Effects of sevoflurane anesthesia on righting reflex and hemolymph gas analysis variables for Chilean rose tarantulas (*Grammostola rosea*)

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**Objective**—To determine the safety, efficacy, and effects on hemolymph gas analysis variables of sevoflurane anesthesia in Chilean rose tarantulas (*Grammostola rosea*).

**Animals**—12 subadult Chilean rose tarantulas of unknown sex.

**Procedures**—Spiders were anesthetized in a custom chamber with sevoflurane (5% in oxygen [1.0 L/min]), then allowed to recover in 100% oxygen. Righting reflex was evaluated every 3 minutes during anesthesia to determine time to anesthetic induction and recovery. Hemolymph samples were collected from an intracardiac location prior to and after induction of anesthesia and evaluated to determine various gas analysis variables.

**Results**—Mean ± SD induction and recovery times were 16 ± 5.91 minutes and 29 ± 21.34 minutes, respectively. Significant differences were detected for \( P_{O_2} \), base excess, and glucose and ionized magnesium concentrations between hemolymph samples obtained before anesthesia and those obtained after induction of anesthesia.

**Conclusions and Clinical Relevance**—Results of this study suggested that the use of sevoflurane as an anesthetic agent for Chilean rose tarantulas was safe and effective. Various hemolymph sample gas analysis values changed during anesthesia. (*Am J Vet Res* 2014;75:521–526)

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Anesthesia is useful for performance of diagnostic procedures and treatments for invertebrates, such as physical examination and biological sample collection. Anesthesia also provides for the safety of invertebrate patients and their handlers during such procedures. Despite their popularity as companion and display animals, the clinical use of anesthesia for arachnid species has been infrequently evaluated. Information regarding anesthesia for arachnids is typically determined from anecdotal reports, rather than scientific analyses. Limited information regarding pharmaceutical anesthesia of theraphosid spiders (ie, tarantulas) is available. Carbon dioxide and isoflurane are effective for anesthesia of Chilean rose tarantulas (*Grammostola rosea*), and isoflurane is also effective for anesthesia of goliath birdeater tarantulas (*Theraphosa blondi*).

Sevoflurane is a polyfluorinated methyl isopropyl ether inhalant anesthetic that has a nonpungent odor, low blood solubility, and minimal cardiovascular and respiratory adverse effects. Sevoflurane is commonly used for anesthesia of humans and veterinary patients; other studies have been conducted to determine the effects of that drug in various domestic and exotic animal species including mice, rats, cats, dogs, pigs, rabbits, guanas, tortoises, and raptor and psittacine birds. To the authors' knowledge, no studies have been conducted to evaluate sevoflurane for any invertebrate species, although the use of that drug in such animals has been anecdotally reported.

The purpose of the study reported here was to determine the efficacy and safety of sevoflurane anesthesia for Chilean rose tarantulas and to identify changes in hemolymph sample gas analysis variables between conscious and anesthetized states of such spiders. The hypotheses were that sevoflurane could be used to safely and effectively anesthetize Chilean rose tarantulas, and that no significant differences in values of hemolymph gas analysis variables would be detected between samples collected when tarantulas were conscious and those collected when they were anesthetized.

**Materials and Methods**

**Animals**—Twelve subadult Chilean rose tarantulas (mean weight, 15.33 g [range, 13 to 18 g]) of
undetermined sex were used in this study. The spiders were determined to be healthy on the basis of results of physical examinations. They were housed individually in plastic enclosures with ground coconut hull substrate, were fed 5 gut-loaded adult crickets (Acheta domestica) weekly, and had access to water ad libitum. The spiders were maintained with a 12-hour light cycle, temperature of 23.9°C, and relative humidity of 75%. Invertebrate species were not included in the University of Illinois Institutional Animal Care and Use guidelines; however, the experiments were performed in accordance with animal welfare considerations similar to those used for vertebrate species.

