FULL PAPER

Wildlife Science

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

Dong-Hyuk JEONG[1], Jeong-Jin YANG[1], Seong-Hoon SEOK[2,3], Dong-Joo SONG[1] and Seong-Chan YEON[2,3]*

[1]Species Restoration Technology Institute of Korea National Park Service, Gurye 542-853, Republic of Korea
[2]Laboratory of Veterinary Surgery and Behavior, College of Veterinary Medicine, Gyeongsang National University, Jinju 52828, Republic of Korea
[3]GyeongNam Wildlife Center, Jinju, Republic of Korea

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 (CO_2) partial pressure and the partial pressure of arterial CO_2, 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

The Asiatic black bear (*Ursus thibetanus*) is a vulnerable species according to the International Union for Conservation of Nature and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (Appendix I), and an endangered species in South Korea. In 2001, the Korean National Institute of Environmental Research reported that only about 20 wild bears remained in South Korea, with less than 10 bears in Jirisan National Park. Although the Asiatic black bear reintroduction project was launched in 2002 to establish a self-sustaining bear population in this park [14], many bears have been killed or injured by poachers since its inception. Despite emergency responses and in-field care by Korea National Park Service staff, some severely injured animals required surgery or other extensive treatment at a special wildlife medical facility. For prolonged procedures, such as surgery, an inhalation anesthesia protocol optimized for Asiatic black bears is necessary.

Isoflurane is halogenated ether used for inhalation anesthesia in conjunction with oxygen. Developed in 1965 and approved in the late 1970's in many countries, it has been widely used in veterinary and human practice owing to its chemical stability and minimal side effects [15]. Because it is not appreciably metabolized, it is non-toxic to the liver and kidneys. Isoflurane does not induce cardiac arrhythmias or sensitize the heart to catecholamines; however, similar to other halogenated gases, it produces dose-dependent hypotension due to peripheral vasodilation [21]. As noted by Caulkett *et al.* [6], isoflurane inhalation is a viable means of maintaining anesthesia in bears as well as humans, and its use in brown bears has been reported [3, 12, 19, 25, 26]. However, there is little information about its use in Asiatic black bears; in particular, the relationship between isoflurane dose and cardiorespiratory response has not been established in this or other bear species. The objective of this study was to determine this relationship and the stable range of isoflurane concentrations in Asiatic black bears immobilized via medetomidine-zolazepam-

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 m^2) 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 (CO$_2$ 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 (SpO$_2$) 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 m*l*/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), SpO$_2$, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean blood pressure (MBP), end-tidal carbon dioxide partial pressure (ETCO$_2$), 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 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, SpO$_2$, ETCO$_2$, TVin, TVex, MVin, MVex, SBP, DBP, 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 m*l* syringes with 18 gauge needle (Nayeon Medical, Seoul, Korea) and immediately measured glucose, blood urea nitrogen (BUN), sodium, chlorine, total carbon dioxide (TCO$_2$), hematocrit (Hct), hemoglobin (Hb), pH, partial pressure of arterial carbon dioxide (PaCO$_2$), bicarbonate (HCO$_3^-$) 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 RTs and cardiovascular and respiratory parameters at each designated ETiso concentration. The Kruskal-Wallis test was used to compare
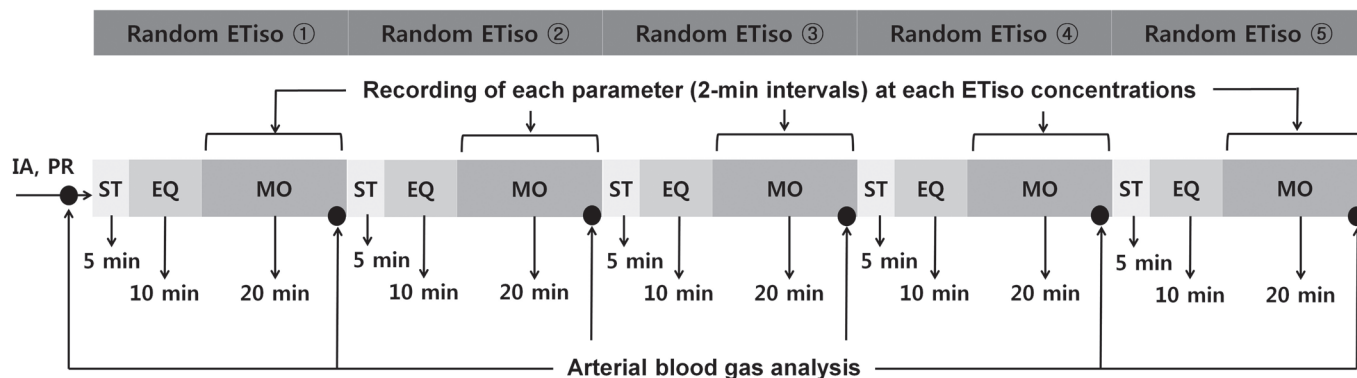
Fig. 1. Representative study design. IA, induction of anesthesia via IM injection of medetomidine, zolazepam and tiletamine; PR, preparation for inhalation anesthesia (transfer to the surgery room, intubation and attachment of monitoring instruments); ETiso, end-tidal isoflurane; ST, stabilization (ETiso changing by no more than ± 0.1% for 5 min); EQ, equilibration (additional 10 min after stabilization); MO, monitoring of parameters (heart rate, respiratory rate, rectal temperature, peripheral capillary oxygen saturation, tidal volume, minute volume and end-tidal carbon dioxide).

