EFFECTS OF DIFFERENT DOSES OF MEDETOMIDINE AND TILETAMINE - ZOLAZEPAM ON THE DURATION OF INDUCTION TIME AND IMMOBILIZATION IN FREE-RANGING YEARLING BROWN BEARS (*URSUS ARCTOS*)

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ABSTRACT

We compared anesthetic protocols with different doses of tiletamine-zolazepam (TZ) combined with medetomidine (M) for 288 yearling brown bear (Ursus arctos) immobilizations with the objective of finding a combination of doses that would provide fast induction with a duration of anesthesia long enough to minimize the need for administering additional drug. The duration of induction time and immobilization was dose-dependent. Increasing the M dose resulted in significantly shorter induction times and a lower probability of giving supplemental drugs. Increasing the TZ dose prolonged duration of anesthesia. For yearling brown bears in Scandinavia, captured shortly after den emergence in April and May, we recommend total dart doses of 1.0 to 1.66 mg M/dart, plus 62.5 to 125 mg TZ/dart, depending on the individual requirements for the length and depth of anaesthesia.

KEYWORDS: brown bear, immobilization, induction time, medetomidine, tiletamine, *Ursus arctos*, yearling, zolazepam

INTRODUCTION

Tiletamine - zolazepam (TZ) combined with medetomidine (M) is recommended for immobilizations providing a dose dependent surgical anaesthetic stage (dose dependent), safe human handling, controllable duration of the immobilization with the option to administer supplemental drugs, an adequate reversal with atipamezole, a wide margin of safety, and low drug volumes suitable for remote darting. Tiletamine-zolazepam (TZ) has been widely used for immobilization of brown bears (*Ursus arctos*), either alone or in combination with xylazine or medetomidine (M) (Cattet et al. 2003; Fahlman et al. 2011). Currently, M and TZ are considered to be the drugs of choice for free-ranging brown bears (Arnemo et al. 2011; Fahlman et al. 2011; Kreeger and Arnemo 2012). Physiologic effects of capture and anesthesia with this combination have been reported in free-ranging brown bears (Fahlman et al. 2011; Fahlman et al. 2010), but the effects of different doses and drug ratios on the duration of induction and immobilization have not been evaluated.

Recommended doses of anesthetic agents for wild animals are usually empirically determined or extrapolated from other species. There are a few reports on controlled clinical trials in captive wildlife (Ryeng et al. 2002; Storms et al. 2006), but these cannot be carried out in free-ranging wild animals, as conditions in the wild are not suitable for controlled clinical studies.

Induction times should preferably be short to minimize stress, the risk of injury, the risk that the bears enter unsuitable terrain, to avoid mother-off-spring separation, and to ensure that the anesthetized individual is clinically monitored as soon as possible. Furthermore, anesthesia duration should preferably be long enough to carry out all the necessary work without having to administer supplemental drugs. Here we report the effects of different doses of M and TZ on induction time and anesthesia duration of free-ranging yearling brown bears.

MATERIALS

Study area

We analyzed data collected in two study areas in Scandinavia from 1992 - 2009. The southern study area, hereafter named the south, was in Dalarna and Gävleborg counties in south-central Sweden (61º N, 15º E, ~13,000 km²). The northern study area, hereafter named the north, was in Norrbotten County in northern Sweden (67º N, 18º E, ~8,000 km²). The rolling landscape in the south is covered by an intensively managed coniferous forest and elevations range from 200 to 1000 m altitude. The northern area is characterised by deep valleys, glaciers and high plateaus ranging up to 2000 m in altitude (Zedrosser et al. 2006). Brown bears were captured shortly after den emergence (Arnemo et al. 2011), in mid-April in the south and at the beginning of May in the north (Zedrosser et al. 2007). Mean yearling litter size is 2.4 and does not differ between the study areas (Swenson et al. 2001; Zedrosser et al. 2009).

