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## USE OF MEDETOMIDINE-ZOLAZEPAM-TILETAMINE WITH AND WITHOUT ATIPAMEZOLE REVERSAL TO IMMOBILIZE CAPTIVE CALIFORNIA SEA LIONS

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**ABSTRACT:** From June 1998 to August 1999, 39 California sea lions (*Zalophus californianus*) were immobilized at a rehabilitation center in northern California (USA) using medetomidine plus zolazepam and tiletamine (MZT), alone and in combination with isoflurane, with atipamezole reversal. Animals were given 70 µg/kg medetomidine with 1 mg/kg of a 1:1 solution of tiletamine and zolazepam intramuscularly. Mean (±SD) time to maximal effect was 5 ± 3 min. At the end of the procedure, animals were given 200 µg/kg atipamezole intramuscularly. Immobilization and recovery times were, respectively, 28 ± 18 and 9 ± 7 min for 15 animals maintained with MZT alone and 56 ± 47 and 9 ± 6 min for 18 animals intubated and maintained with isoflurane. One mortality occurred during anesthesia. Other disadvantages of the MZT combination included some prolonged ataxia, weakness and disorientation during recovery. However, the use of MZT resulted in faster induction and a more reliable plane of anesthesia that was reversible with atipamezole and safer than other previously used intramuscular agents. Physiological parameters including heart rate, respiratory rate, temperature, pulse oximeter saturation, and end-tidal carbon dioxide were monitored.

**Key words:** Anesthesia, atipamezole, California sea lion, end-tidal carbon dioxide, immobilization, isoflurane, medetomidine, tiletamine, *Zalophus californianus*, zolazepam.

### INTRODUCTION

Relatively safe anesthesia in otariids has been accomplished by induction with inhalant anesthetic agents (Heard and Beusse, 1993; Heath et al., 1997; Gales and Mattlin, 1998). However, larger free-ranging otariids especially adult males are not easily restrained for procedures. In addition, many facilities that maintain otariids in permanent display collections do not have adequate space, equipment or necessary personnel to safely capture and restrain the animal for a sufficient duration to allow for induction with an inhalant anesthetic. The immobilization of many large otariids, therefore, still requires the use of intramuscularly (IM) administered anesthetics.

Unfortunately, some of these anesthetic agents, such as zolazepam with tiletamine (ZT), have narrow margins of safety and have resulted in unacceptable mortality rates especially in field conditions when used alone (Loughlin and Spraker, 1989). The use of the α<sub>2</sub>-agonist medetomidine may provide safer anesthesia as compared

to previously used agents because of its reversibility by the α<sub>2</sub>-antagonist atipamezole. In other species, medetomidine is often combined with a more potent anesthetic agent such as ketamine to provide sufficient immobilization (Jalanka and Roeken, 1990; Cullen, 1996).

Recently, the use of medetomidine and ketamine (MK) alone and with isoflurane, with atipamezole reversal was evaluated in a group of California sea lions (*Zalophus californianus*) undergoing rehabilitation at a center in central California (Haulena et al., 2000). It was found that, although the combination was very safe, the time to maximal effect as well as plane of sedation achieved were variable and the required injection volume and drug cost were high.

Medetomidine plus zolazepam and tiletamine (MZT) has been used in free-ranging polar bears (*Ursus maritimus*) to provide safe and effective immobilization (Cattet et al., 1997) that was more reliable than MK (Cattet et al., 1999). The dosage of medetomidine in the MZT combination in polar bears was much less than when it was used with ketamine in the California

sea lion study. It was hypothesized, therefore, that cost and injection volume in sea lions could be reduced while reliability increased if MZT was used instead of MK.

It was the purpose of the current study to describe the efficacy of MZT, alone and in combination with isoflurane, with reversal using atipamezole in California sea lions. The dosages used in the current investigation were derived by halving both the medetomidine dosage that had been previously used in sea lions (Haulena et al., 2000) and the dosage of ZT that has been previously used in otariids (Loughlin and Spraker, 1989; Gage, 1993). It was hypothesized that this dosage would decrease the potential of adverse effects that had been reported for higher dosages of ZT in otariids. This is the first report of the use of MZT in anesthetized pinnipeds.