Experimental protocol—Tarantulas were manually restrained, and hemolymph samples (0.2 mL) were collected from an intracardiac location and immediately transferred to tubes containing lithium heparin. Visible clots were manually removed, and the hemolymph samples were analyzed to determine pH, PO2, PCO2, TCO2, and BE and concentrations of bicarbonate, glucose, sodium, potassium, chloride, and ionized magnesium and calcium with a commercially available blood gas analyzer at the default temperature setting of 37°C. After hemolymph sample collection, spiders were placed in a 4-L custom anesthetic chamber that allowed manipulation of the animals while the chamber was closed. A blunt 14-gauge needle was inserted into the chamber at the level of each spider’s book lungs (approx 1 cm from the floor of the chamber) on the side opposite from the connection port to the anesthetic machine. The needle was used as the connection to a gas monitor for measurement of sevoflurane concentration in the anesthetic chamber.

The spiders were anesthetized with sevoflurane (5% in oxygen [flow rate, 1 L/min]) with a standard anesthetic machine intended for use with small animal patients; the anesthetic chamber was connected between the inspiratory and expiratory portions of the circle system. Spiders were evaluated every 3 minutes for detection of an RR. The RR was scored on an ordinal scale (0 = normal, 1 = attempted but unsuccessful, and 2 = absent). After induction of anesthesia (defined as the number of minutes until RR was not detected during 2 consecutive evaluation times), spiders were removed from the chamber and administration of sevoflurane was discontinued. Another hemolymph sample was collected from an intracardiac location immediately after removal of spiders from the chamber; these hemolymph samples were processed and analyzed by means of the same procedures used for samples obtained before anesthesia. Then, the spiders were returned to the chamber, exposed to 100% oxygen (1 L/min), and evaluated every 3 minutes for detection of an RR until anesthetic recovery (defined as the number of minutes from the end of anesthetic induction to the time the RR score was 0 [normal] during 2 consecutive evaluation times).

Each hemolymph sample collection procedure was completed in <1 minute. The sevoflurane concentration in the anesthetic chamber was recorded at each evaluation time. The temperature range in the chamber during all anesthetic procedures was 22.2° to 22.8°C.  

Statistical analysis—Data were analyzed with a commercially available software program. All data were evaluated for a Gaussian distribution with the Shapiro-Wilk test, skewness, and kurtosis. For determination of the overall anesthesia time, differences between RR scores at each monitoring time were evaluated with the Friedman 2-way ANOVA by ranks. For results with significant differences, the Wilcoxon signed ranks test was used to compare each evaluation time score to the score determined before anesthesia. Additionally, the measured concentration of sevoflurane in the anesthetic chamber during anesthetic induction was compared with the vaporizer setting (5%) at each evaluation time with a 1-sample t test. In addition, the data for each hemolymph sample gas analysis variable before anesthesia and after induction of anesthesia were compared with the Wilcoxon signed rank test or paired t test. Values of P ≤ 0.05 were considered significant.

Results

All spiders survived sevoflurane anesthesia and hemolymph sample collection without apparent adverse effects. As a percentage of body weight, the mean ± SD total amount of hemolymph collected from each spider was 2.35% ± 0.47%. For mammals, the volume of blood collected for testing is typically <1.5% of body weight; collection of a slightly greater amount in spiders in this study was not determined to be associated with any apparent immediate complications. Results of another study of tarantulas indicate that such animals tolerate the collection of similar volumes of hemolymph. The mean ± SD anesthetic induction and recovery times were 16 ± 5.91 minutes and 29 ± 21.34 minutes, respectively. A significant (P < 0.001) difference in the RR interval scores over time was detected. Significant (P = 0.025) differences in RR were detected through 39 minutes after the start of anesthesia, compared with scores determined before anesthesia.

Sevoflurane concentration in the chamber during induction of anesthesia was significantly (P < 0.001) different from the vaporizer setting (5%) through 9 minutes after the start of anesthesia (mean ± SD, 4.39% ± 0.28%). The mean sevoflurane concentration in the chamber was 4.91% ± 0.21% at 12 minutes after the start of anesthesia (approx 98% of the vaporizer setting value). This result was in agreement with the increase in the anesthetic concentration determined by first-order kinetics, in which 95% of the maximum concentration is attained after 3 time constants. Assuming that the volume of the rest of the anesthesia circuit was negligible, the chamber volume of 4 L and the flow rate of 1 L/min used in this study yielded a time constant of 4 minutes; 3 times this time constant was 12 minutes.