Table 1. Dose-response effects of ETiso on temperature and cardiovascular and respiratory parameters following isoflurane anesthesia with spontaneous ventilation in Asiatic black bears

Variable	0.5% ETiso	1.0% ETiso	1.5% ETiso	2.0% ETiso	2.5% ETiso
HR (beats/min)	49.8 ± 11.5	57.0 ± 11.5[a]	64.2 ± 11.3[a,b]	70.7 ± 10.2[a–c]	75.8 ± 9.2[a–d]
RR (breaths/min)	8.8 ± 3.2	10.9 ± 3.8[a]	13.5 ± 4.6[a,b]	15.2 ± 4.4[a,b]	16.5 ± 4.3[a–c]
SBP (mmHg)	205.4 ± 45.5	170.9 ± 39.2[a]	141.6 ± 27.9[a,b]	118.5 ± 27.1[a–c]	101.1 ± 25.1[a–d]
DBP (mmHg)	122.2 ± 17.6	94.2 ± 13.9[a]	73.6 ± 14.5[a,b]	54.2 ± 11.6[a–c]	50.2 ± 14.8[a–d]
MBP (mmHg)	152.6 ± 25.2	121.8 ± 18.5[a]	99.2 ± 13.9[a,b]	81.8 ± 16.7[a–c]	71.9 ± 15.8[a–c]
ETCO$_2$ (%)	4.7 ± 0.82	4.9 ± 0.89	5.3 ± 0.77	5.4 ± 0.3[a,b]	5.5 ± 0.42[a–c]
SpO$_2$ (%)	96.3 ± 1.9	95.7 ± 2.2	95.6 ± 2.6	95.1 ± 2.6[a]	95.5 ± 2.5
RT (°C)	38.1 ± 0.5	38.0 ± 0.5	39.0 ± 0.6	38.1 ± 0.6	38.1 ± 0.6
TVin (ml)	1,428.5 ± 223.5	1,296.6 ± 198.2[a]	1,252.5 ± 222.9[a]	1,293.7 ± 233.8[a]	1,286.5 ± 311.9[a]
TVex (ml)	1,394.3 ± 220.5	1,282.3 ± 207.4[a]	1,238.4 ± 228.5[a]	1,278.1 ± 247.9[a]	1,269.3 ± 316.6[a]
MVin (l/min)	12.0 ± 3.5	13.6 ± 4.1	16.5 ± 5.4[a,b]	19.4 ± 5.4[a–c]	20.8 ± 5.5[a–c]
MVex (l/min)	11.8 ± 3.4	13.6 ± 4.6[a]	16.4 ± 5.9[a,b]	19.3 ± 6.2[a–c]	20.6 ± 6.4[a–c]