#### MATERIALS AND METHODS

From June 1998 to December 1999, 26 female and 13 male California sea lions at a rehabilitation center in northern California (USA), were immobilized for a variety of medical procedures including wound debridement, radiography, sonography, endoscopy, laparoscopy, and orthopedic surgery. The animals ranged in weight from 15 to 220 kg (mean  $\pm$  SD =  $44 \pm 36$  kg) and in age from approximately 1 yr to adult (estimated >5-yr-old). At the time of immobilization many of the animals were affected with a variety of potentially anesthetic-complicating disorders including pneumonia, nephritis and head trauma. Physical status prior to immobilization was scored as good ( $n = 19$ ), fair ( $n = 14$ ), or poor ( $n = 6$ ). Animals in good physical status were bright, responsive, only mildly underweight if at all, and not obviously affected by any condition that would compromise anesthesia. Animals in fair physical condition may have been lethargic, moderately underweight, had an estimated dehydration of up to 5%, or had some suggestion of clinical disease such as mild to moderate dyspnea. Animals in poor condition showed clinical signs such as marked lethargy, weakness, anorexia, estimated dehydration approaching 10%, labored respiration, central nervous system deficits or serum biochemistry values suggestive of renal failure. All animals were weighed within 3 days of immobilization as part of routine husbandry procedures.

Atropine sulfate (Radix Laboratories Inc.,

Eau Claire, Wisconsin, USA) at a dosage of 0.02 mg/kg was given IM to each animal at least 10 min prior to administration of the immobilizing agents. Atropine at one-half of the original dose was repeated at 45 min to one hour intervals or if the animal exhibited bradycardia (heart rate was less than 60 beats per min). Each animal was given approximately 70  $\mu$ g/kg medetomidine (Domitor®, Pfizer Animal Health, Exton, Pennsylvania, USA) and 1 mg/kg of a 1:1 combination of ZT (Telazol®, Fort Dodge Animal Health, Fort Dodge, Iowa, USA) intramuscularly by hand injection using 3, 6 or 12 ml syringes and  $1.2 \times 40$  mm needles (Monoject®, Sherwood Medical, St. Louis, Missouri, USA) with manual restraint. Sites for all IM injections included muscle immediately surrounding the pelvis, femur and tibia. The drugs were administered together in the same syringe.

Time to maximal effect, as defined as the time from injection of MZT to the point after which no noticeable increase in depth of anesthesia took place, was recorded. Maximum plane of anesthesia was scored from Level I to III. Animals at Level I would react to noise, would not lie in place for radiographs, or required physical restraint to accomplish even minor procedures such as venipuncture or masking for gas anesthesia. Animals reaching Level II would display a swallowing reflex and some jaw tone, reaction to deep pain caused by a flipper pinch or lancing of a dermal abscess, but would easily maintain sternal and lateral recumbency for radiographs or ultrasound without additional restraint. Sea lions at Level III could be intubated and showed no swallowing reflex or response to deep pain.

Heart rate and respiratory rate were monitored by stethoscopic chest auscultation and observed thoracic movements respectively. Pulse oximeter saturation (SpO<sub>2</sub>) was monitored using a Vet/Ox 4404® monitor with a standard lingual sensor clip (Heska Corporation, Fort Collins, Colorado, USA) with the clip probe attached to the distal one-third of the tongue. An 8.0 cm long, 1.5 cm diameter plastic hollow tube was placed on opposing canines on one side of the mouth as a gag to prevent pinching of the tongue by the teeth. Esophageal temperature was measured at approximately the level of the heart by an esophageal Vet/Sensor ECG Plus® probe (Heska Corporation) that was also attached to the Vet/Ox 4404®. End-tidal carbon dioxide (EtCO<sub>2</sub>) was measured using a Vet/Cap 7100 Plus® capnography monitor (Heska Corporation) attached to the endotracheal tube via a filter line with a drying tube (Product Number 7102, Heska Corporation, patent pending).

