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THE EFFICACY OF NALBUPHINE, MEDETOMIDINE, AND AZAPERONE IN IMMOBILIZING AMERICAN BISON (*BISON BISON*)

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ABSTRACT: We evaluated a combination of nalbuphine, medetomidine, and azaperone (NalMed-A) in 12 American bison (*Bison bison*) during 13 sedation handling events. The mean (SE) dosage was 0.4 (0.02) mg/kg nalbuphine, 0.08 (0.003) mg/kg medetomidine, and 0.08 (0.003) mg/kg azaperone contained in an average delivery volume of 0.8 mL/100 kg. Two animals required a supplemental dose for safe handling (additive dose used in calculating means) and a third animal was not adequately sedated despite a supplemental dose. Bison immobilized with NalMed-A showed good sedation in 12 of 13 handling attempts. Advantages of this drug combination included a relatively low delivery volume, rapid antagonism, and minimal regulatory burden for component drugs. The most consistent disadvantage was hypoxemia, and oxygen supplementation is recommended when using this sedative combination in bison.

Key words: Azaperone, bison, blood gas, chemical immobilization, medetomidine, nalbuphine.

INTRODUCTION

Capturing and handling American bison (*Bison bison*) presents a variety of inherent challenges that may be best met through the use of remotely delivered immobilizing drugs. Chemical immobilization of bison has evolved appreciably in recent decades. Historically, bison immobilization involved the use of succinylcholine or nicotine alkaloids (Denney and Gill 1970), but these drugs provided variable to poor quality immobilization and had low therapeutic indices. Other drugs used more recently in bison and cattle include various combinations of α_2-adrenoceptor agonists with tiletamine and zolazepam and sometimes ketamine (Caulkett et al. 2000; Hofkes et al. 2005; Re et al. 2013). Bison are most commonly immobilized with carfentanil (Kock and Berger 1987; Haigh and Gates 1995) or thiafentanil (Frey et al. 2013; Clarke et al. 2014); however, regulatory requirements for use and storage of potent opioids limit access to these drugs. An optimal alternative drug combination for field use would offer a high rate of successful, reversible immobilization, a relatively low rate of adverse effects, and a low-volume dose to accommodate remote delivery.

We have evaluated a compounded combination of nalbuphine, medetomidine, and azaperone (NalMed-A, Wildlife Pharmaceuticals, Windsor, Colorado, USA; Wolfe et al. 2014) in hopes of developing a safe and effective chemical immobilization for a wide variety of species. To date, NalMed-A has been used to immobilize elk (*Cervus elaphus nelsoni*), black bears (*Ursus americanus*), and bighorn sheep (*Ovis canadensis*) (Wolfe et al. 2014, 2016; Wolfe and Miller 2016). Advantages of NalMed-A are the relative safety of its component drugs and the exemption of these drugs from oversight by the US Drug Enforcement Administration (DEA), thereby relaxing some regulatory requirements for NalMed-A's use (Wolfe et al. 2014). In elk, NalMed-A produces chemical immobilization characterized by a moderate induction time (5–10 min), acceptable baseline physiologic parameters, and muscle relaxation for up to 50 min (Wolfe et al. 2014). We found NalMed-A immobilization in elk to be subjectively

indistinguishable from immobilization with a combination of butorphanol, azaperone, and medetomidine (BAM). We have seen similar performance of NalMed-A and BAM in bighorn sheep. Bison immobilization with BAM was reliable and repeatable with effective small delivery volumes and good recovery under a variety of field conditions (Shury et al. 2008). It follows that NalMed-A could be useful as a nonscheduled drug combination for bison immobilization.