Values are presented as mean ± SD. ETiso, end-tidal isoflurane; HR, heart rate; RR, respiratory rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; MBP, mean blood pressure; ETCO$_2$, end-tidal carbon dioxide partial pressure; SpO$_2$, saturation of peripheral oxygen; RT, rectal temperature; TVin, inspiratory tidal volume; TVex, expiratory tidal volume; MVin, inspiratory minute volume; MVex, expiratory minute volume. a) $P<0.05$, significant difference compared with the values at 0.5% ETiso. b) $P<0.05$, significant difference compared with the values at 1.0% ETiso. c) $P<0.05$, significant difference compared with the values at 1.5% ETiso. d) $P<0.05$, significant difference compared with the values at 2.0% ETiso.

the blood gas data at each ETiso concentration. Correlations between cardiovascular and respiratory parameters and ETiso concentrations were evaluated by using Pearson correlation analysis. $P<0.05$ was considered statistically significant.

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, ETCO$_2$, TV (TVin + TVex) and MV (MVin + MVex) differed significantly according to ETiso concentration. The SpO$_2$ decreased at ETiso concentration of 2.0% compared with 0.5% ($P=0.029$), 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, $P=0.001$; RR, $P=0.016$), 1.5 (HR, $P=0.001$; RR, $P=0.001$), 2.0 (HR, $P=0.001$; and RR, $P=0.001$) and 2.5% (HR, $P=0.001$; and RR, $P=0.001$) compared with 0.5%, as was ETCO$_2$ 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,

Table 2. Dose-dependent effects of ETiso on blood gas values following isoflurane anesthesia with spontaneous ventilation in Asiatic black bears

Variable	0% ETiso	0.5% ETiso	1.0% ETiso	1.5% ETiso	2.0% ETiso	2.5% ETiso
Glucose (mg/dl)	84.4 ± 17.6	104.6 ± 32.7	102.9 ± 34.2	103.3 ± 33.9	101.6 ± 25.2	100.8 ± 25.4
BUN (mg/dl)	26.3 ± 3.0	25.1 ± 3.8	25.9 ± 4.7	25.2 ± 3.2	26.3 ± 2.6	28.8 ± 8.4
Na (mmol/l)	125.6 ± 3.6	128.0 ±3.7	128.4 ± 4.9	127.3 ± 7.1	126.7 ± 2.9	126.7 ± 3.9
Cl (mmol/l)	114.0 ± 4.2	115.7 ± 5.8	115.0 ± 5.5	115.0 ± 4.3	114.4 ± 2.8	115.7 ± 6.2
TCO_2 (mmol/l)	20.1 ± 4.1	21.9 ± 2.4	22.9 ± 1.7	22.8 ± 1.7	23.4 ± 1.1	23.0 ± 2.1
Hct (%)	47.7 ± 8.2	46.3 ± 8.7	44.9 ± 12.7	44.5 ± 10.8	45.1 ± 9.2	42.2 ± 9.5
Hb (g/dl)	16.2 ± 2.8	15.7 ± 3.0	15.2 ± 4.3	15.1 ± 3.7	15.3 ± 3.1	14.3 ± 3.2
pH	7.296 ± 0.06	7.291 ± 0.04	7.295 ± 0.03	7.274 ± 0.03	7.249 ± 0.05	7.3 ± 0.1
$PaCO_2$ (mmHg)	39.9 ± 12.9	43.2 ± 6.4	44.1 ± 5.8	46.7 ± 5.7	50.6 ± 5.2	51.4 ± 9.2
HCO_3^- (mmol/l)	18.9 ± 4.0	20.7 ± 2.1	21.3 ± 1.6	21.5 ± 1.6	22.1 ± 1.0	22.1 ± 1.6
BEecf (mmol/l)	−7.4 ± 3.6	−6.0 ± 2.1	−5.0 ± 1.63	−5.2 ± 1.9	−5.1 ± 1.7	−5.3 ± 1.6