Of the 39 animals, 17 sea lions (12 females and 5 males) were immobilized with MZT alone. This subset of animals ranged in weight from 16 to 220 kg (mean  $\pm$  SD =  $56 \pm 47$  kg). Preanesthetic condition was scored as good in 11, fair in three, and poor in three of these sea lions. Heart rate was recorded from 10 animals approximately every 7 min and respiratory rate was recorded from 13 animals approximately every 7 min during the immobilization. SpO<sub>2</sub> was recorded from two animals every 5 minutes and EtCO<sub>2</sub> was recorded from three animals approximately every 5 min. Seven of these animals (six females and one male ranging in weight between 23 and 44 kg) were intubated for the duration of the procedure by holding the mouth open with 2 cm wide, 40 cm long nylon straps and visualizing the larynx with a 150 mm McIntosh laryngoscope blade (Rusch Inc., Duluth, Georgia, USA). The cuffed endotracheal tubes (Rusch Inc.) ranged from 7 to 10 mm in diameter. Immobilization time was defined as the time from first observed maximal effect to the time of atipamezole (Antisedan®, Pfizer Animal Health) injection at the end of the procedure.

The remaining 22 sea lions (14 females and eight males) were intubated and maintained with isoflurane (AErrane®, Fort Dodge Animal Health) for procedures that were expected to be longer than 30 min or of a more invasive nature such as laparoscopy or orthopedic surgery. This group of sea lions ranged in weight from 15 to 114 kg (mean  $\pm$  SD =  $35 \pm 23$ ). Preanesthetic condition was good in eight, fair in eleven, and poor in three of the animals. If these animals could not be intubated after being given MZT they were given 3 to 5% isoflurane with oxygen flow at 2 to 5 L/min through a mask (Jorgensen Laboratories Inc., Loveland, Colorado, USA) placed over the animal's muzzle until intubation could be accomplished. Cuffed endotracheal tube diameter in this group ranged from 7.0 to 16.0 mm. All 22 individuals were maintained with 0 to 3% isoflurane and a reduced oxygen flow of 2 to 3 L/min delivered through a precision Fluotec II vaporizer in a standard, semi-closed, small animal rebreathing system (VMS®, MDS Matrx, Orchard Park, New York, USA). The animals were maintained at anesthetic Level III for the remainder of the procedure.

Heart rate was recorded from 21 animals, respiratory rate and SpO<sub>2</sub> were recorded from 16 sea lions, and temperature and EtCO<sub>2</sub> were recorded from five animals. If SpO<sub>2</sub> fell below 85%, the sea lions were manually ventilated by squeezing the rebreathing bag. Immobilization time was recorded as the time from first observed maximal effect after MZT injection to

injection of atipamezole. Atipamezole was given immediately after turning off isoflurane gas at the end of the procedure for all but one animal. One animal was given atipamezole 36 min after MZT due to bradycardia and dilating pupils and maintained for the remaining 135 min with 1 to 1.5% isoflurane. Animals were allowed to recover on room air while still intubated and extubated once a swallowing reflex was re-established and the animals attempted to cough the endotracheal tube out of the trachea.

At the end of the procedure, 15 animals that had been given MZT only and 18 that also had received isoflurane gas were given 200 µg/kg atipamezole IM in the muscle overlying the pelvis, femur, and tibia. Recovery time was recorded as the time elapsed from injection of atipamezole to the ability to stand on front flippers and begin locomotion.

Statistical analysis was performed using Instat version 2.01 (GraphPad Software, San Diego, California, USA). Differences between time and physiologic parameters were calculated using a two-tailed Student *t*-test and was considered significant at  $P < 0.05$ . If calculated means had significantly different standard deviations ( $P < 0.05$ ), then a non-parametric Mann-Whitney test was used.

## RESULTS

Injection of MZT resulted in rapid (Table 1) and reliable immobilization with a majority of animals reaching a plane of anesthesia sufficient to allow intubation (Table 2). Only two animals did not reach an anesthetic plane higher than Level I and one of these moved during the MZT injection which caused a partial loss of an unknown quantity of the anesthetic. Of the animals that were given a preanesthetic score of good, six (32%) reached up to Level II, and 13 (68%) reached Level III. Of the sea lions in fair preanesthetic condition, one (7%) reached only Level I, three (22%) reached up to Level II, and 10 (71%) reached Level III. For the animals that were in poor condition, one (17%) reached only Level I, three (50%) reached up to Level II, and two (33%) reached Level III. There did not appear to be any direct relationship between preanesthetic score and plane of anesthesia reached. Overall, 37 (95%) of all the animals reached at least Level II, a plane of

TABLE 1. Ranges and means  $\pm$  SD of recorded time parameters describing the immobilization and reversal characteristics of medetomidine-zolazepam-tiletamine (MZT) and atipamezole, used alone or with isoflurane, in California sea lions (*Zalophus californianus*).