Nalbuphine hydrochloride is an opiate receptor kappa agonist/partial mu antagonist with analgesic and sedative effects related to compounds such as butorphanol. However, unlike butorphanol, abuse potential with nalbuphine appears quite low, and consequently this opioid is not scheduled by the DEA (2013). Nalbuphine is a potent analgesic and also was considered a good adjunct for anesthesia in horses (Kulkarni et al. 2015), cattle (Coetzee et al. 2013), and dogs (Wagner et al. 2008). Medetomidine is a potent α2-adrenoceptor agonist with sedative and analgesic properties. Medetomidine provides smooth induction and good muscle relaxation that can be potentiated by the effects of opioids like nalbuphine and butorphanol and can be antagonized with atipamezole (Wolfe et al. 2008, 2014). Azaperone, a butyrophenone, is a short-acting neuroleptic sedative. Although similar to acetylpromazine, Hughes et al. (1977) found that azaperone was more effective in reducing stress in domestic sheep. These antianxiety properties can be particularly useful for captive handling, as well as free-range capture (Ebedes 1992; Wolfe and Miller 2016). Here, we report the results of a pilot study to assess the potential efficacy of NalMed-A in bison immobilization.

MATERIALS AND METHODS

We used captive adult bison housed at the US Department of Agriculture Animal and Plant Health Inspection Service, Colorado State University Wildlife Research Facility in Fort Collins, Colorado, US (40°34′54″N, 105°08′49″W). Elevation was approximately 1,519 m. Animals were housed in outdoor enclosures and fed a standard diet consisting of a grass hay/alfalfa mix and free access to mineral blocks. Animals were not fasted before handling. The study plan was approved by the Colorado State University Animal Care and Use Committee (protocol 15-5561A)

The NalMed-A solution was premixed for a final concentration of 40 mg/mL nalbuphine, 10 mg/mL azaperone, and 10 mg/mL medetomidine (Wildlife Pharmaceuticals Inc., Windsor, Colorado, USA). Before sedation, animals were herded into a handling pen and then moved individually into a hydraulic squeeze chute (Silencer, Stapleton, Nebraska, USA), where they were weighed on a walk-on scale, except for two cows with calves whose weights were estimated. Each animal was hand-injected intramuscularly with drug in the hindquarter while restrained in a squeeze chute, except for the two cows with calves that were darted in their respective paddocks (air-powered rifle, Dan-Inject™, Børkop, Denmark; 3cc Pneu-Dart type U, Pneu-Dart, Williamsport, Pennsylvania, USA). Animals were released from the chute within a few minutes of intramuscular injection.

The initial dosage was based on previous data in elk (Wolfe et al. 2014), adjusted using the specific metabolic energy cost (SMEC; kcal/kg per day) calculated as $SMEC = K(M_{kg}^{-0.25})$, where K is a taxonomically dependent constant based on mean core body temperature (K=70 for placental mammals) and M is body mass (Hunter and Isaza 2008). The initial dosage of NalMed-A was 0.23 mg/kg nalbuphine, 0.06 mg/kg azaperone, and 0.06 mg/kg medetomidine. We subsequently titrated dosing in increments of 20% of the initial dose until adequate sedation was observed.

Induction was measured as the time (minutes) from drug administration to level 1 (no change, not reported), level 2 (partial sedation, ataxia), level 3 (sternal recumbency), and level 4 (complete sedation). Handling time was measured from level 4 to when the antagonist was administered. Once sedated, bison were blindfolded, and vital data, including temperature, pulse rate, and respiration rate, were measured at about 5-min intervals. However, because the vital data remained fairly consistent throughout sedation, we report only the first and last measurements. Percent oxygen saturation (SpO_2) was measured with a pulse oximeter using a lingual clip on the prepuce or vulva (Nellcor, Covidien, Boulder, Colorado, USA, or Masimo Rad, Masimo Corp., Irvine, California, USA). If the SpO_2 was below 80%, then the animal was considered hypoxic and was supplemented with intranasal oxygen (3 L/min). Anaerobic blood samples were collected in self-filling arterial syringes (Pro-Vent, Smiths Medical, Keen, New Hampshire, USA) from the auricular artery and immediately processed with an Element POC rapid blood analyzer (Heska, Loveland, Colorado, USA) to measure blood

gases, acid-base status, and selected hematologic variables. Partial pressure of arterial oxygen (PaO_2), partial pressure of arterial carbon dioxide ($PaCO_2$), and pH were corrected to the rectal temperature (Kelman and Nunn 1966). Blood samples were collected at two time points: the first within 25 min after recumbency, denoted as sampling period 1 (S1), and the second within 26–60 min after recumbency (S2). For comparison, three awake bison were also sampled (auricular artery) in the squeeze chute with the head gate and halter for restraint. Animals given supplemental oxygen were reported separately.