Values are presented as mean ± SD. BUN, blood urea nitrogen; Na, sodium; Cl, chlorine; TCO_2, total CO_2; Hct, hematocrit; Hb, hemoglobin, $PaCO_2$, partial pressure of carbon dioxide; HCO_3^-, bicarbonate; BEecf, base excess in the extracellular fluid.

P=0.001; and RR, P=0.001) and 2.5% (HR, P=0.001; and RR, P=0.001) vs. 1.0% and at 2.0 (HR, P=0.002; and RR, P=0.1) and 2.5% (HR, P=0.001; and RR, P=0.001) vs. 1.5%, and $ETCO_2$ was significantly higher at 2.0 (P=0.001 and P=0.015, respectively) and 2.5% (P=0.001 and P=0.001, respectively) vs. 1.0 and 1.5%. MBP was significantly lower at 1.0 (P=0.001), 1.5 (P=0.001), 2.0 (P=0.001) and 2.5% (P=0.001) vs. 0.5% and at 1.5 (P=0.001), 2.0 (P=0.001 and P=0.001, respectively) and 2.5% (P=0.001 and P=0.001, respectively) vs. 1.0 and 1.5%. TVin was significantly lower at 1.0 (P=0.007), 1.5 (P=0.001), 2.0 (P=0.005) and 2.5% (P=0.003) vs. 0.5%, whereas MVin was significantly higher at 1.5 (P=0.001 and P=0.002, respectively), 2.0 (P=0.001 and P=0.001, respectively) and 2.5% (P=0.001 and P=0.001, respectively) vs. 0.5 and 1.0% (Table 1).

The mean blood gas values of the bears at the different ETiso concentrations remained within physiological ranges [5, 10, 11, 24] throughout the anesthetic procedure and did not significantly differ (Table 2). The $PaCO_2$ showed a tendency to increase as the ETiso concentration increased.

ETiso concentration correlated strongly and positively with HR, RR and MVin (r=0.656, 0.565 and 0.562, respectively; P<0.01), strongly and negatively with MBP (r= −0.830, P=0.001), positively with TCO_2, $PaCO_2$ and HCO_3^- (r=0.381, 0.476, and 0.426, respectively; P<0.05) and negatively with pH and $ETCO_2$ (r= −0.397 and −0.392, respectively; P<0.05; Fig. 2).