Time parameter	Number of animals	Range (min)	Mean $\pm$ SD (min)
Time to maximal effect <sup>a</sup>	39	3–14	5 $\pm$ 3
Immobilization time <sup>b</sup> (MZT only)	15	10–62	28 $\pm$ 18
Recovery time <sup>c</sup> (MZT only)	15	2–26	9 $\pm$ 7
Immobilization time <sup>b</sup> (maintained with isoflurane)	18	21–219	56 $\pm$ 47
Recovery time <sup>c</sup> (maintained with isoflurane)	18	4–25	9 $\pm$ 6

<sup>a</sup> Time from injection of MZT to maximum observable effect.

<sup>b</sup> Time from maximum observable effect to injection of atipamezole.

<sup>c</sup> Time from injection of atipamezole to ability to stand on front flippers and begin locomotion.

sedation that was sufficient to carry out most non-invasive diagnostic procedures.

There was no significant difference in recovery time after injection of atipamezole between sea lions given MZT alone and those maintained with isoflurane (Table 1). Prolonged recovery (over 20 min) was noted in two animals given MZT only (Table 2) and three animals maintained with isoflurane (Table 3). Preanesthetic condition was rated as good in both of the animals given MZT only. For the three animals also given isoflurane, preanesthetic condition was fair in one and poor in the other two. Immobilization times, as de-

finied in Table 1, in the two sea lions given MZT only were 10 min and 21 min and, though the 10 min time was the shortest immobilization time recorded for the current study, no obvious associations between recovery and immobilization times were evident from the rest of the data. However, it is not inconceivable that blood and/or tissue levels of ZT were still high enough after only 10 minutes to prolong recovery after the medetomidine had been reversed in that animal.

Four animals, one after being given MZT only and three maintained with isoflurane, were euthanized prior to recovery

TABLE 2. Characteristics and results of immobilization of 39 California sea lions (*Zalophus californianus*) using medetomidine-zolazepam-tiletamine (MZT) and reversal with atipamezole.

Characteristic or result	Number of animals
Animals reaching only anesthetic Level I <sup>a</sup>	2 of 39 (5%)
Animals reaching up to anesthetic Level II <sup>b</sup>	12 of 39 (31%)
Animals reaching anesthetic Level III <sup>c</sup>	25 of 39 (64%)
Total intubated	29
Intubated after MZT only	22 of 29 (76%)
Intubated after masking with isoflurane	7 of 29 (24%)
Bradycardia (minimum heart rate <50 beats/min)	0 of 17 (0%)
Prolonged recovery time (>20 min)	2 of 15 (13%)
Poor recovery <sup>d</sup>	2 of 15 (13%)
Euthanized based on findings during procedure	1 of 17 (6%)
Mortalities due to anesthetic complications	1 of 17 (6%)

<sup>a</sup> Animals at Level I would react to noise, would not lie in place for radiographs, or required physical restraint to accomplish even minor procedures such as venipuncture or masking for gas anesthesia.

<sup>b</sup> Animals reaching Level II would display a swallowing reflex and some jaw tone, reaction to deep pain caused by a flipper pinch, but would easily maintain sternal and lateral recumbency for radiographs or ultrasound without additional restraint.

<sup>c</sup> Sea lions at Level III could be intubated and showed no response to deep pain.

<sup>d</sup> Poor recoveries were characterized by ataxia, disorientation, weakness and head tremors for up to 20 min after standing.



TABLE 3. Characteristics and results of immobilization of 22 California sea lions (*Zalophus californianus*) using medetomidine-zolazepam-tiletamine (MZT) with isoflurane and reversal with atipamezole.