Descriptive statistical values for hemolymph sample gas analysis variables were summarized (Table 1). Values for PO2 and glucose and ionized magnesium concentrations were significantly higher in hemolymph samples obtained after induction of anesthesia than they were in samples obtained before anesthesia; BE was significantly lower in hemolymph samples ob-
Table 1—Values of hemolymph gas analysis variables for samples collected from an intracardiac location from 12 subadult Chilean rose tarantulas (Grammostola rosea) at rest prior to anesthesia and after induction of anesthesia with sevoflurane (5% in oxygen).

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Sample*</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Range</th>
<th>Distribution</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>C</td>
<td>7.480</td>
<td>0.069</td>
<td>7.479</td>
<td>7.303 to 7.570</td>
<td>G</td>
<td>0.13</td>
</tr>
<tr>
<td>Po2 (mm Hg)</td>
<td>1</td>
<td>157.41</td>
<td>28.74</td>
<td>168.50</td>
<td>90.3 to 178.8</td>
<td>NG</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>190.71</td>
<td>21.97</td>
<td>196.85</td>
<td>142.1 to 210.9</td>
<td>NG</td>
<td>—</td>
</tr>
<tr>
<td>Pco2 (mm Hg)</td>
<td>C</td>
<td>14.14</td>
<td>3.85</td>
<td>13.80</td>
<td>9.60 to 23.10</td>
<td>G</td>
<td>0.79</td>
</tr>
<tr>
<td>Tco2 (mmol/L)</td>
<td>C</td>
<td>10.73</td>
<td>1.35</td>
<td>10.30</td>
<td>8.40 to 12.60</td>
<td>G</td>
<td>0.33</td>
</tr>
<tr>
<td>HCO3 (mmol/L)</td>
<td>C</td>
<td>10.31</td>
<td>1.24</td>
<td>9.90</td>
<td>8.10 to 12.10</td>
<td>G</td>
<td>0.28</td>
</tr>
<tr>
<td>BE (mmol/L)</td>
<td>1</td>
<td>−13.11</td>
<td>1.14</td>
<td>−13.10</td>
<td>−14.5 to −11.10</td>
<td>G</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>−14.11</td>
<td>1.09</td>
<td>−14.30</td>
<td>−15.40 to −11.90</td>
<td>G</td>
<td>—</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>1</td>
<td>9.00</td>
<td>3.55</td>
<td>9.00</td>
<td>4.00 to 14.00</td>
<td>G</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>11.75</td>
<td>3.66</td>
<td>12.00</td>
<td>7.00 to 18.00</td>
<td>G</td>
<td>—</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>C</td>
<td>210.75</td>
<td>11.33</td>
<td>208.60</td>
<td>194.50 to 231.20</td>
<td>G</td>
<td>0.70</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>C</td>
<td>3.93</td>
<td>0.57</td>
<td>3.77</td>
<td>3.30 to 4.96</td>
<td>G</td>
<td>0.48</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>C</td>
<td>180.42</td>
<td>11.01</td>
<td>180.95</td>
<td>165.30 to 200.40</td>
<td>G</td>
<td>0.55</td>
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<tr>
<td>Magnesium (mmol/L)</td>
<td>1</td>
<td>0.48</td>
<td>0.012</td>
<td>0.049</td>
<td>0.25 to 0.68</td>
<td>NG</td>
<td>0.042</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.57</td>
<td>0.11</td>
<td>0.61</td>
<td>0.33 to 0.86</td>
<td>NG</td>
<td>—</td>
</tr>
<tr>
<td>Calcium (mmol/L)</td>
<td>C</td>
<td>1.79</td>
<td>0.13</td>
<td>1.78</td>
<td>1.62 to 2.02</td>
<td>G</td>
<td>0.092</td>
</tr>
</tbody>
</table>

Values of P ≤ 0.05 were considered significant.
*Data are indicated for hemolymph samples obtained before anesthesia (sample 1), for samples obtained after induction of anesthesia (sample 2), or for all samples obtained before and after induction of anesthesia (combined data; C).