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 $ETCO_2$ and $PaCO_2$. 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 ml 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 ml 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 $PaCO_2$ in arterial blood via blood gas analysis. The $PaCO_2$ represents the rate and depth of breathing and the amount of metabolic CO_2 production [7]. In previous studies of dogs, cats and humans, the $PaCO_2$ 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 $PaCO_2$ or HCO_3^- level between different MACs in iguanas [20]. In our study, increases in ETiso concentration were accompanied by statistically
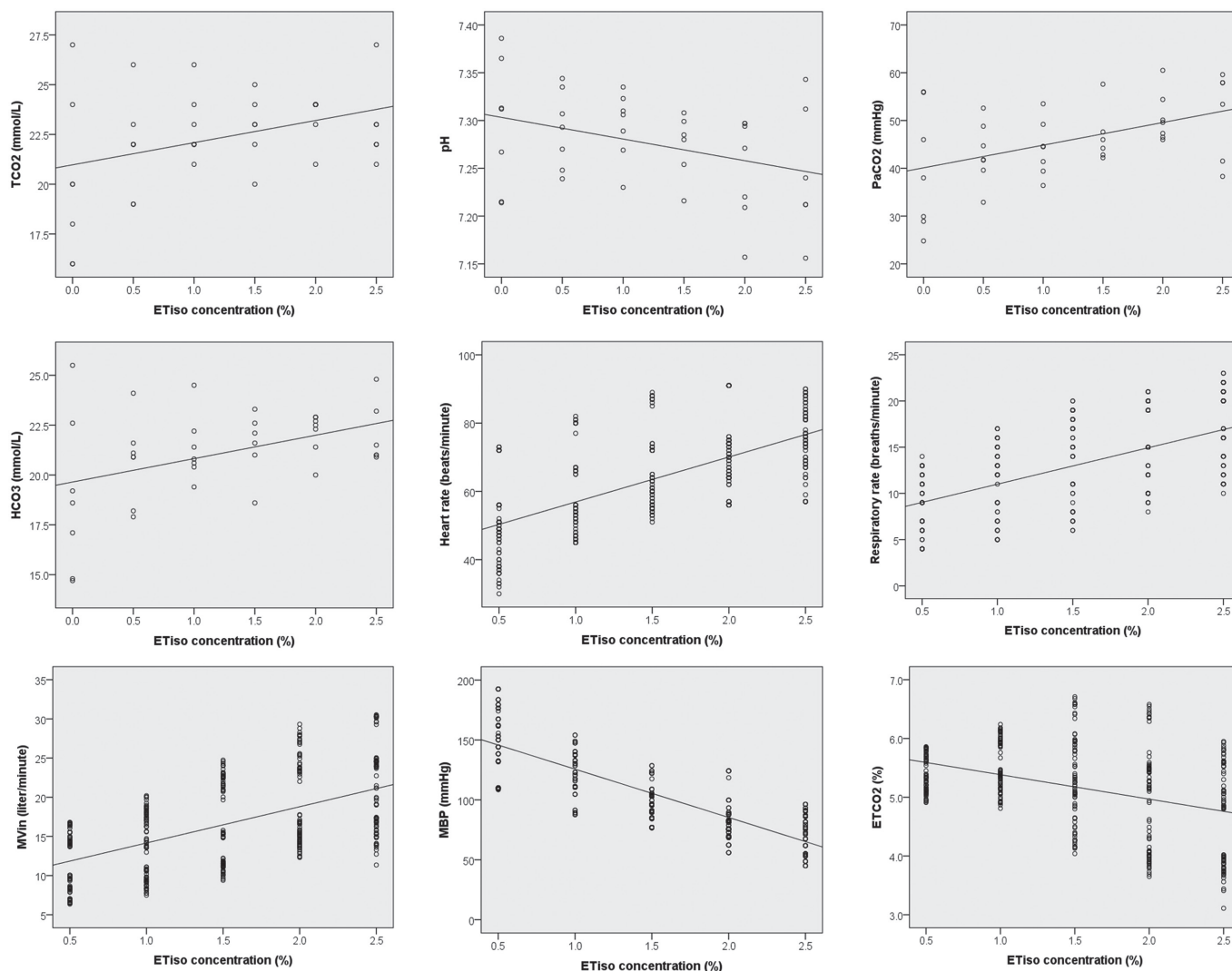
Fig. 2. Scatter plots of Pearson correlation between ETiso concentration and cardio-respiratory parameters, between ETiso concentration and blood gas values. (TCO_2, total carbon dioxide, $r=0.381$, $P=0.015$; pH, $r=-0.367$, $P=0.02$; $PaCO_2$, partial pressure of carbon dioxide, $r=0.476$, $P=0.002$; 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, $r=-0.392$, $P=0.001$)

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

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 SpO_2 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, $ETCO_2$, $PaCO_2$ and MV, and dose-dependent decreases in MBP and TV. RT and blood gas values, excepting those CO_2 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.

