Cardiorespiratory effects of isoflurane in Asiatic black bears (*Ursus thibetanus*) anesthetized with intramuscular medetomidine and zolazepam/tiletamine

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**ABSTRACT.** The objective of this study was to determine the dose-dependent effects of isoflurane on various cardiovascular parameters and the stable range of isoflurane concentrations in Asiatic black bears (*Ursus thibetanus*). Seven Asiatic black bears were intramuscularly injected with medetomidine, zolazepam and tiletamine (MZT) to induce anesthesia, and anesthesia was maintained by administering isoflurane in 100% oxygen (4 l/min) without mechanical ventilation. Several cardiovascular parameters were measured at five end-tidal isoflurane concentrations (0.5, 1.0, 1.5, 2.0, and 2.5%). Blood was collected from the femoral artery before administration of isoflurane and after each administration for immediate blood gas analysis. Isoflurane produced dose-dependent increases in heart rate, respiratory rate, minute volume, end-tidal carbon dioxide (CO₂) partial pressure and the partial pressure of arterial CO₂, and dose-dependent decreases in non-invasive blood pressure and tidal volume. Rectal temperature, oxygenation and acid-base balance were unaffected by isoflurane. All parameters in this study were in a clinically acceptable range at all times. The data show that the combination of MZT and isoflurane is suitable for general anesthesia in Asiatic black bears with spontaneous breathing during prolonged procedures. End-tidal isoflurane concentrations of 0.5 to 2.5% can be used in Asiatic black bears without adverse side effects.

**KEY WORDS:** anesthesia, Asiatic black bear, cardiorespiratory, immobilization, isoflurane
tiletamine (MZT) administration.

MATERIALS AND METHODS

Animals

The study protocol was approved by the Animal Care and Research Committee of the Species Restoration Technology Institute, Korea National Park Service (SRTI number 15-010). Seven clinically healthy male adult Asiatic black bears (age range, 7–15 years and weight range, 130–150 kg) housed at the Species Restoration Technology Institute were included in this study. The bears had been released into the wild for recovery of the bear population, but were subsequently moved to a captive facility because of various conflicts with humans.

Each bear was housed individually in a small pen (approximately 3 m × 4 m × 3 m) with a cement floor. The bears spent 4–6 hr per day in a semi-natural enclosed field (2,880 m²) that resembled the wild bear habitat. They were fed acorns, chestnuts, fruits, vegetables, sweet potatoes and commercial feed (Omnivore Diet Dry®, ZuPreem, Mission, KS, U.S.A.) twice a day based on seasonal calorie requirements. Feeding was discontinued during the hibernation period (mid-December to early March) in accordance with the unique ecology and physiology of bears.

Immobilization and monitoring

The bears were fasted for 24 hr before anesthesia. To allow the bears to be transported to the surgery room, they were received an intramuscular injection of 2 mg/kg zolazepam/tiletamine (Zoletil 50®, Virbac Co., Ltd., Fort Worth, TX, U.S.A.) and 0.04 mg/kg medetomidine (Domitor®, Pfizer Inc., New York, NY, U.S.A.) using a dart gun (CO₂ PI, Dan-Inject, Børkop, Denmark). After transportation to the surgery room, they were restrained on a surgery table in dorsal recumbency and intubated with a 10-mm (inner diameter) endotracheal tube with an inflatable cuff (Sheridan endotracheal tube, Jorvet, Loveland, CO, U.S.A.). The tube was connected to a semi-closed re-breathing system (Multiplus-MEVD, Royal med, Bucheon, Korea) with a spirometer sensor (D-lite®, GE Healthcare Life Science Co., Ltd., Seoul, Korea), and pop-off valve was opened. The isoflurane (Terrell®, Piramal Critical Care Inc., Bethlehem, PA, U.S.A.) in 100% oxygen (4 l/min) was administered. At the same time, a limb leads (three leads) electrocardiogram (ECG) device was attached to pads on the right and left forelegs and the left hind leg in order to record a lead III ECG, and a temperature probe was inserted more than 20 cm into the rectum. A non-invasive blood pressure cuff (Dura-cuff® REF 2204® or 2205®, GE Healthcare Life Science Co., Ltd.) was attached to a foreleg after measurement of its circumference, and a peripheral capillary oxygen saturation (SpO₂) sensor (OXY-E-UN®, GE Healthcare Life Science Co., Ltd.) was attached to the tongue.

