Venous blood gas analytes during isoflurane anesthesia in black-tailed prairie dogs (Cynomys ludovicianus)

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Objective—To describe changes in venous blood gas analytes during isoflurane anesthesia in black-tailed prairie dogs (Cynomys ludovicianus).

Design—Prospective study.

Animals—16 black-tailed prairie dogs.

Procedures—Black-tailed prairie dogs were placed in an anesthesia chamber for induction of general anesthesia, which was maintained with isoflurane in oxygen delivered via mask. Immediately following anesthetic induction, a venous blood sample was obtained from the medial saphenous vein; a second venous blood sample was obtained just prior to anesthetic gas shutoff. An evaluation of venous blood gas analytes was performed on each sample. General linear mixed models with repeated measures were used for data analyses.

Results—Median anesthetic time was 90 minutes (range, 60 to 111 minutes). A significant increase from immediately after induction to completion of anesthesia was observed in P\textsubscript{CO\textsubscript{2}} and mean blood chloride ion, BUN, and creatinine concentrations. A decrease in P\textsubscript{O\textsubscript{2}}, mean blood pH, and anion gap was observed from induction of anesthesia to completion. No significant differences during anesthesia were observed in mean base excess or blood bicarbonate, sodium, potassium, calcium, magnesium, blood glucose, lactate, and total CO\textsubscript{2} concentrations. No complications occurred during or after anesthesia for any animal.

Conclusions and Clinical Relevance—Examination of prairie dogs often requires general anesthesia, with isoflurane currently the inhalation agent of choice. Results suggested respiratory acidosis and relative azotemia may occur during isoflurane anesthesia of prairie dogs. Given the increased risk associated with anesthesia in small mammals and the propensity for respiratory disease in prairie dogs, insight into physiologic changes associated with isoflurane anesthesia in healthy prairie dogs can aid in perioperative evaluation and anesthetic monitoring in this rodent species. (J Am Vet Med Assoc 2015;247:404–408)
Anesthesia and experimental protocol—Food and water were withheld from each prairie dog for 3 to 5 hours prior to the start of the procedure, and the prairie dogs were kept in climate-controlled conditions indoors in small animal carriers until the start of the procedure. Each prairie dog was placed in a small animal carrier completely covered with a large, clear plastic bag for induction of anesthesia with circulation of 5% isoflurane in 2 L of oxygen/min. Following anesthetic induction, anesthesia was maintained by means of 2.5% isoflurane in 1.5 L of oxygen/min delivered via a small face mask and nonrebreathing circuit. The animals were allowed to breathe spontaneously. Body temperature was monitored rectally with a hand-held digital thermometer and maintained with a warm water blanket and heat packs. Vital signs were monitored every 2 to 3 minutes with a stethoscope and a Doppler ultrasound machine, and oxygen saturation was measured with a pulse oximeter, with the probe placed on the foot pad of a pelvic limb.

Handling and examination of animals—Following induction of anesthesia, each animal was weighed with a digital scale and a complete physical examination performed. Complete blood count, serum biochemical analysis, whole body radiography, and echocardiography were also performed, and all animals were deemed healthy.

Blood sample collection and analysis—Immediately following induction of general anesthesia, animals were placed in lateral recumbency to obtain the first venous blood sample. This blood sample was obtained prior to the complete physical examination and diagnostic imaging described. The hair on the medial aspect of 1 hind limb was clipped and the skin aseptically prepared. A 0.5-mL sample of venous blood was obtained from the medial saphenous vein with a pre-heparinized 1-mL syringe and a 25-gauge, 0.5 × 16-mm needle and immediately placed into a tube containing lithium-heparin. The blood sample was directly processed with an electrolyte and chemistry analyzer. The same technique was used to obtain a second venous blood sample from the other hind limb at the conclusion of the anesthesia procedure, immediately prior to cessation of isoflurane administration. Each venous blood sample was analyzed for blood pH, P CO 2 , P O 2 , O 2 saturation, anion gap, and concentrations of bicarbonate, base excess, sodium, chloride, free calcium, free magnesium, glucose, lactate, BUN, creatinine, and total CO 2 . Additionally, the duration of anesthesia, body temperature, age, weight, and sex of each prairie dog were recorded.

Following the second blood sample collection, each animal received a 40-mL bolus of lactated Ringer’s solution, SC; the anesthetic gas was turned off; and the prairie dogs were maintained with oxygen delivered via mask until purposeful movement was observed. Once awake, the prairie dogs were placed in a heated incubator.