Capture and handling

All bears were captured as a part of a long-term project on brown bear ecology in Scandinavia (Swenson et al. 2001; e.g., Swenson et al. 1995; Zedrosser et al. 2009). Yearlings accompanying their radio-marked mothers were darted from a helicopter using a remote drug delivery system (Dan-Inject®, DK-7080 Børkop, Denmark). The standard capture procedure was to first immobilize the yearling offspring and then the mother (Fahlman et al. 2011). For yearlings we used 1.5 ml dart syringes with 1.5 x 25 mm barbed needles with different doses and ratios of medetomidine (M) (Domitor® 1 mg/ml or Zalopine 10 mg/ml, Orion Pharma Animal Health, Turku, Finland) and tiletamine-zolazepam (TZ) (Zoletil® 500 mg dry powder, Virbac, Carros, France) (Table 1). Tiletamine-zolazepam is commercially available only as premixed drug combination in a ratio of 1:1. All following dose information will therefore imply that both drugs are in an equal proportion. Dose is expressed as mg per animal or mg per kg bodyweight. Induction time was defined as the time from dart injection until an individual was immobilized without movement. If an individual showed no or only slight

signs of anaesthesia within 5-10 minutes after receiving the first dart, a second dart with the same dose was administered (Fahlman et al. 2011). Handling time was the period between the animal being immobilized without movement until administration of the antidote. This period was influenced by amounts of samples taken, litter size, terrain conditions and helicopter landing possibilities. Rectal temperature, pulse rate, and respiratory rate were measured throughout the immobilization, and a pulse oximeter (Nellcor® NP-20, Nellcor Inc., Pleasanton, CA, USA) with the sensor (VetSat®) clipped to the tongue measured hemoglobin oxygen saturation (Arnemo et al. 2011). Not all physiologic measurments were recorded contiuously nor were they recorded at specific time intervals for most captures before 2006. We introduced intranasal oxygen therapy in 2006 to prevent or treat hypoxemia during immobilization (Fahlman et al. 2010). We have implanted intraperitoneal radiotransmitters (Telonics®, model IMP/400/L HC) in all female yearlings in the south since 1997 (Arnemo et al. 2011). Supplemental drugs were defined as additional drugs administered to extend the period of immobilization.We used atipamezole (Antisedan® 5 mg/ml, Orion Pharma Animal Health) administered intramuscularly at 5 mg per mg of medetomidine for reversal (Arnemo et al. 2011). All captures and handling conformed to the current laws regulating the treatment of animals in Sweden and was approved by the Ethical Committee on Animal Experiments, Uppsala, Sweden.

Data analysis

We limited statistical analysis to yearlings immobilized with the first dart; captures with failed darts or multiple darts were not included. Sample sizes differed between analyses due to missing data. To avoid co-linearity among variables and because a given ratio of M:TZ will result in different amounts of drug injected dependent upon the body weight of a given bear, we did not use the ratio of M:TZ but rather the interaction M mg/kg * TZ mg/kg to evaluate the combined effect of the two drugs. The variables mg/kg M and mg/kg TZ were normalized

with a mean of zero and a variance of one (Zuur et al. 2007). We used a two sample *t*-test to compare the differences in body mass between the study areas and to evaluate if it was necessary to control for the effects of study area in our analyses.

We carried out four analyses. We evaluated whether the individual handling times differed among years with a general linear model, because sampling procedures changed between the years over the course of our long-term study. In this analysis we controlled for the effect of litter size on handling time.

We evaluated which factors affected the length of induction time (in minutes) with a Poisson distributed generalized linear model. We tested the effect of the following variables on induction time: dose of M in mg/kg, dose of TZ in mg/kg, the interaction between these two variables, and capture order (as factor; whether an individual was captured as first, second or third offspring in a litter).