Normal saline (Techflex 2-NS, JW Pharmaceutical, Seoul, Korea) was administered intravenously at a dose of 5 ml/kg/hr via the cephalic vain. Spontaneous respiration was maintained without mechanical ventilation during anesthesia, and the following parameters were measured by using a multiple functional patient monitor (Datex-Ohmeda S/5, GE Healthcare Life Science Co., Ltd., Helsinki, Finland): end-tidal isoflurane concentration (ETiso), heart rate (HR), respiratory rate (RR), rectal temperature (RT), SpO₂, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), end-tidal carbon dioxide partial pressure (ETCO₂), inspired tidal volume (TVin), expired tidal volume (TVex), inspired min volume (MVin) and expired min volume (MVex). At the end of the procedure, 0.2 mg/kg atipamezole (Antisedan®, Pfizer Inc.) was injected IM to reverse the anesthesia. And, all of anesthesia related times were recorded (Set up time, time from intramuscular injection of MZT to intubation, which included induction time by MZT and transport time to surgery room; total anesthesia time, time from the intubation to the cessation of isoflurane administration; recovery time, time from the intubation to the cessation of isoflurane administration to raising of the head).

Study design and experimental paradigm

After inducing anesthesia via MZT injection, five end-tidal isoflurane (ETiso) concentrations (0.5%, 1.0%, 1.5%, 2.0% and 2.5%) were administered to each bear in a random order determined by using a Latin square design (Fig. 1). Administration of each ETiso concentration was followed by 10 min of equilibration. Stability was defined as a change in the ETiso concentration of no more than ± 0.1% for 5 min. HR, RR, RT, SpO₂, ETCO₂, TVin, TVex, MVin, MVex, SBP, DPB, MBP and the ECG reading were then recorded at 2-min intervals for 20 min at each ETiso concentration. Before administration of isoflurane and at 19 min of each ETiso concentration, a blood sample was collected from the femoral artery, which was accessed by palpation, for blood gas analysis. The samples were collected anaerobically in pre-heparinized 3 ml syringes with 18 gauge needle (Nayeon Medical, Seoul, Korea) and immediately measured glucose, blood urea nitrogen (BUN), sodium, chloride, total carbon dioxide (TCO₂), hematocrit (Hct), hemoglobin (Hb), pH, partial pressure of arterial carbon dioxide (PaCO₂), bicarbonate (HCO₃⁻) and base excess in the extra cellular fluid (BEecf) by using a portable analyzer (iSTAT, Abaxis Inc., Union City, CA, U.S.A.). During the procedures, it was injected 0.1 mg/kg of meloxicam (Metacam®, Boehringer Ingelheim Co., Ltd., Ingelheim am Rhein, German) and 5 mg/kg cefazolin (Cefazolin®, Yuhan Co., Ltd., Seoul, Korea) intravenously.

Statistical analysis

Total 350 data (70 data from 7 bears at each isoflurane concentration ×5 different isoflurane concentration) were used for statistical analysis. All statistical tests were performed by using statistics program (IBM SPSS Statistics 18 software®, Foster City, CA, U.S.A.). Repeated measures analysis of variance and Tukey multiple comparisons were used to compare RIs and cardiovascular and respiratory parameters at each designated ETiso concentration. The Kruskal-Wallis test was used to compare
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RESULTS

Mean set up time was 26 ± 4 min (time from injection of MZT to complete induction of anesthesia, 13 ± 2 min; transport time to surgery room, 7 ± 1 min; and time from arriving at surgery room to intubation, 6 ± 1 min), total mean anesthesia time was 175 ± 4 min, and mean recovery time was 12 min ± 3 min. There were no side effects, such as vomit, excessive salivation, hyperthermia and conversion, during the anesthesia and no re-sedation after recovery. Besides, all bears could be transferred to the surgery room without additional injection of MZT.