Statistical analysis—A general linear mixed model was fitted to each response variable. In all cases, the statistical model included the fixed effect of time (immediately after induction of anesthesia vs immediately prior to gas shutoff). For each venous blood gas analyte, age, body temperature, body weight, and duration of anesthesia were evaluated as potential explanatory covariates. Random effects in the linear predictor included zoological collection as a blocking factor and animal nested within to account for repeated measures over time for each individual. In this study, any variability due to sex was confounded with zoological collection; therefore, it was not possible to test sex separately in the statistical model. Variance components were estimates based on the residual maximum likelihood. The Kenward-Roger procedure was used to estimate degrees of freedom and make the corresponding adjustments in estimated SEs. Model assumptions were evaluated by checking externally Studentized residuals and were considered to be appropriately met.

Model fitting was performed with statistical software implemented by means of the Newton-Raphson method with ridging as the optimization technique. Pairwise comparisons were conducted to assess changes in venous blood gas analytes during isoflurane anesthesia between immediately after induction of anesthesia and immediately prior to gas shutoff. Tukey-Kramer or Bonferroni adjustments, as appropriate in each case, were implemented to prevent inflation of type I error due to multiple comparisons. For all comparisons, a value of P < 0.05 was considered significant.

Results

Sixteen black-tailed prairie dogs were included in this study, including 10 sexually intact males and 6 sexually intact females. Median weight was 791 g (1.88 lb; range, 382 to 1,200 g [1.31 to 2.63 lb]), and median age was 6 months (range, 6 to 54 months). Median anesthetic procedure time was 90 minutes (range, 60 to 111 minutes). There were no complications observed during anesthesia or recovery. For the purpose of reporting and because of the small sample size of this study, analyte data were pooled across sex and age groups. Descriptive statistics were calculated for observations collected for venous blood gas analytes immediately following induction of isoflurane anesthesia and immediately prior to anesthetic gas shutoff (Table 1).

During isoflurane anesthesia (ie, from immediately after induction to immediately prior to gas shutoff), a significant increase was observed in P CO 2 (estimated mean ± SE increase, 3.7 ± 1.6 mm Hg; P = 0.040), mean ± SE blood chloride ion concentration (1.6 ± 0.7 mmol/L; P = 0.028), mean ± SE BUN concentration (1.8 ± 0.6 mg/dL; P = 0.012), and mean ± SE creatinine concentration (0.08 ± 0.03 mg/dL; P = 0.032). A significant decrease was observed in P O 2 (estimated mean ± SE decrease, 34.6 ± 13.5 mm Hg; P = 0.020), mean ± SE blood pH (0.050 ± 0.016; P = 0.009), and mean ± SE anion gap (3.1 ± 1.2 mmol/L; P = 0.018). No significant difference was observed from induction of anesthesia to time immediately prior to anesthetic gas shutoff in mean blood bicarbonate, mean blood base excess, mean blood sodium concentration, mean blood potassium concentration, mean blood calcium concentration, mean blood magnesium concentration, mean blood glucose concentration, mean blood lactate concentration, and total CO 2 content.
Discussion

In the present study of 16 healthy adult prairie dogs undergoing isoflurane anesthesia, a significant increase in \( P_{\text{CO}_2} \) and mean blood chloride ion, BUN, and creatinine concentrations, with a significant decrease in \( P_{\text{O}_2} \), mean blood pH, and anion gap, was observed from induction of anesthesia to completion. These changes were indicative of respiratory acidosis and relative azotemia. Animals remained normoglycemic and no complications were observed.

Evaluation of venous blood gas analytes during general anesthesia is important for evaluation of overall health status, diagnosis of many diseases, assessment of acid-base status, and evaluation of respiratory function.\(^{11,12}\) Results of the present study indicated that isoflurane anesthesia is associated with an increase in \( P_{\text{CO}_2} \) in prairie dogs. Anesthesia causes respiratory depression and a decrease in respiratory rate.\(^{13}\) When respiratory rate is decreased, there is a resultant decrease in respiratory minute ventilation, leading to hypoventilation.\(^{14}\) In addition, isoflurane anesthesia has been associated with a decreased tidal volume and relaxation of the intercostal muscles.\(^{15}\) These physiologic responses can contribute to reduced alveolar ventilation and increased end-tidal \( P_{\text{CO}_2} \) concentration.\(^{15}\) The reduced respiratory rate, combined with the decrease in tidal volume, depression of the ventilatory control centers, and relaxation of intercostal muscles, may explain the elevation in \( P_{\text{CO}_2} \) observed in this study.

