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EFFECTS OF TOTAL INTRAVENOUS ANESTHESIA ON HEMATOLOGY AND BIOCHEMISTRY VALUES DURING HEALT CHEK IN BROWN BEARS (URSUS ARCTOS)

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ABSTRACT

The present study was conducted to define the physiological responses of captive brown bears *(Ursus arctos)* immobilized by standardized total intravenous anesthesia protocol (TIVA), during routine health check. Hematology and biochemistry parameters (WBC, Lymph, Mon, Gran, Lymph %, Mon %, Gran %, RBC, HGB, HCT, PLT, ALT, AST, ALP, GLU, TP, ALB, UREA, CREAT, GLB) were evaluated in nine brown bears, anesthetized with total intravenous anesthesia protocol for ninety minutes during the health check. The animals were kept in the „Park for Dancing Bears“ Belitza, Bulgaria. A standardized premedication protocol of tiletamine HCl and zolazepam HCl (Zoletil 100® Virbac, France) at 1 mg/kg, medetomidine HCl at 0.003 mg/kg (3 mcg/kg) and butorphanol tartrate at 0.05 mg/kg (50 mcg/kg) administered intramuscularly. Anesthesia was induced intravenously with a combined bolus of ketamine at 2 mg/kg and propofol at 2 mg/kg, and maintained with a constant rate infusion (CRI) of ketamine at 0,8 mg/kg/h and propofol 0,04 at mg/kg/min**.** Overall results compared to baseline levels did not present statistically significant changes, to the exception of GLU, and CREAT. In conclusion, the research anesthetic protocol is an inexpensive and relatively safe method for various manipulations and procedures in the brown bear.

Key words: Ursus arctos, TIVA, Hematology, biochemistry

INTRODUCTION

Bears are anesthetized for a variety of reasons, they may be anesthetized by wildlife managers for translocation and/or marking. Bears may be anesthetized for research purposes or for medical management. The article discusse the anesthesia of bears for therapeutic manipulation and prophylactic treatment that routinely requires anesthesia. The focus is on changes in some haematology and biochemistry values during anesthesia. Safety of both the patient and personnel requires the patient to remain on a stable anesthetic level with minimal effects on the baseline physiological parameters. There is a relevant risk to the staff while working with this species and in-depth knowledge of the methods of anesthesia is mandatory. Different anesthetic combinations have been used in brown bears in the past. The combination of zolazepam and tiletamine (Zoletil 100 ®) is widely used, despite the absence of an antagonist and often observed prolonged effect, with recovery times reaching up to several hours (Boever et al.1977, Schobert E. 1987, Bush et al., 1980). The combination zolazepam - tiletamine - medetomidine is also an effective anesthetic protocol for brown bears (Arnemo, J. M., 2001). Medetomidine is often used in combination with other drugs such as ketamine and butorphanol for anesthesia of different non-domestic species (Chittick et al., 2001, Larsen et al, 2003, Hahn et al., 2005). As with zolazepam-tiletamine, adding medetomidine as an alpha-2-agonist helps to reduce the dose of other drugs, such as ketamine and butorphanol, administered in a single anesthetic protocol included in the scheme. Butorphanol, a morphinian-type opioid with partial agonist and antagonist activity, provides analgesia and

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moderate tranquilizing effects and can be included in anesthetic protocols of bears as a part of an anesthetic approach (Wolfe et al 2008).

The understanding of haematological and biochemical changes during anesthesia is an important tool to assess health of animals and understand the impact of anesthetics on the individual and population.

Various factors may affect the biochemical and haematological variables and should be considered when anesthetizing patients. Extrinsic factors include factors that may stress the animal, anesthesia whereas intrinsic factors are associated with host characteristics and concomitant illnesses.

Only few studies have evaluated biochemistry and haematology in European brown bears (Pearson et al., 1972, Fahlman et al., 2011), and the published data are mostly based on low numbers of animals or specimen from captive bears, both of which may not be representative of brown bears in general. The objectives of this study were to establish changes for standard haematological and biochemical variables in free-ranging brown bears immobilised with - tiletamine-zolazepam-medetomidine-butorphalol - ketamine and proprfol.

MATERIALS AND METHODS

The goal of this study is to determine how a TIVA anesthetic protocol influences haematology and biochemistry values during anesthesia in brown bears.