Dose-response effects of isoflurane on temperature and cardiovascular and respiratory parameters

The mean values for HR, RR, blood pressure, ETCO₂, TV (TVin + TVex) and MV (MVin + MVex) differed significantly according to ETiso concentration. The SpO₂ decreased at ETiso concentration of 2.0% compared with 0.5% (P<0.01), although clinical relevant hypoxia was not detected. The RT remained constant throughout the entire procedure. The HR and RR were significantly higher at ETiso concentrations of 1.0 (HR, P<0.01; RR, P<0.016), 1.5 (HR, P<0.01; RR, P<0.001), 2.0 (HR, P<0.01; and RR, P<0.001) and 2.5% (HR, P<0.001; and RR, P<0.001) compared with 0.5%, as was ETCO₂ at 2.0 (P<0.001) and 2.5% (P<0.001; Table 1). HR and RR were also significantly higher at 1.5 (HR, P<0.001; and RR, P<0.001), 2.0 (HR, P<0.05) and 2.5% (P<0.05).
Table 2. Dose-dependent effects of ETiso on blood gas values following isoflurane anesthesia with spontaneous ventilation in Asiatic black bears

<table>
<thead>
<tr>
<th>Variable</th>
<th>0% ETiso</th>
<th>0.5% ETiso</th>
<th>1.0% ETiso</th>
<th>1.5% ETiso</th>
<th>2.0% ETiso</th>
<th>2.5% ETiso</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>84.4 ± 17.6</td>
<td>104.6 ± 32.7</td>
<td>102.9 ± 34.2</td>
<td>103.3 ± 33.9</td>
<td>101.6 ± 25.2</td>
<td>100.8 ± 25.4</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>26.3 ± 3.0</td>
<td>25.1 ± 3.8</td>
<td>25.9 ± 4.7</td>
<td>25.2 ± 3.2</td>
<td>26.3 ± 2.6</td>
<td>28.8 ± 8.4</td>
</tr>
<tr>
<td>Na (mmol/l)</td>
<td>125.6 ± 3.6</td>
<td>128.0 ± 3.7</td>
<td>128.4 ± 4.9</td>
<td>127.3 ± 7.1</td>
<td>126.7 ± 2.9</td>
<td>126.7 ± 3.9</td>
</tr>
<tr>
<td>Cl (mmol/l)</td>
<td>114.0 ± 4.2</td>
<td>115.7 ± 5.8</td>
<td>115.0 ± 5.5</td>
<td>115.0 ± 4.3</td>
<td>114.4 ± 2.8</td>
<td>115.7 ± 6.2</td>
</tr>
<tr>
<td>TCO2 (mmol/l)</td>
<td>20.1 ± 4.1</td>
<td>21.9 ± 2.4</td>
<td>22.9 ± 1.7</td>
<td>22.8 ± 1.7</td>
<td>23.4 ± 1.1</td>
<td>23.0 ± 2.1</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>47.7 ± 8.2</td>
<td>46.3 ± 8.7</td>
<td>44.9 ± 12.7</td>
<td>44.5 ± 10.8</td>
<td>45.1 ± 9.2</td>
<td>42.2 ± 9.5</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>16.2 ± 2.8</td>
<td>15.7 ± 3.0</td>
<td>15.2 ± 4.3</td>
<td>15.1 ± 3.7</td>
<td>15.3 ± 3.1</td>
<td>14.3 ± 3.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.296 ± 0.06</td>
<td>7.291 ± 0.04</td>
<td>7.295 ± 0.03</td>
<td>7.274 ± 0.03</td>
<td>7.249 ± 0.05</td>
<td>7.3 ± 0.1</td>
</tr>
<tr>
<td>PaCO2 (mmHg)</td>
<td>39.9 ± 12.9</td>
<td>43.2 ± 6.4</td>
<td>44.1 ± 5.8</td>
<td>46.7 ± 5.7</td>
<td>50.6 ± 5.2</td>
<td>51.4 ± 9.2</td>
</tr>
<tr>
<td>HCO3− (mmol/l)</td>
<td>18.9 ± 4.0</td>
<td>20.7 ± 2.1</td>
<td>21.3 ± 1.6</td>
<td>21.5 ± 1.6</td>
<td>22.1 ± 1.0</td>
<td>22.1 ± 1.6</td>
</tr>
<tr>
<td>BEecf (mmol/l)</td>
<td>−7.4 ± 3.6</td>
<td>−6.0 ± 2.1</td>
<td>−5.0 ± 1.63</td>
<td>−5.2 ± 1.9</td>
<td>−5.1 ± 1.7</td>
<td>−5.3 ± 1.6</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD. BUN, blood urea nitrogen; Na, sodium; Cl, chlorine; TCO2, total CO2; Ht, hematocrit; Hb, hemoglobin; PaCO2, partial pressure of carbon dioxide; HCO3−, bicarbonate; BEecf, base excess in the extracellular fluid.