G = Gaussian distribution. NG = Non-Gaussian distribution.
— = Not determined.

Discussion

Results of the present study suggested that the use of sevoflurane for anesthesia of Chilean rose tarantulas was safe and effective. After a short anesthetic induction time, the duration of immobilization was adequate for performance of clinical procedures typically performed for theraphosid spiders, such as physical examination, biological sample collection, or transponder implantation.20 Although the concentration of sevoflurane and the anesthetic method used in this study would likely be adequate for performance of clinical procedures, they may be insufficient for procedures that require complete immobilization of spiders. Anecdotal information suggests that concentrations of isoflurane > 3% may be required for research procedures that require complete immobilization of spiders; similar concentrations of sevoflurane may be required for such procedures. A direct method for delivery of inhalant anesthetics to the spiracles and book lungs of spiders may provide complete immobility.

Results of other studies in which isoflurane anesthesia of G. rosea was evaluated indicate shorter anesthetic induction and recovery times than those found for sevoflurane anesthesia of tarantulas in the present study. However, differences in anesthetic agents and methods make direct comparisons among these findings difficult. The shortest induction and recovery times for spiders anesthetized with isoflurane were determined in one of those other studies: a 1-L anesthetic chamber was used in that study, and monitoring of the RR was performed by shaking the chamber. Such a disturbance would likely increase the activity level of a spider as it attempted to compensate. Compared with a resting state, the spiracles (external openings to the book lungs) in spiders increase in size by approximately 26 times to allow increased respiratory gas diffusion after exercise.21 The increased diffusion of isoflurane in such a situation would likely account for the more rapid induction and recovery.

The custom anesthetic chamber17 used in the present study was chosen because it allowed for manipulation of the spiders for monitoring of an RR without having to open the chamber. This helped to minimize exposure of personnel to sevoflurane. Inhalation anesthetics have various adverse effects in humans, including hepatic toxic effects, mutagenicity, and teratogenicity.22 However, the large size of the anesthetic chamber required to provide sealed access ports, in combination with the gas flow rate of 1 L/min, likely affected the rate of increase of sevoflurane concentration and thus the time to anesthetic induction of spiders in the present study. To compensate for this, the gas flow rate from the anesthetic machine could have been increased. Another technique that could have been used to rapidly deliver inhalation anesthetic to spiders was the use of a small, flexible facemask placed over the entire opisthosoma (the posterior body segment) or the ventral aspect of the opisthosoma for access to the spiracles and book lungs.23 This technique would allow manipulation of spiders for determination of an RR and would decrease exposure of personnel to anesthetic.

The mechanism of action of sevoflurane and other inhalation anesthetics is unknown, although results of other studies of invertebrate species suggest various possibilities. In nematodes (Caenorhabditis elegans), volatile anesthetics affect presynaptic neurotransmitter...
release, ion channels and other cell membrane proteins of neurons, and oxidative phosphorylation in mitochondria. Similar results have been determined for fruit flies (Drosophila melanogaster). Interestingly, modulation of GABA receptors is believed to be a substantial component of the mechanism of action of volatile anesthetics. This may occur in spiders; results of a recent study indicate GABAergic neurons are detected in multiple areas of the CNS of barn spiders (Araneus caviatus).