Study site and animals

The study includs eight captive brown bears located at the “Four Paws” park for dancing bears near Belica, Bulgaria. The park is located in the Rila Mountains, near Belica, and covers an area of 12 ha at an altitude of 1350 m. The survey was conducted in September 2017. Bears are free-range within the park area and are fed in specifically designated areas (zones) where food is provided in feeders. At the time of the study none of the bears were in hibernation. Age, sex and body mass of all subjects are represented in Table 1. The bears are raised free in the park area and twelve hours prior to anesthesia patients were sequestered in individual holding pens to facilitate pre-anesthetic manipulation and ensure fasting. The bears were anesthetized for a routine dental examination and procedures, ophtalmological examinations, etc. Food was withheld 12 h before the start of anesthesia.

Table 1. Age, sex and weight of the bears

№	Age	Sex	Weight (Kg)
1	23	M	149
2	26	F	133
3	19	M	222
4	17	F	120
5	21	F	111
6	19	F	100
7	25	F	150
8	17	F	119

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Sampling procedure

Blood for haematological analyses was collected from the jugular vein using a 4 mL vacutainer system with K2EDTA (ethylenediaminetetraacetic acid) as anticoagulant (Vacutest® KIMA srl. ARZERGRANDE- Italy). The blood was kept refrigerated until shipment, and kept cool by cooling elements during the transport to the Clinical Chemistry Laboratory. The time from sampling to analysis was 24 hours.

Blood for biochemistry was collected in 4 mL tubes with gel and clot activating factor (Vacutest® KIMA srl. ARZERGRANDE- Italy). The blood tubes were kept at room temperature for 1–2 hours to ensure complete clotting, and then centrifuged at 2000 g for 10 minutes to separate the serum. The serum was stored in 2 mL Eppendorf tubes and kept at −20°C until shipment to the Laboratory. The blood tubes were kept cool by ice packs during shipment.

Sampling steps

Blood samples were obtained in 7 steps. First sampling was after premedication with intramuscular injection of tiletamine HCl and zolazepam HCl (Zoletil 100® Virbac, France) + medetomidine HCl (Sedin®, Vet Farma, Spain) + butorphanol tartrate (Butomidor®, Richterharma AG). After that every 15 minutes a new blood sample was obtained and total of 7 samples for each animal were collected till the 90th minute.

Laboratory analyses

The haematology profile included white blood cell (WBC) count, red blood cell (RBC) count, lymphocytes count, granulocytes count, monocytes count, haemoglobin, haematocrit, platelet count and differential count (percentage of total) on lymphocytes, granulocytes and monocytes. The BC-2800Vet Hematology Analyser (Mindray, China) was used for the haematological analyses. The biochemical profile included alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), total protein (TP), urea (BUN), creatinine, glucose and two protein fractions; albumin and globulin and the albumin-globulin ratio. All clinical chemistry analyses were carried out with an BA-88A® Semiautomatic Chemistry System (Mindray, China).

Anesthetic protocol

Premedication consisted of a combined intramuscular injection of tiletamine HCl and zolazepam HCl (Zoletil 100® Virbac, France) at 1 mg/kg, medetomidine HCl (Sedin®, Vet Farma, Spain) at 0.003 mg/kg (3mcg/kg) and butorphanol tartrate (Butomidor®, Richterharma AG) at 0.05 mg/kg (50mcg/kg). Premedication was administered by distant sedation dart (DAN-INJECT ApS Sellerup Skovvej 116 DK-7080 Børkop - Denmark) and syringes (arrows) using a 1.2 mm x 38 mm needle and 1.5 ml or 3 ml syringes. An 18G (Neotec Medical Industries Ltd. Singapore) intravenous catheter was placed in both the cephalic and medial saphenous vein. Anesthesia was induced using ketamine HCl (Ketaminol®, Intervet - Holand) at 2 mg/kg administered intravenously into the cephalic vein and an intravenous bolus of propofol (2,6-disopropylphenol) (Norofol® Norbrook, Northern Ireland) at 2 mg/ kg administered in the medial saphenous vein, for reasons of chemical incompatibility between propofol and ketamine. A surgical plane of anesthesia was maintained using an intravenous CRI of NaCl 0.9% (10 ml/ kg/h) + ketamine HCl at a rate of 0.8 mg/ kg/h and propofol CRI at 0.04 mg/kg/min. All animals were intubated and ventilated with

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oxygen set at 5-6 L/min. Patients were given intravenous NaCl 0.9% at 10 ml/ kg/h while under anesthesia.