DISCUSSION

In veterinary practice, isoflurane is the most widely used inhalant anesthetic, because of its stability, potency, lack of metabolism and safety [7, 9]. Its use in brown bears [12, 19, 25, 26] and American black bears [3] has been reported. However, these studies did not focus on isoflurane, but rather on surgical and other procedures. Thus, to our knowledge, our study is the first to specifically examine the effects of isoflurane-mediated anesthesia in bears. Because it is a respiratory depressant, isoflurane decreases the TV and causes hypoxemia and hypercapnia. It also decreases blood pressure [8, 9, 13]. In dogs anesthetized with isoflurane, nitrous oxide and thiopental under controlled ventilation, MBP and cardiac output decreased as the minimum alveolar concentration (MAC) increased [1]. In vultures anesthetized with isoflurane under controlled ventilation, HR increased and MBP decreased in a manner dependent on increases in the MAC [17]. On the other hand, myocardial function was not appreciably depressed, and HR was maintained or even increased in dogs receiving isoflurane; hence, cardiac output and blood flow were essentially unaffected [7, 9]. Therefore, the dose-dependent effects of isoflurane on cardiovascular function may vary according to species.

In this study, isoflurane dose-dependently increased HR and decreased MBP, perhaps by causing vasodilation, which would reduce systemic vascular resistance and the reflex stimulus of the pressure receptors [18, 22]. Isoflurane also increased RR and MV and decreased TV; this may reflect the inhibition of ventilatory function by increasing ETiso concentrations. That depressed ventilation reduces the TV is supported by accompanying increases in the ETCO2 and PaCO2. In addition, decreases in ventilation and the TV may have resulted in corresponding increases in the RR as a compensatory means of maintaining normal ventilation, and the increase of the RR would in turn increase the MV. In general, the TV is 7–15 ml/kg, and the MV is 100–300 ml/kg/min in dogs [28]. Extrapolating these values to animals, weighing 156 kg, which is the mean weight of the bears in this study, yields a TV of 1,092–2,340 m/l and an MV of 15.6–46.8 l, which are similar to the actual values in our study (1,238.4–1,428.5 m/l and 12.0–20.8 l, respectively). Therefore, compared with dogs, the TV and MV of the Asiatic black bears in our study appear to be in an acceptable range.

Ventilation is best monitored by measuring the PaCO2 in arterial blood via blood gas analysis. The PaCO2 represents the rate and depth of breathing and the amount of metabolic CO2 production [7]. In previous studies of dogs, cats and humans, the PaCO2 increased when the isoflurane concentration increased [2, 7]. On the other hand, in horses anesthetized with thiopental and isoflurane, it decreased when the MAC increased [16], and there were no significant differences in the PaCO2 or HCO3− level between different MACs in iguanas [20]. In our study, increases in ETiso concentration were accompanied by statistically
insignificant increases in the PaCO$_2$, HCO$_3^-$ and TCO$_2$ levels, and significant increases in the ETCO$_2$, but not by changes in RT. These results may reflect the dose-dependent effects of isoflurane on hypoventilation in Asiatic black bears, although the arterial pH did not change significantly.