We evaluated which factors affected the probability (0 = no, 1 = yes) of administering additional drugs to a yearling with a binomial generalized linear model. We tested the effect of the following variables: dose of M in mg/kg, dose of TZ in mg/kg, the interaction between these two variables, capture order, litter size (as factor variable), handling time, and whether a radio-transmitter was implanted or not (as binomial variable, no = 0, yes = 1).

We evaluated which factors affected the time (in minutes) after which additional drugs had to be administered during captures with a general linear model. We tested the effect of the following variables: dose of M in mg/kg, dose of TZ in mg/kg, the interaction between these two variables, capture order, litter size, handling time, and whether a radio-transmitter was implanted or not.

We carried out model selection in all analyses using the *drop1*-function (e.g., Zuur et al. 2009) in the statistical software R 2.12.0 (R-Development Core Team 2010). The level for statistical significance was set to be $P \leq 0.05$, and *p*-values $P < 0.1$ were considered statistically suggestive.

RESULTS

We captured 387 yearling brown bears during 1992-2009. Of these, 85% (328) were captured after one dart injection, 13% (52) required two darts, 2% (6) required 3 darts, and 0.3% (1) required 4 darts. We observed an overall capture mortality rate of 0.005% (n = 2; one yearling died due to dart trauma, the other due to shock/circulatory failure). Due to missing data, 40 yearling captures with one dart injection had to be excluded from further analyses. The litter size of the captured yearlings ranged from 1-3 cubs; 141 yearlings were either singletons or captured as first sibling of the litter, 104 were captured as second sibling of a litter, and 43 yearlings were captured as third sibling of a litter (n = 288).

Yearling body mass ranged from 8-45 kg and did not differ between the study areas (north: 22.2 ± 6.0 kg (mean ± SD), south: 22.5 ± 6.1 kg, two-sample t-test, t = -0.417, df = 286, P = 0.677, n = 288). Therefore, we pooled the data from both study areas for further analyses.

The handling time of individuals increased significantly with litter size (Table 2), however it did not vary among years of the study period (P = 0.612). The mean overall handling time of all yearlings in litters of size 1 was 93 ± 32 minutes, in litters of size 2, 105 ± 27 minutes, and in litters of size 3, 112 ± 28 minutes (n = 288).

Induction time decreased significantly with an increasing dose of M mg/kg (i.e., faster induction time with higher dose of M), and increased significantly with an increasing dose of TZ mg/kg in relation to M mg/kg (i.e., the more M mg/kg in relation to TZ mg/kg, the longer the induction time) (Table 3). Capture order had no significant effect on induction time (P = 0.751), and was removed to obtain the final model.

The probability that an additional dose had been administered increased significantly with handling time, but decreased suggestively with an increasing dose of M mg/kg (Table 4). None of the variables capture order (P = 0.966), whether or not a radio-transmitter was implanted (P = 0.939), M mg/kg*TZ mg/kg (P = 0.250), litter size (P = 0.222), and TZ mg/kg

(P = 0.209) had a significant effect on the probability that an additional dose had been administered, and were removed in that order to obtain the final model.

The time after which additional drugs had been administered increased significantly with increasing TZ mg/kg, and increased significantly with a decreasing dose of M mg/kg in relation to TZ mg/kg (i.e., the more TZ mg/kg in relation to M mg/kg, the later an additional dose was needed) (Table 5). The variable M mg/kg was not significant by itself (P = 0.905), but was retained in the final model because it was part of a significant interaction. None of the variables capture order (P = 0.253), whether or not a radio-transmitter had been implanted (P = 0.841), and litter size (P = 0.160) had a significant effect on the time after which additional drugs had been administered, and were removed in that order to obtain the final model.