The expected PaO_2 range for the study location was determined by calculating the partial pressure of alveolar oxygen (PAO_2) from the alveolar gas equation: $PAO_2=F_iO_2(P_{ATM}–P_{H20})–(PaCO_2/RQ)$, where F_iO_2 is the fraction of inspired O_2 (0.21), P_{ATM} is atmospheric pressure (639 mm Hg), P_{H20} is water vapor pressure at 37 C (47 mm Hg), $PaCO_2$ is assumed to be 35–45 mm Hg, and RQ is the respiratory quotient (assumed to be 0.8 for herbivores).

Antagonists were administered intramuscularly by hand syringe at a rate of 5 mg atipamezole/mg medetomidine and 150 mg naltrexone/animal (total dose/animal). Recovery was measured as time (minutes) from atipamezole administration to level 1 (no change, not reported), level 2 (increased respiration), level 3 (able to hold head up), and level 4 (able to stand). Animals were observed immediately, and at 2 and 24 h postanesthesia for adverse effects.

The quality of immobilization was rated subjectively on a continuous scale (level 1=no sedation, level 2=shows signs of sedation but not recumbent, level 3=recumbent but able to stand with stimulation, level 4=recumbent but responsive to stimulation, level 5=good level of sedation for sampling and handling) for each bison with respect to ease of routine handling and sampling. Responses to mild stimuli, including a loud noise, repositioning of the animal, ear tagging, and venipuncture, were used to assess level of sedation. In two animals, additional handling included hoof trim and, for one bull, electroejaculation. Safety of immobilization with NalMed-A in bison was evaluated by heart rate, respiration rate, blood oxygenation, and lack of regurgitation.

RESULTS

Twelve adult bison were used in 13 sedation events to evaluate NalMed-A with a mean (SD) dosage of 0.4 (0.02) mg/kg nalbuphine, 0.08 (0.003) mg/kg medetomidine, and 0.08 (0.003) mg/kg azaperone contained in an average delivery volume of 0.8 mL/100 kg. Bison reached level 4 in 12 of 13 attempts. Two animals that were hand-injected in the chute required a supplemental dose for adequate sedation. One of those animals reached level 4 but was given a supplemental dose by hand injection during sampling. The second animal was given a supplemental dose by pole syringe (Dan-Inject) after it reached level 3. We used the cumulative dose for calculation of means. One attempt failed to produce adequate sedation despite supplemental dosing. This individual did not reach level 4 and was not considered adequately sedated for safe handling, because it was ataxic and would lie down but would get up with stimulation. We had given this bison an initial dose of 0.3 mg/kg nalbuphine, 0.08 mg/kg medetomidine, and 0.08 mg/kg azaperone and then supplemented with 10% of the original dose by volume. Subsequent handling of that same animal using a higher starting dosage of 0.4 mg/kg nalbuphine, 0.09 mg/kg medetomidine, and 0.09 mg/kg azaperone resulted in good sedation. Quality of immobilization was scored at 5 (out of 5) for 12 sedation events and at 3 for the animal that was not immobilized. Three animals responded with ear twitch in response to ear-tagging; however, there was no movement in response to electroejaculation in one bull or to hoof trimming with an electric grinder in two other animals. Mean induction time to level 3 was 8 (1) min for all animals (including individuals that required supplemental dosing). The mean induction time for 11 of the animals that reached level 4 was 11.5 (1.3) min, and the time to standing was 4 (1) min (Table 1). We initially estimated the NalMed-A dosage in bison based on previous experience with other species; however, two animals required supplemental dosing for safe sedation, and a third animal never reached complete sedation. Therefore, supplemental dosing was included in calculating mean dose, resulting in a slightly higher recommended dosage than for other species studied to date. The extended induction time for one bison was not recorded and therefore was not included in the means for level 4 induction. The average handling time