A significant decrease in the venous \( P_{\text{O}_2} \) during anesthesia was observed in this study. A reduction in the \( P_{\text{O}_2} \) may be a result of hypoventilation with increasing \( P_{\text{CO}_2} \) contributing to a progressive decrease in the \( P_{\text{O}_2} \).\(^{14}\) Although the magnitude of the change in mean values for both of these parameters was small (Table 1), the range of values was large; therefore, the impact that a rising \( P_{\text{CO}_2} \) may have had on the \( P_{\text{O}_2} \) via its interaction in the alveolar-gas equation and a prairie dog’s subsequent ability to oxygenate fully should not be overlooked. The \( P_{\text{O}_2} \) is one of the key local factors in regulating tissue blood flow.\(^{14}\) Prolonged exposure to drugs such as anesthetic agents can blunt or completely abolish the reflex activity of the baroreceptors and chemoreceptors as well as the vascular response to sympathetic stimulation.\(^{14}\) This results in poor compensatory responses and alterations in normal tissue blood flow.\(^{14}\) These physiologic responses may also explain the decrease in venous \( P_{\text{O}_2} \) during anesthesia observed in this study. Considering that we evaluated venous rather than arterial blood samples, the decline in \( P_{\text{O}_2} \) during anesthesia may reflect greater oxygen extraction occurring at the tissue level.\(^{16}\) Reductions in cardiac output, hypotension, a state of relative hypovolemia, and decreased tissue perfusion are conditions that might increase the likelihood for a higher oxygen extraction ratio that would be reflected in a decline in venous \( P_{\text{O}_2} \).

### Table 1—Venous blood gas analytes recorded immediately after induction of isoflurane anesthesia (time 0) and immediately prior to anesthetic gas shutoff (time 1) in black-tailed prairie dogs (\textit{Cynomys ludovicianus}; \( n=16 \)) from 2 zoological collections.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Time</th>
<th>Median</th>
<th>Interquartile range</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_{\text{H}} ) (mm Hg)</td>
<td>0</td>
<td>37.45</td>
<td>35.00 to 39.85</td>
<td>32.00 to 42.50</td>
</tr>
<tr>
<td>( P_{\text{O}_2} ) (mm Hg)</td>
<td>0</td>
<td>178.89</td>
<td>150.75 to 209.50</td>
<td>135.50 to 225.50</td>
</tr>
<tr>
<td>( P_{\text{CO}_2} ) (mmol/L)</td>
<td>0</td>
<td>3.87</td>
<td>3.00 to 5.00</td>
<td>0.50 to 12.70</td>
</tr>
<tr>
<td>( \text{pH} )</td>
<td>0</td>
<td>7.41</td>
<td>7.35 to 7.55</td>
<td>7.30 to 7.58</td>
</tr>
<tr>
<td>( \text{BIC} ) (mmol/L)</td>
<td>0</td>
<td>32.95</td>
<td>30.15 to 35.60</td>
<td>27.50 to 41.60</td>
</tr>
<tr>
<td>( \text{HCO}_3^- ) (mmol/L)</td>
<td>0</td>
<td>32.11</td>
<td>28.10 to 36.60</td>
<td>24.00 to 46.00</td>
</tr>
<tr>
<td>( \text{K}^+ ) (mmol/L)</td>
<td>0</td>
<td>4.79</td>
<td>4.35 to 5.25</td>
<td>3.80 to 8.00</td>
</tr>
<tr>
<td>( \text{Na}^+ ) (mmol/L)</td>
<td>0</td>
<td>139.63</td>
<td>135.50 to 144.90</td>
<td>130.00 to 149.00</td>
</tr>
<tr>
<td>( \text{Glucose} ) (mg/dL)</td>
<td>0</td>
<td>3.59</td>
<td>3.15 to 4.20</td>
<td>1.50 to 8.80</td>
</tr>
<tr>
<td>( \text{Lactate} ) (mg/dL)</td>
<td>0</td>
<td>1.71</td>
<td>1.20 to 2.30</td>
<td>0.60 to 4.60</td>
</tr>
</tbody>
</table>