DISCUSSION

The duration of induction is important for safety reasons, but it is also important to minimize the excitement stage of anesthesia, with all its side effects (Kreeger and Arnemo 2012). Inductions that are too short, due to over dose or poor body condition and health status, may lead to cardiovascular or respiratory collapse (Frey and Löscher 2002). The yearlings showing the shortest induction times were those with either higher doses of all three drugs, MZT, or those with a lower ZT dose and a higher M dose (Table 1). This agrees with the general knowledge about the reduction of each drug component using balanced anesthesia (i.e. a combination of TZ with M reduces the effective dose of TZ by as much as 75%) described by Cattet et al. (1997). A reduction of ZT is preferable, as T cannot be antagonized and therefore causes prolonged recoveries. Higher doses of M may cause problems with increased vascular resistance due to alpha 2 adrenergic receptor occupation (Caulkett et al. 1999)et al. 1999) and a ceiling effect might be reached at higher plasma concentrations, with no further sedative effects (Kuusela et al. 2000). This has not been documented in bears, however. We recorded an overall capture mortality rate of 0.005% for the yearling captures,

reflecting the wide safety margin of this drug combination and the ability of using a wide range of doses without adverse effect. Wide safety margins are important in immobilizing wildlife in general, as exact body mass cannot be determined from a distance.

The three dart doses that had the fastest induction times, had a range of M doses between 1.0 to 1.66 mg/dart (mean mg/kg body weight (BW) range = 0.04 - 0.10 mg/kg), and ZT doses of 62.5 to 125 mg/dart (mean mg/kg BW = 3.15 – 5.61 mg/kg) for brown bear yearlings immobilized shortly after den emergence in April and May (Table 1).

Many factors must be considered when deciding a dart dose, weight varies with season; bears weigh more during autumn then after leaving the den in spring, therefore autumn doses should be higher than spring-doses we report here. In a stressed animal with an activated fight and flight response, higher doses of immobilizing drugs are required than in calm and naïve animals. Animals undergoing surgery require analgesics (pain medication) and a deeper plane of anesthesia compared to animals immobilized for non-invasive procedures, such as radio-collaring or body measurements. When prolonged procedures are planned, one should consider administering higher doses of TZ, to increase the duration of anesthesia. It is also important to consider the physiological effects that the drug combination and doses used will have on the animal, and monitor the animal's physiological condition during anesthesia as standard procedure. Our data suggest that high doses of M in relation to ZT increased induction times and the duration of immobilization. However, hypoxemia is a common side effect in brown bears anesthetized with MZT at the doses we suggest, and the degree of hypoxemia may be related to the dose of M (Fahlman et al. 2011). Intranasal oxygen therapy should be provided when using this protocol to increase the safety for the anesthetized bears (Fahlman et al. 2010).

ACKNOWLEDGEMENTS

We thank A. Söderberg and R. Franzén for help with the capture of bears. The Scandinavian Brown Bear Research Project was funded by the Swedish Environmental Protection Agency, the Norwegian Directorate for Nature Management, the Swedish Association for Hunting and Wildlife Management, WWF Sweden, and the Research Council of Norway. All capture and handling was approved by the appropriate authority and ethical committee (Djuretiska nämden i Uppsala, Sweden). This is scientific paper no. 132 from the Scandinavian Brown Bear Research Project.

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Table 1: Doses and ratios of medetomidine (M), tiletamine-zolazepam (TZ), body mass, induction time, and time after which additional drugs had been administered to free-ranging yearling brown bear immobilized in Sweden during 1992-2009. Induction time is defined as the time from darting to until an individual was immobilized without movement. Induction time and time until additional drugs were administered are presented as mean time (standard deviation, SD) and median time (range) in minutes, *n* is the number of individuals per group.