Statistical analysis

The collected data was analyzed by one-way ANOVA/LSD (Statmost for Windows, DataMost Corp.) to assess the effect of time on the monitored parameters in TIVA. Differences were considered statistically significant at $p \leq 0.05$ - *, $p \leq 0.01$ - ** , $p \leq 0.001$ - **.

RESULTS

The results confirmed that in the brown bear (*Ursus arctos*) TIVA anesthetic protocol allows various procedure and treatments of significant duration with a minimal anesthetic risk for the patient. Overall results compared to baseline levels did not present statistically significant changes, to the exception of PLT, GLU, and CREAT. Table 2 presents the results of the haematological test of animals during anesthesia.

Table 2 Haematological values of a brown bears (n=8) during anesthesia

Sampling steps	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	6th sampling	7th sampling
WBC x 10^9/L	8,82±2.1	7,5±1.5	7,9± 1,2	8,5±1,6	8,2±0.77	8,3±1,1	8,4±1,2
Lymph x 10^9/L	3,8± 2	3,2±1,6	2,7±1,2	3±1,6	2,9±2.3	3,6±1,3	3,2±1
Mon x 10^9/L	1,4±0,4	1,2±0,4	1,1±0,4	1,2±0,4	1±0,14	1,2±0,5	1,2±0,4
Gran x 10^9/L	3,5±2,2	3,1±1,6	4±2	4,2±2,3	4,3±3,2	2,8±1	3±1,2
Lymph %	34±16	32±11	26±11	27±13	27±21	35± 17	36±16
Mon %	16±2,3	15,5±3,2	14,3±3,8	13,9±3,4	11,9±2,8	11.2±2.3	12.1±2.2
Gran %	40±6.4	42,4±21,8	48,8±22,2	48,2±23,9	50,7±34,1	49,1±12,3	47,2±13,3
RBC x 10^{12}/L	6,6±0,64	7,1±1,8	6,4±0,7	6,8±1	6,7±0,4	6,4±1.1	6.2±0,9
HGB g/L	157±15,5	163±40	151±16	159±20	158±7	151±10,4	150±12.7
HCT %	49±4	52±12,3	47±5	50±7	49±6	48±9,2	46±5.7
PLT fL	381±174	556±81	567±116	559±155	534±152	502±86	498±99

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Table 3 presents the results of the biochemical test of animals during anesthesia.

Table 3 Biochemical values of a brown bears (n=8) during anesthesia

Sampling steps	1st sampling	2nd sampling	3rd sampling	4th sampling	5th sampling	6th sampling	7th sampling
ALT U/L	19,8±12,4	23,1±3,3	25,5±3,8	24,8±3,5	25±1,4	28±2,3	29,3±3,3
AST U/L	45,1±16,4	34,8±24,8	23,8±22,3	22,3±12,3	24,5±8,3	29±16,3	28,1± 9,3
ALP U/L	23,67±24,3	29,3±14	7,1±5,6	4,6±4,3	4,2±4	9±6.6	8±7,7
GLU mmol/L	3,1±2,9	3,7±2,5	4,2± 3,1	4,9±4	5,5± 3,3	6±4,4*	6,3±4.1*
TP g/L	75±6,75	79±11,2	76±11,9	83±10	77±11,3	80,3±11	80,1±12,5
ALB g/L	39±6,8	36,2±7.2	34±5,07	37,2±6,8	40±6,6	40,1±7.3	40,7±10,1
GLB g/L	35,6±4	42,8±6,5	42,4±6,2	46,1±9,8	52,2±7,8	47,2±7,5	48±9,3
UREA mmol/L	4,3±1,8	6,4±3,8	5,9±3,3	6,2±3,3	5,7±3,8	3,2±2	4±2,1
CREAT µmol/L	60,2±32,7	62,9±19,6	46,6±8,1	57,4±38	38,05±13*	38,5±13*	37,9±11*

p≤0.05 *

DISCUSSION

This study provides thoroughgoing report on hematology and biochemistry parameters for captive brown bears. The use of a standardized total intravenous anesthesia protocol using a multimodal pharmaceutical approach allows for lower drug doses and minimization of individual drug-related side effects. Stress increases the release of glucocorticoid hormones which results in increased gluconeogenesis and decreases use of glucose and decreased sensitivity to insulin, (Reeder et al. 2005). This results in increased blood glucose levels during stress and anesthesia. The anaesthetics used can potentially affect the blood variables. Medetomidine has been shown to increase the blood glucose level in different species of animals (Arnemo et al. 1999, Soveri et al. 1999, Ambrisko et al. 2002).

