rattlesnakes \( (Crotalus viridis) \) for 3 different inhalant anesthetics.

<table>
<thead>
<tr>
<th>Event (min)</th>
<th>Isoflurane</th>
<th>Sevoflurane</th>
<th>Desflurane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of pressure response</td>
<td>5 ± 2</td>
<td>2 ± 1</td>
<td>5 ± 4</td>
</tr>
<tr>
<td>Loss of righting reflex</td>
<td>3 ± 1</td>
<td>3 ± 3</td>
<td>2 ± 1</td>
</tr>
<tr>
<td>Intubation</td>
<td>7 ± 1</td>
<td>6 ± 1</td>
<td>5 ± 1</td>
</tr>
<tr>
<td>Return of pressure response</td>
<td>12 ± 11</td>
<td>20 ± 12</td>
<td>8 ± 4</td>
</tr>
<tr>
<td>Return of righting reflex</td>
<td>28 ± 15</td>
<td>16 ± 10</td>
<td>12 ± 5</td>
</tr>
<tr>
<td>Extubation</td>
<td>23 ± 10</td>
<td>33 ± 13</td>
<td>21 ± 3</td>
</tr>
<tr>
<td>Total anesthetic time</td>
<td>11 ± 1</td>
<td>15 ± 3</td>
<td>15 ± 4</td>
</tr>
</tbody>
</table>
specific anatomic differences. In chelonians and other reptilian species, inhalation anesthesia is challenging as a result of breath holding and intracardiac right-to-left shunting.\textsuperscript{15,20,21,25,26} Reptiles have a lower cardiac index than mammals, which may slow movement of anesthetic gas; however, they also have higher alveolar anesthetic partial pressures, resulting in a faster induction of anesthesia.\textsuperscript{15,27,28} The right-to-left shunting in noncrocodilian reptiles reduces the effective cardiac output for distribution of anesthetic-rich blood, and movement of inhaled anesthetics from the lungs to the blood can be impeded by large ventilation-perfusion mismatches.\textsuperscript{15,29,30} Lastly, the core body temperature of snakes could change the blood-gas partition coefficient of inhaled anesthetics if it is below their optimal temperature zone.\textsuperscript{1,15} Snakes in the present study were maintained at the same temperature for the duration of the study, eliminating temperature as a difference in inhalant gas recovery. Future studies should evaluate variations in cardiac index between different reptilian species, right-to-left shunting for ventilation-perfusion mismatch, and the effect of temperature differences in relation to inhalant anesthetics.

On the basis of the findings in the present study, Semmes-Weinstein monofilament threads may be a useful, noninvasive method for assessment of pressure response in both venomous and nonvenomous reptilian species. Monofilament threads were also used successfully for assessment of anesthetic depth in garter snakes anesthetized with intracoelomic administration of alfaxalone.\textsuperscript{16}

In the present study, isoflurane and sevoflurane in prairie rattlesnakes provided effective anesthesia while animals were at their optimal temperatures. Long-term use of inhalant anesthetics in rattlesnakes requires further research. Under the conditions of the present study, the use of desflurane in rattle-snakes, and potentially other vipers, is not recommended. Isoflurane and sevoflurane are effective for anesthetizing venomous snakes for quick, nonpainful procedures.

Footnotes

c. Baseline tactile monofilaments, Fabrication Enterprises, White Plains, NY.
d. Soma Technology, Henry Schein Medical, Melville, NY.
e. SURFLO, Terumo Medical Corp, Somerset, NJ.
g. Patterson Veterinary Supply Inc, Columbus, Ohio.
h. RSstudio Desktop, version 1.1.463, Rstudio Inc, Boston, Mass.

References


From this month’s AJVR

Comparison of histomorphometric characteristics of dorsal colon and pelvic flexure biopsy specimens obtained from horses with large colon volvulus that underwent resection

Liara M. Gonzalez et al

OBJECTIVE
To determine the degree of histomorphometric damage in dorsal colon and pelvic flexure biopsy specimens (DCBSs and PFBSs, respectively) obtained from horses with large colon volvulus (LCV) and assess the accuracy of predicting short-term outcome for those horses on the basis of DCBS or PFBS characteristics.

ANIMALS
18 horses with ≥ 360° LCV that underwent large colon resection.

PROCEDURES
During surgery, biopsy specimens from the dorsal colon resection site and the pelvic flexure (when available) were collected from each horse. Interstitial-to-crypt (I:C) ratio (ratio of the lamina propria space occupied by the interstitium to that occupied by crypts), hemorrhage within the lamina propria (mucosal hemorrhage score [MHS] from 0 to 4), and percentage losses of glandular and luminal epithelium were determined in paired biopsy specimens and compared to determine optimal cutoff values for calculating the accuracy of DCBS and PFBS characteristics to predict short-term outcome (survival or nonsurvival after recovery from surgery).

RESULTS
Paired biopsy specimens were obtained from 17 of the 18 horses. The I:C ratio and percentage glandular epithelial loss differed between DCBSs and PFBSs. For DCBSs, an I:C ratio ≥ 0.9 and MHS ≥ 3 each predicted patient outcome with 77.8% accuracy. For PFBSs, an I:C ratio ≥ 1 and MHS ≥ 3 predicted patient outcome with 70.6% and 82.4% accuracy, respectively.

CONCLUSIONS AND CLINICAL RELEVANCE
Although different, histomorphometric measurements for either DCBSs or PFBSs could be used to accurately predict short-term outcome for horses with LCV that underwent large colon resection, and arguably, PFBSs are easier to collect. (Am J Vet Res 2020;81:899–903)