Characteristic or result	Number of animals
Total intubated and maintained with isoflurane	22
Intubated after MZT only	16 of 22 (73%)
Intubated after masking with isoflurane	6 of 22 (27%)
Bradycardia (minimum heart rate <50 beats/min)	1 of 18 (6%)
Prolonged recovery time (>20 min)	3 of 18 (17%)
Poor recovery <sup>d</sup>	3 of 18 (17%)
Euthanized based on findings during procedure	3 of 22 (14%)
Mortalities due to anesthetic complications	0 of 22 (0%)

<sup>a</sup> Poor recoveries were characterized by ataxia, disorientation, weakness and head tremors for up to 20 min after standing.

based on findings during the procedure. Reasons for euthanasia included: multiple fractures with severe osteomyelitis and poor prognosis, severe peritonitis and septicemia, chronic hind-end paralysis, and metastatic carcinoma.

There were no statistical differences detected in any of the recorded physiologic parameters between animals given MZT only and those maintained with isoflurane (Table 4). Recorded EtCO<sub>2</sub> values were higher than expected based on values from awake domestic animals (Hendricks, 1995).

Potential complications of immobilization of both groups of sea lions including slow recoveries, poor recoveries and bradycardia were recorded (Table 2 and 3). The animal that developed bradycardia

and was reversed early was given a good preanesthetic score. Signs of poor recovery included ataxia, disorientation, weakness and head tremors for up to 20 min after standing. The two animals given MZT only that showed poor recoveries were in good and fair preanesthetic condition. Preanesthetic scores of good, fair and poor were given to the three animals maintained with isoflurane that had poor recoveries.

One animal that was given MZT developed apnea approximately 37 min after injection of MZT. No heart beat could be detected shortly afterwards. The animal was intubated and intermittent positive pressure ventilation and thoracic compressions were initiated. The animal never responded to intravascular nor to intracardiac injections of atropine, doxapram (Do-

TABLE 4. Ranges and means  $\pm$  SD of recorded values of physiologic parameters in California sea lions (*Zalophus californianus*) after injection with medetomidine-zolazepam-tiletamine (MZT) alone and in combination with isoflurane.

Physiologic parameter	Number of animals	Number of recordings	Range	Mean $\pm$ SD
Heart rate (beats/min) <sup>a</sup>	10	35	57–140	81 $\pm$ 21
Respiratory rate (breaths/min) <sup>a</sup>	13	39	3–32	16 $\pm$ 8
Pulse oximeter saturation (%) <sup>a</sup>	2	12	73–98	91 $\pm$ 7
End-tidal carbon dioxide (mmHg) <sup>a</sup>	3	15	41–74	64 $\pm$ 9
Heart rate (beats/min) <sup>b</sup>	21	165	49–120	79 $\pm$ 13
Respiratory rate (breaths/min) <sup>b</sup>	16	122	5–36	18 $\pm$ 7
Temperature (C) <sup>b</sup>	5	62	35.5–39.8	37.8 $\pm$ 1.1
Pulse oximeter saturation (%) <sup>b</sup>	16	116	70–98	87 $\pm$ 7
End-tidal carbon dioxide (mmHg) <sup>b</sup>	5	67	40–88	63 $\pm$ 12

<sup>a</sup> MZT only.

<sup>b</sup> MZT in combination with isoflurane.

pram-V®, Fort Dodge), and epinephrine (Phoenix Pharmaceuticals, Inc., St. Joseph, Missouri, USA). Pupils became fixed and dilated 6 minutes after the apnea had been detected and the animal was pronounced dead. This individual was an adult male and was the largest animal included in the study (220 kg). Preanesthetic score was poor due to open-mouth breathing, marked weight-loss, 5% dehydration, and unilateral front-end lameness associated with a 15 cm diameter abscess on the front flipper. Post mortem examination showed moderate verminous pneumonia associated with *Parafilaroides decorus*, moderately enlarged thoracic lymph nodes, severe pyloric ulceration, and severe cellulitis of the left front flipper. Death was primarily attributed to anesthetic complications.

#### DISCUSSION

In comparison to other agents that have been previously used, there are several advantages to the use of MZT to immobilization California sea lions. The reversibility of medetomidine with atipamezole as well as the decreased amount of ZT that is required when medetomidine is added may decrease prolonged recovery times and mortality rates that have been reported for some other agents such as ZT alone (Loughlin and Spraker, 1989).