Total dose M+ZT (mg)	Ratio M:ZT	Dose M (mg/kg)	Dose TZ (mg/kg)	Body mass (kg)	Mean time (SD)	Median time (range)	*n*	Mean time (SD)	Median time (range)	*n*
1.25+62.5	1:50	0.04-0.16	1.8-7.8	8-35	3.2 (1.9)	3 (1-9)	125	71.9 (30.2)	77.0 (5-116)	19
1.66+83.3	1:50	0.06-0.17	3.0-8.8	9.5-28	3.3 (1.9)	3 (1-9)	26	57.4 (31.8)	48.5 (28-116)	8
1.00+100	1:100	0.02-0.10	2.3-10.0	10-44	4.5 (1.6)	5 (1-7)	11	81.7 (40.4)	63.0 (54-128)	3
1.00+125	1:125	0.02-0.06	2.9-7.8	16-43	3.1 (1.8)	3 (1-9)	71	86.2 (32.0)	96.0 (9-130)	11
0.75+125	1:167	0.02-0.05	3.8-8.3	15-33	4.4 (1.7)	4 (2-8)	16	111.0 (15.5)	111.0 (92-130)	4
0.50+125	1:250	0.01-0.05	3.0-12.5	10-42	3.6 (2.7)	3 (1-11)	39	69.8 (28.9)	65.5 (34-115)	12

Table 2: Significant results of a generalized linear model testing whether individual handling times (i.e. how long an individual was handled after immobilization without movement until the administration of a reversal drug) of 288 yearling brown bears differed among years in Sweden during 1992-2009. The effect of the following variables was tested: year (as factor), and litter size (as factor, with the effect of litter size 1 set to 0), i.e. if an individual had been captured as part of a litter consisting of either one, two, or three yearlings. β = logistic regression coefficient, SE = standard error, z = z-value, P = significance level.

Variables	β	SE	z	P
Litter size 1	0	0		
Litter size 2	11.622	6.845	1.698	0.091
Litter size 3	18.947	6.932	2.733	0.007

Table 3: Significant results of a generalized linear model testing which factors affected the length of induction time (in minutes) for captures of 288 yearling brown bears in Sweden during 1992-2009. Induction time is defined as the time from darting until the animals was immobilized without movements. The effect of the following variables was tested: dose of medetomidine (M) in mg/kg, dose of tiletamine-zolazepane (TZ) in mg/kg, the interaction between M mg/kg * TZ mg/kg, and capture order (whether an individual was captured as first, second or third offspring in a litter). β = logistic regression coefficient, SE = standard error, z = z-value, P = significance level.

Variables	β	*SE*	z	P
M mg/kg	-0.112	0.040	-2.850	0.004
TZ mg/kg	-0.026	0.033	-0.788	0.431
M mg/kg * TZ mg/kg	0.070	0.031	2.277	0.023

Table 4: Significant results of a generalized linear model testing which factors affect the probability (binomial, with 0 = no, 1 = yes) of whether additional drugs had been administered during captures of 240 yearling brown bears in Sweden during 1992-2009. The effect of the following variables was tested: dose of medetomidine (M) in mg/kg, the overall time (in minutes) an individual was handled, capture order (whether an individual was captured as first, second, or third offspring in a litter), litter size, and whether or not an radio-transmitter had been implanted. β = logistic regression coefficient, *SE* = standard error, *z* = *z*-value, *P* = significance level.

Variables	β	*SE*	*z*	*P*
Handling time	0.028	0.006	-5.889	<0.001
M mg/kg	-0.302	0.166	-1.826	0.068

Table 5: Significant results of a generalized linear model testing which factors affect the time after which additional drugs had been administered during captures of 52 yearling brown bears in Sweden during 1992-2009. The effect of the following variables was tested: dose of medetomidine (M) in mg/kg, dose of tiletamine-zolazepam (TZ) in mg/kg, the interaction M mg/kg * TZ mg/kg, the overall time (in minutes) an individual was handled, capture order (whether an individual was captured as first, second or third offspring in a litter), litter size, and whether or not an radio-transmitter had been implanted. β = logistic regression coefficient, *SE* = standard error, *t* = *t*-value, *P* = significance level.

Variables	β	*SE*	*t*	*P*
M mg/kg	-0.480	4.022	-0.119	0.906
TZ mg/kg	11.672	4.931	2.367	0.021
M mg/kg * TZ mg/kg	-7.627	2.776	-2.748	0.008