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\( \text{CO}_2 \) may be a result of hypoventilation with increasing \( \text{CO}_2 \) contributing to a progressive decrease in the \( \text{CO}_2 \). Although the magnitude of the change in mean values for both of these parameters was small (Table 1), the range of values was large; therefore, the impact that a rising \( \text{CO}_2 \) may have had on the \( \text{CO}_2 \) via its interaction in the alveolar-gas equation and a prairie dog’s subsequent ability to oxygenate fully should not be overlooked. The \( \text{CO}_2 \) is one of the key local factors in regulating tissue blood flow. Prolonged exposure to drugs such as anesthetic agents can blunt or completely abolish the reflex activity of the baroreceptors and chemoreceptors as well as the vascular response to sympathetic stimulation. This results in poor compensatory responses and alterations in normal tissue blood flow. These physiologic responses may also explain the decrease in venous \( \text{CO}_2 \) during anesthesia observed in this study. Considering that we evaluated venous rather than arterial blood samples, the decline in \( \text{CO}_2 \) during anesthesia may reflect greater oxygen extraction occurring at the tissue level. Reductions in cardiac output, hypotension, a state of relative hypovolemia, and decreased tissue perfusion are conditions that might increase the likelihood for a higher oxygen extraction ratio that would be reflected in a decline in venous \( \text{CO}_2 \).
The venous pH was significantly reduced during isoflurane anesthesia in this study. As modeled on the basis of the Henderson-Hasselbalch equation, blood pH depends on 2 major components: a ventilatory component (\(P_{CO_2}\)) and a metabolic component (bicarbonate concentration).\(^9\) We suspect that the decrease in blood pH found in this study may have been a result of acidemia from changes in ventilation (ie, from the observed increase in \(P_{CO_2}\)). These findings suggest that respiratory acidosis occurs during isoflurane anesthesia in prairie dogs. No significant difference was observed in measured mean venous bicarbonate concentration during isoflurane anesthesia in this study. Bicarbonate is used by the body to neutralize the hydrogen ions that produce acidosis.\(^13\) Metabolic compensation for respiratory acidosis occurs in 2 steps.\(^16\) Initially, cellular buffering occurs that elevates plasma bicarbonate concentration slightly, and hours later, renal compensation begins, whereby both renal excretion of carbonic acid and bicarbonate reabsorption are increased.\(^16\) The lack of evidence for changes in bicarbonate concentration during isoflurane anesthesia despite evidence of respiratory acidosis may be explained by the physiologic delay in the renal compensatory mechanism.\(^13\) We suggest that the relatively short anesthetic episode (median, 90 minutes; range, 60 to 111 minutes) experienced by these prairie dogs would likely have been inadequate for renal compensation to be evident.

A significant decrease was observed in measured mean anion gap during isoflurane anesthesia in this study. Anion gap is a measure of the difference between cations and anions and is calculated as the difference between the sum of sodium and potassium ion concentrations and the sum of chloride and bicarbonate ion concentrations.\(^17\) This value represents the ions that are not measured directly, including calcium, sulfates, phosphates, lactates, ketoacids, and protein. Anion gap provides additional information when classifying the type of acid-base disorder.\(^17\) In other species, serum anion gaps that are below lower limits or are negative are infrequently reported;\(^16\) however, some negative values were noted for the prairie dogs in this study. Low values for anion gap have been associated with laboratory error, underestimation or overestimation of certain electrolytes, hypoalbuminemia, alkalosis with positive base excess, uremia, and respiratory acidosis.\(^17\)\(^18\) Respiratory acidosis was noted in anesthetized prairie dogs in the present study, which could explain the lower anion gaps observed.