TABLE 1. Induction and reversal times (recorded to the nearest minute) for American bison (*Bison bison*) sedated with a combination of nalbuphine, medetomidine, and azaperone. For induction: level 2 = ataxia; level 3 = sternal recumbency; level 4 = complete sedation (not responsive to stimuli). For reversal: level 2 = increased respiration; level 3 = sternal recumbency; level 4 = standing.

	Induction		Reversal	
Sedation level	*n*	Min (SE)	*n*	Min (SE)
2	12	4 (0.3)	12	2 (0.4)
3	13	8 (1.1)	12	3 (0.6)
4	11[a]	12 (1.3)	12	4 (1.0)

[a] A total of 12 bison reached level 4, but times were only recorded for 11 of them.

TABLE 2. Vital measurements of 12 American bison (*Bison bison*) sedated with a combination of nalbuphine, medetomidine, and azaperone during 13 trials. Vital measurements remained stable throughout sedation. Only the means obtained during the first measurement (S1; mean 18 min, SE 2 min postinjection) and the last measurement (S2; mean 33 min, SE 3 min postinjection) are reported.

	S1		S2	
Parameter	*n*	Mean (SE)	*n*	Mean (SE)
Temperature (C)	12	39.8 (0.5)	8	39.7 (0.6)
% Oxygen saturation	10	79 (2)	10	81 (1)
Heart rate (beats/min)	10	54 (5)	10	54 (5)
Respiratory rate (breaths/min)	12	33 (5)	10	36 (5)

for the 12 animals that reached level 4 was 31 (4) min.

Vital rates are shown in Table 2, and blood gases are presented in Table 3. Most animals were considered hypoxic (SpO_2<80%) and remained so throughout the anesthetic period despite oxygen supplementation. Mean PaO_2 from S1 demonstrated mild hypoxemia (PaO_2=58–68 mm Hg), whereas mean PaO_2 of S2 was within the expected range of PaO_2 (68–80 mm Hg) at the elevation of the study site (Fig. 1). Animals given supplemental oxygen were still considered mildly hypoxemic (66 mm Hg); however, paired samples pre- and postoxygen supplementation were not available for comparison to evaluate efficacy of supplementation. Partial pressure of arterial oxygen was low (21–29 mm Hg) in animals sampled in the chute but increased to within normal range (35–45 mm Hg) at later sampling points. Bison sampled at S1 had a normal pH, whereas bison sampled at S2 had mild acidosis. The level of lactate decreased during anesthesia, whereas bicarbonate increased (Table 3). In contrast, blood gases of unsedated bison sampled in the chute indicated a low pH (acidosis).

Rectal temperatures were elevated in some individuals before and during sedation. Two animals that were darted in pens had rectal temperatures of 39 C and 39.1 C. Conversely, one animal held in the chute had a rectal temperature of 41.4 C (it was not sedated because of elevated temperature), indicating that elevated temperatures in other animals might have been due in part to moving the animals through the pen and chute system.

DISCUSSION

The sedative combination NalMed-A provided a safe and effective chemical immobilization in 12 of 13 bison. One animal was not effectively immobilized for safe handling initially; however, at subsequent handling, this individual was effectively sedated when we used a higher dose. Although respiratory rates were within normal limits, the most significant side effect was hypoxemia. Two animals were given oxygen by nasal insufflation, which improved but did not resolve the hypoxia when given at a rate of 2 L/min (time was not recorded for duration of oxygen supplementation). A higher rate of oxygen flow may have improved the response. Shury et al. (2008) reported similar results (SpO_2=75–96%) in bison immobilized with BAM. Caulkett (2014) indicated that bison are prone to hypoxemia during general anesthesia. When comparing medetomidine-tiletamine-zolazepam (MZT) to xylazine-tiletamine-zolazepam (XTZ), Caulkett et al. (2000) indicated that hypoxemia and hyper-