REFERENCES

1. Bang, S. H., Kwak, I. Y. and Kim, Y. L. 1987. The cardiovascular effects of isoflurane with increment of minimum alveolar concentration in dogs. *Korean J. Anesthesiol.* **20**: 304–313. [CrossRef]
2. Basak, B. K. 1984. Isoflurane as a general anesthetic: Will it displace all other volatile anesthetics? *J. Am. Assoc. Nurse Anesth.* **52**: 614–618.
3. Boone, W. R., Catlin, J. C., Casey, K. J., Dye, P. S., Boone, E. T. and Schuett, R. J. 1999. Live birth of a bear cub following nonsurgical embryo collection. *Theriogenology* **51**: 519–529. [Medline] [CrossRef]
4. Burton, S. A., Lemke, K. A., Ihle, S. L. and Mackenzie, A. L. 1997. Effects of medetomidine on serum insulin and plasma glucose concentrations in clinically normal dogs. *Am. J. Vet. Res.* **58**: 1440–1442. [Medline]
5. Caulkett, N. A. and Cattet, M. R. 1997. Physiological effects of medetomidine-zolazepam-tiletamine immobilization in black bears. *J. Wildl. Dis.* **33**: 618–622. [Medline] [CrossRef]
6. Caulkett, N., West, G. and Heard, D. 2007. Bears. pp. 409–415. *In*: Zoo Animal and Wildlife Immobilization and Anesthesia (West, G., Heard, D. and Caulkett, N. eds.), Blackwell Publishing, Ames.
7. Clarke, K. W., Trim, C. M. and Hall, L. W. 2014. Veterinary Anaesthesia, 11th ed., Elsevier Health Sciences, Edinburgh.
8. Clark, M. A., Finkel, R., Rey, J. A. and Whalen, K. 2012. Anesthetics. pp. 133–150. *In*: Lippincott Illustrated Reviews: Pharmacology, 5th ed. (Harvey, R. A. ed.), Wolters Kluwer/Lippincott Williams & Wilkins, Philadelphia.
9. Eddie, C. R. 2011. Cardiovascular disease. pp. 200–219. *In*: BSAVA Manual of Canine and Feline Anesthesia and Analgesia (Chris, S. and Tanya, D. N. eds.), British Small Animal Veterinary Association, Quedgeley.
10. Evans, A. L., Sahlén, V., Støen, O. G., Fahlman, Å., Brunberg, S., Madslien, K., Fröbert, O., Swenson, J. E. and Arnemo, J. M. 2012. Capture, anesthesia, and disturbance of free-ranging brown bears (*Ursus arctos*) during hibernation. *PLoS ONE* **7**: e40520. [Medline] [CrossRef]
11. Fahlman, A., Arnemo, J. M., Swenson, J. E., Pringle, J., Brunberg, S. and Nyman, G. 2011. Physiologic evaluation of capture and anesthesia with medetomidine-zolazepam-tiletamine in brown bears (*Ursus arctos*). *J. Zoo Wildl. Med.* **42**: 1–11. [Medline] [CrossRef]
12. Friedrich, K., Pazzaglia, L., Valente, M., Costamagna, G., Wibbelt, G., Kretschmar, P., Hofer, H. and Seet, S. 2009. Laparoscopic ovariohysterectomy in a brown bear (*Ursus arctos*) with pyometra. pp. 85–89. *In*: Proceedings of the International Conference on Diseases of Zoo and Wild Animals, Beekse Bergen.
13. Ian, S. 2006. Adverse drug reactions. pp. 311–322. *In*: Foundations of Anesthesia (Hugh, C. H. and Philip, M. H. eds.), Mosby Elsvier, Phildelphia.
14. Jeong, D. H., Yang, D. H. and Lee, B. K. 2010. Re-introduction of the Asiatic black bear into Jirisan National Park, South Korea. pp. 254–258. *In*: UCN Global Re-introduction Perspectives 2010. (Prital, S. ed.), IUCN/SSC/ Re-introduction Specialist Group, Abu Dhabi.
15. Jeong, S. M. 2002. Effects of electroacupuncture on minimum alveolar concentration of isoflurane and cardiovascular system in isoflurane anesthetized dogs. *J. Vet. Sci.* **3**: 193–201. [Medline]
16. Johnson, C. B. and Taylor, P. M. 1998. Comparison of the effects of halothane, isoflurane and methoxyflurane on the electroencephalogram of the horse. *Br. J. Anaesth.* **81**: 748–753. [Medline] [CrossRef]
17. Kim, Y. K., Lee, S. S., Suh, E. H., Lee, L., Lee, H. C., Lee, H. J. and Yeon, S. C. 2011. Minimum anesthetic concentration and cardiovascular dose-response relationship of isoflurane in cinereous vultures (*Aegypius monachus*). *J. Zoo Wildl. Med.* **42**: 499–503. [Medline] [CrossRef]
18. Ko, S. H., Lee, M. S., Lee, J. R. and Song, H. S. 2000. The effect of age on the cardiovascular responses induced by high concentration of isoflurane. *Korean J. Anesthesiol.* **38**: 212–219. [CrossRef]
19. Mama, K. R., Steffey, E. P. and Withrow, S. J. 2000. Use of orally administered carfentanil prior to isoflurane-induced anesthesia in a Kodiak brown bear. *J. Am. Vet. Med. Assoc.* **217**: 546–549, 503. [Medline] [CrossRef]
20. Mosley, C. A., Dyson, D. and Smith, D. A. 2004. The cardiovascular dose-response effects of isoflurane alone and combined with butorphanol in the green iguana (*Iguana iguana*). *Vet. Anaesth. Analg.* **31**: 64–72. [Medline] [CrossRef]
21. Park, J. Y., Oh, S. J., Lee, H. B. and Jeong, S. M. 2015. Effects of isoflurane/Remifentanil and isoflurane/fentanyl anesthesia in beagle dogs. *J. Vet. Clin.* **32**: 148–153. [CrossRef]
22. Souza, A. P., Guerrero, P. N., Nishimori, C. T., Paula, D. P., Santos, P. S., de Rezende, M. L. and Nunes, N. 2005. Cardiopulmonary and acid-base effects of desflurane and sevoflurane in spontaneously breathing cats. *J. Feline Med. Surg.* **7**: 95–100. [Medline] [CrossRef]