During recovery from sevoflurane anesthesia, most of the drug is likely removed from a spider's body by means of diffusion through the book lungs. In humans, a small fraction (3% to 5%) of sevoflurane is metabolized in the liver by cytochrome P450 2E1. Spiders may metabolize sevoflurane with a similar mechanism; cytochrome P450 has been detected in another species of arachnid, the 2-spotted spider mite (Tetranychus urticae). The respiratory physiology of arachnids, particularly the function of hemocyanin (the oxygen-binding molecule), has been evaluated in other studies. Hemolymph gas analysis values for theraphosid spiders, primarily Euryypelma californicum, have been evaluated in other studies; however, these spiders were likely misidentified Texas brown tarantulas (Aphonopelma hentzi). In a resting state at 22.5°C, arterial (ie, collected from an intracardiac location) samples obtained from such spiders have variable PO2 values that range from 15 to 40 mm Hg, or approximately 40 mm Hg at 25°C. Values are considerably lower than those determined for hemolymph samples obtained from spiders in the present study before anesthesia. Because the default analyzer temperature (37°C) was used in this study, a temperature correction value of 6% for each 1°C difference in ambient temperature may be used; however, even after such correction, values in that other study and those determined in the present study were not similar. The reason for this discrepancy was not identified but may have been attributable to differences in methods or biological differences between species.

The significant increase in PO2 detected between hemolymph samples collected before anesthesia and those collected after induction of anesthesia for spiders in this study was not surprising. Arachnid respiration occurs by passive diffusion of gases across membranes of the book lungs. The use of 100% oxygen for delivery of an inhalation anesthetic agent increases the oxygen concentration gradient across the book lung membranes, which increases the amount of oxygen entering the hemolymph circulation. The PO2 values for hemolymph samples collected from spiders during anesthesia in this study were similar to those for such samples collected from goliath birdeater tarantulas during isoflurane anesthesia in another study (median value, 200 mm Hg; range, 147.8 to 348.0 mm Hg) by use of similar methods. To the authors' knowledge, the present study and that other study are the only 2 in which the effects of anesthesia on hemolymph sample gas analysis variables for spiders were determined.

The PCO2 values did not change significantly during sevoflurane anesthesia of spiders in this study. With consideration of the SD of these values, data determined in this study were similar to those determined for T blondi (mean ± SD, 20.3 ± 3.9 mm Hg) in another study. Values were also similar to those determined for E californicum (mean ± SD, 14.09 mm ± 0.76 mm Hg) acclimated to an ambient temperature of 30°C in another study. Hemolymph gas analysis values vary with ambient temperature; PCO2 values for E californicum are significantly lower (mean ± SD, 7.00 ± 0.86 mm Hg) at an acclimation ambient temperature of 15°C.

In hemolymph samples collected from E californicum in a previous study, mean measured concentrations of CO2 range from 10.68 to 14.71 mmol/L; these values are independent of acclimation ambient temperatures and are only slightly higher than values of bicarbonate concentrations. These findings are similar to those for T blondi and the Chilean rose tarantulas of the present study, except that the effects of multiple ambient temperatures were not evaluated in the present study and that other study.

The pH of theraphosid hemolymph samples has been determined in other studies for E californicum, T blondi, and Aphonopelma echnium (reported for the previously used species name, Dugesiella echina). Mean pH values for these animals range from 7.44 to 7.81, which was consistent with the value determined in the present study for G rosea.

The changes in BE and concentrations of glucose and ionized magnesium detected between hemolymph samples obtained before anesthesia and those obtained after induction of anesthesia in the present study were small. Although results were significant, these changes likely have minimal biological importance. Changes in hemolymph ionized magnesium concentrations were not likely attributable to sevoflurane. In human children and adults, sevoflurane anesthesia does not cause changes in serum ionized magnesium concentration. Significant increases in serum ionized magnesium concentration were detected in another study for cats and dogs undergoing sevoflurane anesthesia for performance of surgical procedures; however, this result was confounded by the fact that fluids with a low concentration of magnesium were administered during the procedures. More likely, the increase in hemolymph sample ionized magnesium concentration detected in the present study was attributable to activity of the spiders. Concentrations of ionized magnesium in hemolymph samples of E californicum increase from 0.4 mmol/L to approximately 0.7 mmol/L after exhaustive exercise (ie, 3 minutes of continuous movement). Although the spiders in the present study did not have changes in hemolymph sample ionized magnesium concentration that were as high as those detected for spiders in that other study, the spiders in the present study seemed to be calm and had a low amount and intensity of activity during the procedures. The amount of change in hemolymph sample ionized magnesium concentrations in tarantulas may be proportional to their amount and intensity of activity.