TABLE 3. Arterial blood gas and acid-base results collected from the auricular artery of American bison (*Bison bison*) sedated with a combination of nalbuphine, medetomidine, and azaperone. All arterial blood samples were collected from bison handled through a chute system. No blood samples were taken from darted animals. S1 = the first measurement (mean 18 min, SE 2 min postinjection); S2 = the last measurement (mean 33 min, SE 3 min postinjection). Time was not recorded for the duration of oxygen supplementation.

	Timing of samples							
	Awake in chute (n=3)		S1 (n=7)		S2 (n=5)		Supplemented with O_2 (n=2)	
Parameter[a]	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range	Mean (SE)	Range
pH	7.24 (0.02)	7.20–7.26	7.41 (0.01)	7.36–7.43	7.33 (0.03)	7.23–7.38	7.22 (0.02)	7.20–7.24
$PaCO_2$ (mm Hg)	25.0 (2.6)	21.0–29.0	40.9 (1.7)	35.0–48.0	45.4 (1.6)	41.0–51.0	43.0 (2.0)	41.0–45.0
PaO_2 (mm Hg)	98.3 (4.4)	90.0–105.0	61.9 (4.5)	49.0–71.0	69.6 (6.3)	64.0–94.0	66.5 (2.5)	64.0–69.0
PAO_2 (mm Hg)	93.1 (2.9)	88.1–98.1	74.6 (2.3)	64.3–80.6	67.6 (2.0)	60.6–73.1	70.6 (2.5)	68.1–73.1
$cHCO_3$ (mmol/L)	11.6 (2.2)	9.4–13.7	20.9 (2.1)	8.4–30.2	24.7 (2.7)	18.4–33.9	17.1 (1.4)	15.7–18.5
BE(b) (mmol/L)	−13.1 (2.2)	−10.9–15.3	−3.1 (2.0)	−15.0–6.0	−0.4 (2.8)	−7.6–8.0	−9.0 (1.7)	−10.7–7.3
Lactate (mmol/L)	18.0 (2.0)	16.0–20.0	9.7 (2.0)	2.0–20.0	7.6 (2.3)	1.2–12.9	15.1 (0.6)	14.4–15.7
AGapK (mmol/L)	32.5 (2.5)	30.0–35.0	19.9 (1.9)	8.4–30.2	18.0 (1.8)	1.2–12.9	25.5 (1.5)	24.0–27.0

[a] $PaCO_2$ = partial pressure of arterial carbon dioxide; PaO_2 = partial pressure of arterial oxygen; PAO_2 = partial pressure of alveolar oxygen calculated at 1,519 m (639 mm Hg) using the animal's measured arterial carbon dioxide, fraction of inspired oxygen of 0.21, and a respiratory quotient of 0.8; $cHCO_3$ = bicarbonate; BE(b) = base excess; AGapK = anion gap.

carbia were major adverse effects for both drug combinations.

The mean level of lactate was elevated at all sampling points, particularly for those animals sampled in the chute. Blood samples were not collected from darted animals for comparison, but further evaluation of hematologic parameters in darted animals is warranted.

The mean PaO_2 of unsedated animals sampled in the handling chute was higher than expected at the study elevation, likely because of stress associated with handling and hyperventilation, as evidenced by low mean $PaCO_2$. At S1, sedated bison were considered mild to moderately hypoxemic, but PaO_2 values increased at S2 to mildly hypoxemic or normal. Presumably this was from a combination of stimulation of the animal for procedures and partial metabolism of the immobilization drugs. Low pH in animals sampled in the chute was likely due to handling stress and lactic acidosis, as evidenced by the high mean lactate as well as the high rectal temperature. The slightly low pH in animals sampled 26–60 min postrecumbency indicated mild acidosis despite a compensatory increase in bicarbonate. Mean $PaCO_2$ in this group was at the high end of the normal range, suggesting a possible mild respiratory acidosis associated with hypoventilation.