Different studies have looked at the effects of ketamine or tiletamine/zolazepam on blood variables in the literature. The effect of ketamine in dogs has been linked to effects caused by stress, with increased plasma values of glucose and cortisol.

During the study we found that the creatinin values decreased throughout sampling steps of the study. Creatinine values have been reported to decrease during immobilisation (Brannon RD 1985). The observed changes in the other parameters are not statistically significant and do not deviate significantly from the normal ones.

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CONCLUSIONS

In conclusion, the research anesthetic protocol is an inexpensive and relatively safe method for various manipulations and procedures in the brown bear. The main hematology and biochemistry parameters are not significantly influenced by this anesthesia protocol proposed in this study, which can even be used during surgical procedures of short or medium duration.

REFERENCES

1. Ambrisko TD, Hikasa Y. Neurohormonal and metabolic effects of medetomidine compared with xylazine in beagle dogs. Can J Vet Res. 2002; 66:42–49.
2. Arnemo JM, Ranheim B. Effects of medetomidine and atipamezole on serum glucose and cortisol levels in captive reindeer (*Rangifer tarandus tarandus*) Rangifer. 1999;19:85–89. doi: 10.7557/2.19.2.284.
3. Arnemo, J. M., Brunberg S., Ahlqvist P., Franze´n R., Friebe A., P. Segerstro¨M., So¨derberg A., Swenson J. E. Reversible immobilization and anesthesia of free-ranging brown bears (Ursus arctos) with medetomidine-tiletamine-zolazepam and atipamezole: a review of 575 captures. Proc. Am. Assoc. Zoo Vet., Am. Assoc. Wildl. Vet., Am. Assoc. Reptile Amphibian Vet., Natl. Assoc. Zoo Wildl. Vet. Joint Conf. Pp. 2001; 234–236.
4. Boever, W. J., Holden J., Kane K. K. Use of Telazol TM (CI-744) for chemical restraint and anesthesia in wild and exotic carnivores. Vet. Med. Small Anim. Clin. 1977; 72: 1722–1725.
5. Brannon RD. Serum chemistry of central and northern Alaska grizzly bears. J Wildl Manage. 1985;49:893–900. doi: 10.2307/3801365.
6. Bush M., Custer R., Smith E. Use of dissociative anesthetics for the immobilization of captive bears: blood gas, hematology and biochemistry values. J. Wildl. Dis. 1980;16: 481–489.
7. Chittick, E., Horne W., Wolfe B., Sladky K., Loomis M., Cardiopulmonary assessment of medetomidine, ketamine, and butorphanol anesthesia in captive Thomson's gazelles (Gazella thomsani). J. Zoo and Wildl. Med. 2001; 32: 168–175.
8. Fahlman A, Arnemo JM, Swenson JE, Pringle J, Brunberg S, Nyman G. Physiologic evaluation of capture and anesthesia with medetomidine-zolazepam-tiletamine in brown bears (*Ursus arctos*) J Zoo Wildl Med. 2011;42:1–11. doi: 10.1638/2008-0117.1.
9. Hahn, N., Eisen R. J., Eisen L., Lane R. Ketamine-medetomidine anesthesia with atipamezole reversal: Practical anesthesia for rodents under field conditions. Lab Animal 2005; 34: 48–51.
10. Larsen, R. S., Loomis M. R., Kelly B. T., Sladky M. K., Stoskopf H., Horne W. A. Cardiorespiratory effects of medetomidine- butorphanol, medetomidine butorphanoldiazepam, and medetomidine-butorphanol-ketamine in captive red wolves (Canis rufus). J. Zoo and Wildl. Med. 2002; 33: 101–107.

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11. Pearson AM, Halloran DW. Hematology of the brown bear (*Ursus arctos*) from southwestern Yukon Territory, Canada. Can J Zool. 1972;50:279–286. doi: 10.1139/z72-038.

12. Reeder DM, Kramer KM. Stress in free-ranging mammals: Integrating physiology, ecology, and natural history. J Mammal. 2005;86:225–235. doi: 10.1644/BHE-003.1.

13. Schobert E. Telazol use in wild and exotic animals. Vet. Med. 1987; 82: 1080–1088.

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

15. Wolfe LL., Goshorn C.T., Baruch-Mordo S. Immobilization of Black Bears (Ursus americanus) with a Combination of Butorphanol, Azaperone, and Medetomidine J. Wildl. Dis. 2008; 44(3): 748–752.