In this study, a relative increase in BUN concentration and creatinine concentration was observed during anesthesia in prairie dogs. There are several reports of serum and plasma biochemistry parameters in prairie dogs. According to a recent report by Keckler et al,\(^19\) the prairie dogs in our study had both relative and absolute azotemia on the basis of measured BUN and creatinine concentration (Table 1) at the end of isoflurane anesthesia. Keckler et al\(^19\) reported a normal mean BUN concentration of 26 mg/dL (range, 22 to 31 mg/dL). Immediately prior to anesthetic gas shutoff, prairie dogs in this study had a mean ± SD BUN concentration of 36.25 ± 7.66 mg/dL (median, 34.5 mg/dL; range, 27 to 52 mg/dL), and values were significantly (\(P = 0.012\)) different, compared with the BUN concentration immediately following induction of isoflurane anesthesia. Keckler et al\(^19\) reported a normal mean creatinine concentration of 0.6 mg/dL (range, 0.4 to 0.8 mg/dL). Immediately prior to anesthetic gas shutoff, prairie dogs in our study had a mean ± SD creatinine concentration of 0.74 ± 0.27 mg/dL (median, 0.6 mg/dL; range, 0.3 to 1.3 mg/dL), and values were significantly (\(P = 0.032\)) different, compared with the creatinine concentration measured immediately following induction of isoflurane anesthesia. Also, absolute azotemia in these prairie dogs while under isoflurane anesthesia was suggested when BUN and creatinine concentrations from our study were compared with physiologic data for prairie dogs previously reported by Broughton.\(^20\) Regardless, BUN and creatinine concentrations measured and compared at the beginning and the end of isoflurane anesthesia suggested that animals in our study had relative azotemia. It is unknown whether the elevations in BUN and creatinine concentration were a result of dehydration, an effect of isoflurane on hemodynamics, or a combination of both.\(^21\) Anesthesia decreases vascular tone and cardiac output.\(^21\) These changes result in a decrease in blood flow to the tissues, leading to decreased perfusion.\(^21\) Decreased renal perfusion results in a reduction in the glomerular filtration rate and an increase in the BUN and creatinine concentrations.\(^21\) Additionally, food and water were withheld from the prairie dogs in this study for approximately 3 to 5 hours from capture to the end of the procedure. Although kept in climate-controlled conditions, this might have contributed to some mild dehydration. Regardless of the cause of the elevations in BUN and creatinine concentrations, we suggest that this finding emphasizes the importance of fluid therapy when an animal is placed under general anesthesia. By providing fluid therapy, the vascular volume is increased and perfusion improved, helping to combat relative azotemia.\(^21\) Subcutaneous fluids were provided at the end, and not before or during the procedure, to avoid potentially influencing the results of the final venous blood gas sample. With all small mammals such as rodents, it is usually advisable to administer warmed fluids at the end of anesthesia to provide some fluid supplementation in the immediate postoperative period.\(^22\)

There was no evidence for changes in blood glucose concentrations in prairie dogs during anesthesia in this study. Often during such a procedure, there are concerns about withholding food from small rodents and the possibility of this predisposing them to hypoglycemia.\(^24\) Small mammals have higher metabolic rates and smaller glycogen reserves, which can predispose them to hypoglycemia if preanesthetic food withholding is prolonged.\(^23\)\(^24\) Regardless of the duration of anesthesia (median, 90 minutes; range, 60 to 111 minutes) in this study, hypoglycemia was not detected.

Venous blood samples were evaluated in this study. Arterial blood samples provide information primarily regarding pulmonary function, whereas mixed venous samples provide information on whole body acid-base status and overall cardiac performance and perfusion.\(^9\) Evaluation of venous blood can also be useful to moni-
tor trends in overall perfusion and acid-base status in small animals. However, in reality, obtaining an arterial sample in a prairie dog is not practical, and it would likely only be possible to obtain a sample surgically for research purposes.

In this study, anesthesia was induced solely with isoflurane in oxygen because this is common practice for clinical evaluation of prairie dogs. A previous study that examined the risk of anesthetic death in small mammals reported rates 10% to 22% higher than that for dogs and cats. Undetected respiratory disease should be considered as a potential contributing factor in some of these anesthetic deaths and contributing to the higher mortality rate. Respiratory disease is also commonly reported in prairie dogs. All animals in this study were stable under isoflurane anesthesia with no complications, regardless of the duration of the procedure, suggesting that isoflurane may be considered both an effective and safe method of general anesthesia in healthy prairie dogs, consistent with recommendations from others.

The main limitations of this study included the small sample size, the uncertain age of the animals (estimated by the keepers), and the possibility of subclinical illness, despite the thorough clinical evaluation. In addition, the prairie dogs were from 2 zoological collections, with each collection containing a single sex. This could raise a potential issue of sex confounded with zoological collection; however, no evidence for sex differences was apparent for any of the venous blood gas analytes considered. Further, we note that the initial blood sample was collected with animals under anesthesia, although it was immediately following anesthetic induction; however, it must be considered that this may have affected the values observed in this study. We must also consider that the biochemical analyzer used in this study has not been validated for use with prairie dog blood, given that this is the first study to examine it. The changes in blood gas analytes during isoflurane anesthesia in prairie dogs documented in this report may improve knowledge of the physiology and anesthetic management of black-tailed prairie dogs.

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