The decrease in BE detected between hemolymph samples collected before anesthesia and those collected after induction of anesthesia in this study may also have been attributable to activity of the spiders. During movement, leg extension in spiders is facilitated...
by hydraulic force as muscles in the prosoma (anterior body segment) contract the dorsal and ventral aspects of the exoskeleton. 43 This process generates high pressures (up to 300 mm Hg) 44 relative to the combined pressure from the opisthosoma and the heart within it (approx 80 mm Hg). 43 As a result, perfusion and oxygenation of the prosoma cease, with anaerobic energy metabolism consequently occurring. 43 Lactate produced in E californicum during exhaustive exercise is associated with decreases in hemolymph pH and bicarbonate concentration. 37 Similar changes in hemolymph pH and bicarbonate concentration were not detected for spiders in the present study. The amount and intensity of activity of the tarantulas in this study may not have been high enough to cause a significant change in the concentrations of hemolymph lactate and bicarbonate. Minor changes in circulating concentrations of these molecules could have stimulated additional regulatory mechanisms to which changes in BE could be attributed.

The increase in hemolymph glucose concentrations detected in this study between samples obtained from spiders before anesthesia and those obtained after induction of anesthesia may have been attributable to various factors. Increases in hemolymph sample total sugar concentrations are detected in A domesticus captured and anesthetized with carbon dioxide gas for performance of sham (ie, water) injection. 45 Similar findings for hemolymph total sugar concentrations are detected for cockroaches (Periplaneta americana) anesthetized with various gases (carbon dioxide, nitrogen, and diethyl ether fumes). 49 Sevoflurane anesthesia impairs use of glucose in mammals, leading to hyperglycemia. 46 However, stress and excitement can also cause hyperglycemia in mammals. 48 In P americana, handling alone increases hemolymph sample sugar concentrations. 46 In addition to the effects of anesthesia and handling stress, performance of hemolymph sample collection may also have affected glucose concentrations in samples obtained from tarantulas in the present study. Interestingly, self-induced trauma (ie, appendage autotomy) increases sugar concentrations and blood collection by means of traumatic appendage injury decreases sugar concentrations in hemolymph of the crustacean arthropod Ocypode platytarsis. 47

Hemolymph sample glucose and ion concentrations for various theraphosid spider species have been determined in other studies. 18, 36–38 Values of hemolymph sample glucose, sodium, potassium, and chloride concentrations are similar among these species; values of hemolymph ionized magnesium and calcium concentrations vary among such spider species. The hemolymph sample concentrations of glucose and ions determined for G rosea spiders in the present study were similar to values determined for spiders in those other studies, except for potassium and chloride concentrations. The hemolymph sample potassium concentrations were higher and the chloride concentrations were lower for spiders in the present study versus spiders in the other studies. 18, 36–38 Such differences may be attributable to biological differences among species. However, it was more likely that such differences were attributable to differences in methods among studies (eg, use of flame spectrophotometry versus use of electrode potentiometry for determination of values).

Pharmaceutical anesthesia of invertebrates is a relatively recent phenomenon. Thus, there is still a lack of information regarding the physiologic consequences of anesthesia in these animals. It is unknown if characteristics of anesthesia for invertebrates are comparable to those for vertebrate species. However, results of the present study suggested that veterinarians and biologists should consider the use of sevoflurane for anesthesia of tarantulas when performing clinical examinations and diagnostic tests. Use of sevoflurane for anesthesia seemed to be safe, provided a suitable duration of anesthesia, and had minimal effects on hemolymph sample biochemical and gas analysis variables for Chilean rose tarantulas. Additional studies are warranted to determine whether sevoflurane can be safely used for complete immobilization of spiders.

References
31. Nentwig W. The species referred to as Eurypelma californicum (Theraphosidae) in more than 100 publications is likely to be Aphonopelma hentzi. J Arachnol 2012;40:128–130.