23. Soveri, T., Sankari, S., Salonen, J. S. and Nieminen, M. 1999. Effects of immobilization with medetomidine and reversal with atipamezole on blood chemistry of semi-domesticated reindeer (*Rangifer tarandus tarandus L.*) in autumn and late winter. *Acta Vet. Scand.* **40**: 335–349. [Medline]

24. Teisberg, J. E., Farley, S. D., Nelson, O. L., Hilderbrand, G. V., Madel, M. J., Owen, P. A., Erlenbach, J. A. and Robbins, C. T. 2014. Immobilization of grizzly bears (*Ursus arctos*) with dexmedetomidine, tiletamine, and zolazepam. *J. Wildl. Dis.* **50**: 74–83. [Medline] [CrossRef]

25. Tuttle, A. D., MacLean, R. A., Linder, K., Cullen, J. M., Wolfe, B. A. and Loomis, M. 2009. Acquired arteriovenous fistula in a grizzly bear (*Ursus arctos horribilis*). *J. Zoo Wildl. Med.* **40**: 193–195. [Medline] [CrossRef]

26. Witz, M., Lepage, O. M., Lambert, C., Couillerot, D., Fau, D., Rachail, M. and Troncy, E. 2001. Brown bear (*Ursus arctos arctos*) femoral head and neck excision. *J. Zoo Wildl. Med.* **32**: 494–499. [Medline] [CrossRef]

27. Yang, J. J. 2015. Blood chemistry and hematology of the Asiatic black bear (*Ursus thibetanus ussuricus*) in South Korea. Thesis, Master of Veterinary Medicine, Jeju National University.

28. Yves, M. and Paul, C. 2011. Patient monitoring and monitoring equipment. pp. 61–78. *In*: BSAVA Manual of Canine and Feline Anesthesia and Analgesia, 2nd ed. (Chris, S. and Tanya, D. N. eds.), British Small Animal Veterinary Association, Quedgeley.