Compared to a similar group of sea lions given MK (Haulena et al., 2000), the use of MZT in sea lions produced faster and deeper anesthesia. In addition, the required volume and cost was less with MZT than with MK. For example, for a 75 kg animal, the injection volume of MZT was approximately 6 ml, compared to over 10 ml when MK was used. The cost of MZT and atipamezole to immobilize a 75 kg sea lion would be approximately US\$65.00 as opposed to US\$95.00 for MK and atipamezole at current market prices.

Prolonged recovery and poor recovery characteristics noted in some animals as well as the one mortality indicate that caution should be used with this anesthetic combination in California sea lions. This

may be especially true for animals that are in poor preanesthetic condition as was the animal that died. However, there was no obvious association between duration or quality of recovery and preanesthetic condition or length of immobilization. It may also be possible that larger animals may be more at risk since the animal that died was the largest individual in the study. However, because there was only one death and only two animals that weighed over 100 kg, no such conclusions can be drawn from the current study. The number of poor recoveries and mortalities was greater than in a similar group of sea lions given MK (Haulena et al., 2000). However, from previously published anesthetic mortality rates in immobilized otariids (Bester, 1988; Gales, 1989; Loughlin and Spraker, 1989; Boyd et al., 1990; Work et al., 1993; Sepulveda et al., 1994; Heath et al., 1996), MZT appears to be as safe or safer than other injectable agents that have been used, though not as safe as MK (Haulena et al., 2000).

The use of  $\alpha_2$ -agonists has been cautioned in diving or carbon dioxide tolerant species (Sedgwick, 1999). Widely ranging SpO<sub>2</sub> and EtCO<sub>2</sub> values reported in animals given MZT alone as well as those maintained with isoflurane support that recommendation. However, the specific effects of medetomidine on the diving reflex and the resultant physiological consequences in diving mammals is an area requiring further study. It is suggested that individuals be prepared to intubate and assist pulmonary ventilation if considering the use of MZT in sea lions. The large range in SpO<sub>2</sub> and EtCO<sub>2</sub> values in this study may also have been a consequence of disease status in addition to possible effects of the agents used. Pulmonary perfusion and gas exchange may be affected by diseases such as verminous pneumonia as caused by *Parafilaroides decorus* that are relatively common in California sea lions undergoing rehabilitation (Gage et al., 1993). There were no obvious trends in the relationship between preanesthetic

condition and SpO<sub>2</sub> or EtCO<sub>2</sub> levels. In dogs, awake EtCO<sub>2</sub> levels are usually between 35 to 46 mmHg (Hendricks, 1995). However, because high EtCO<sub>2</sub> values in anesthetized sea lions may be a reflection of carbon dioxide tolerance and, therefore, expected, it is difficult to interpret their physiological significance. More research into the repercussions of rising EtCO<sub>2</sub> levels in marine mammals is required before the usefulness of this parameter can be evaluated.

Medetomidine, a highly selective and potent  $\alpha_2$ -agonist, produces sedation and analgesia by stimulating central receptors. Reported side-effects may include significant cardiovascular changes such as bradycardia and vasoconstriction and decreased respiratory rate (Cullen, 1996). In the current study, bradycardic episodes were recorded in 1 animal. Since bradycardia is a characteristic of the "diving response" in marine mammals (Kooyman et al., 1981), it is unknown whether the bradycardia was due to medetomidine or was a result of anesthesia inducing a diving reflex in the sea lions. Though some reports contraindicate the use of atropine with medetomidine (Cullen, 1996), atropine was administered in these animals as the risk due to stimulating the diving reflex was thought to outweigh the risks associated with medetomidine interactions.

Disadvantages of MZT with atipamezole reversal in sea lions included poor recovery in some animals and a single mortality that may have been due to the anesthetic that was administered. However, MZT appears to be safer than some other previously used agents and results in a faster induction time and reliable plane of anesthesia that is administered by the intramuscular route. The use of MZT in sea lions requires a smaller injection volume and is less expensive than when MK is used. MZT can be combined with isoflurane for longer or more invasive procedures.

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