In general, acidemia is defined as a pH <7.35 and is considered marked when the pH exceeds 7.25. Hypocapnia, mild hypercapnia and marked hypercapnia are defined as a PaCO$_2$ <35 mmHg, 45–60 mmHg and >60 mmHg, respectively [10]. In brown bears anesthetized with carfentanil and isoflurane for 3 hr, the mean pH, PaCO$_2$ and HCO$_3^-$ were 7.21, 54.6 mmHg and 21.5 mmol/l, respectively [19]. Although within a clinically acceptable range, these values indicated mild acidemia and hypercapnia according to the above definitions. Direct comparison of the blood gas values in this study with those in others is limited by the paucity of information about MZT-isoflurane anesthesia in bears. However, the blood gas values in this study appear to be in a clinically acceptable range when compared with those in studies of other types of anesthesia in bears [5, 10, 19, 24]. In those studies, the values during the anesthesia were as follows: pH, 7.2–7.36; PaCO$_2$, 36–59.2 mmHg; HCO$_3^-$, 16.4–21.5 mmol/l; glucose, 61–127 mg/dl; hematocrit, 39–41%; and base excess in the extracellular fluid, −3.2 to −6.2 mmol/l, excepting some extreme values. However, differences in species, drugs, doses, ventilation conditions, oxygen supply and capture methods make comparisons difficult.

It is impossible to anesthetize the bears with isoflurane after simple sedation, and they have to be immobilized previously and deeply by remote delivery system, because bear is a ferocious animal. Thus, in this study, it was used MZT which is one of the best intramuscular combinations in bear anesthesia, although it has cardiorespiratory adverse effects, such as hypertension and hypoxemia [6]. In the present study, the highest MBP was 152.6 ± 25.2 mmHg in ETiso 0.5% and it was probably not hypertensive, because Caulkett et al. [6] and Caulkett & Cattet [5] mentioned mean blood pressure (150 mmHg) in polar bears.

Fig. 2. Scatter plots of Pearson correlation between ETiso concentration and cardio-respiratory parameters, between ETiso concentration and blood gas values. (TCO$_2$, total carbon dioxide, $r$=−0.381, $P$=0.015; pH, $r$=−0.367, $P$=0.02; PaCO$_2$, partial pressure of carbon dioxide, $r$=0.476, $P$=0.002; HCO$_3^-$, bicarbonate, $r$=0.426, $P$=0.006; Heart rate, $r$=0.656, $P$=0.001; Respiratory rate, $r$=0.565, $P$=0.001; MVin, inspired minute volume, $r$=−0.562, $P$=0.001; MBP, mean blood pressure, $r$=−0.830, $P$=0.001; and ETCO$_2$, end-tidal carbon dioxide partial pressure, $r$=−0.392, $P$=0.001)
immobilized with zolazepam-tiletamine was stable and over 200 mmHg in American black bears was hypertensive. However, considering that some papers reported hypertension in polar bears and American black bears immobilized with MZT [6, 11], there may have been hypertension before inhalation of isoflurane. There was a statistical decrease of SpO₂ in ETiso 2.0% compared with ETiso 0.5%, but the degree of decrease was clinically slight and maintained about 95% or more at each ETiso concentration. Thus, the bears didn’t seem to have severe hypoxemia which has been often cited in MZT combination, although did not measure partial pressure of oxygen by blood gas analysis. Increase of blood glucose was reported in reindeers [23] and dogs [4] immobilized with medetomidine. However, there was no increase of glucose, and the values were within normal range of Asiatic black bear (84.4–104.6 mg/dl in this study; and 73–118 mg/dl, 25–75% quartiles in [27]).

In conclusion during spontaneous ventilation in Asiatic black bears, isoflurane caused dose-dependent increases in HR, RR, ETCO₂, PaCO₂ and MV, and dose-dependent decreases in MBP and TV. RT and blood gas values, excepting those CO₂ related, were unaffected by isoflurane. All parameters in this study were in the clinically acceptable range at all times, and isoflurane offsets the increase in blood pressure induced by MZT. We suggest that the combination of MZT and isoflurane can effectively maintain anesthesia during long procedures in Asiatic black bears without mechanical ventilation. ETiso concentrations of 0.5% to 2.5% can be applied to Asiatic black bears without side effects, and 1.0–1.5% is the recommended concentration. These results will be helpful to people who study or care for bears, as well as to the Asiatic black bear reintroduction program in Korea.

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