Mean induction time was comparable to that when using BAM in bison (14.4 min, range 10–18 min; Shury et al. 2008) but slower than that reported for other drug combinations. Caulkett et al. (2000) reported induction times of 8.8 min (2.1 SD) and 5.5 min (1.08 SD) when comparing the MZT and XZT combinations, respectively, in wood bison (*Bison bison athabascae*). When using carfentanil and xylazine, Haigh and Gates (1995) reported induction times of 6.5 (0.4) min in 107 wood bison darted from a helicopter, and Kock and Berger (1987) reported induction times of 9.3 (1.3) min in American bison. Reversal was comparable to 4.1 (±0.6) min reported by Kock and Berger (1987). Caulkett et al. (2000) reported rapid reversal of MZT at 1.7 (±0.82) min and somewhat longer with XZT at 11.8 (9.65) min. On reversal, bison remained calm with mild signs of sedation after they were standing but were mobile when stimulated. Compared with previous experiences with NalMed-A in elk (Wolfe et al. 2014), proper estimation of the initial drug dose in bison seems especially

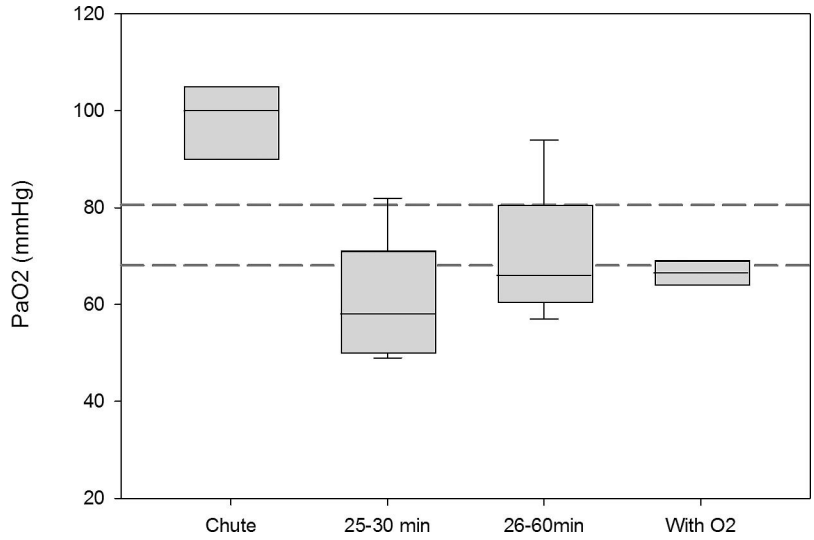

FIGURE 1. Partial pressure of arterial oxygen (PaO_2) for American bison (*Bison bison*) immobilized with a combination of nalbuphine, medetomidine, and azaperone. Dotted lines delimit the range of expected PaO_2 calculated based on the partial pressure of alveolar oxygen at an elevation of 1,519 m (atmospheric pressure=639 mm Hg), assuming a respiratory quotient of 0.8, a fraction of inspired oxygen of 0.21, and a normal partial pressure of arterial carbon dioxide range of 35–45 mm Hg. Our calculation of the expected PaO_2 did not account for a normal alveolar-arterial gradient (unknown for bison), which would lower the true expected PaO_2.

important in assuring successful immobilization, and relying on supplemental dosing may be ineffective. We subjectively noted that animals that became anxious in the handling chute system were more difficult to sedate. Therefore, this combination may be better suited for ground darting or for captive situations in which animals can remain relatively calm before immobilization.

Overall, the advantages of NalMed-A in bison include good quality sedation with a relatively low delivery volume, rapid and smooth antagonism, and no DEA regulatory scheduling of the drugs in this combination. The most common disadvantages were somewhat slow induction, need for careful dosing, and hypoxemia. Oxygen supplementation and close monitoring of respiratory parameters are recommended with this sedative combination in bison and other species.

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