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CAPTURE OF WOOD BISON (*BISON BISON ATHABASCAE*) USING CARFENTANIL-BASED MIXTURES

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ABSTRACT: Between 1986 and 1991, 155 wood bison (*Bison bison athabascae*) (33 adult females, 92 adult males, twelve 6 mo-old calves, eighteen 1 to 2 mo-old calves) in the Mackenzie Bison Sanctuary, Northwest Territories, Canada, and adjacent area were captured by dart immobilization. Initial trials with carfentanil, xylazine and R51163 as immobilizing agents were conducted. Subsequently, carfentanil alone, or in combination with xylazine, was used. Small doses of xylazine were used when required to control head and hind limb movement of recumbent bison. The mean dose of carfentanil used was 7.0 μg/kg. Narcotic antagonists used were naltrexone, naloxone and M5050. Narcotic recycling was seen in animals treated with naloxone and low doses of naltrexone. Furthermore recycling was suspected in the deaths of several animals treated with these antagonist regimes. No recycling was seen when doses of naltrexone in excess of 90:1 naltrexone : carfentanil were used. We recommend using a naltrexone : carfentanil dose in excess of 125:1 to ensure uneventful recovery.

Key words: Wood bison, immobilization, *Bison bison athabascae*, carfentanil, xylazine, naloxone, naltrexone, diprenorphine, recycling.

INTRODUCTION

The Mackenzie wood bison (*Bison bison athabascae* Rhoads 1897) population is the largest continuous population of free-roaming bison in Canada and the largest population of this subspecies. In 1987, it was estimated to number in excess of 1,700 animals (Gates and Larter, 1990). The population occupies an area exceeding 8,000 km² west of Great Slave Lake and north of the Mackenzie River (61°25′N, 117°40′W) in the Northwest Territories, Canada (Gates and Larter, 1990). The population is free of both bovine tuberculosis and brucellosis which are enzootic to bison herds in the greater Wood Buffalo National Park (WBNP) area (Tessaro et al., 1993).

Since 1986, a number of studies requiring immobilization of bison have been conducted on a range of topics including population dynamics (Gates and Larter, 1990), movements and distribution (Larter and Gates, 1990), diet and habitat selection (Larter and Gates, 1991), breeding behavior (Komers, 1992), assessment of reproductive status (Haigh et al., 1991), and disease status (Tessaro et al., 1993). The studies required the development of a safe and effective protocol for chemical immobilization of wood bison ranging in size from neonates (40 kg, 0 to 1.5 mo old) to mature males (>900 kg) under a wide range of climatic conditions. Our objective was to evaluate the effect of carfentanil, xylazine, and RR51163 as immobilizing agents, and naloxone, naltrexone and diprenorphine as opioid antagonists for wood bison.

MATERIALS AND METHODS

The studies were conducted for 1 to 2-wk periods between 1986 and 1991. In winter (December to February), animals were darted from a Bell Jet Ranger helicopter using a Zoolu darting rifle (Zoolu Arms of Omaha, Omaha, Nebraska, USA) firing 3.0 ml Palmer darts (Palmer Chemical & Equipment Co., Douglasville, Georgia, USA) modified by the use of a Zoolu tail piece, or a Pneudart rifle with 3.0 ml Pneudart syringes (Pneudart Inc., Williamsport, Pennsylvania, USA). In summer (June and July) all animals were immobilized after ground stalks or from ambush. Most animals were darted with the same rifle and dart configurations as above but five adult males approached directly were injected with plastic darts via blow-gun (Zoolu) from ranges of 13 to 18 m.

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Initially, for immobilization, we used a mixture of the opioid carfentanil (Janssen Pharmaceutica, Beerse, Belgium) and the serotonin antagonist R51163 (Tameridome, Wildlife Laboratories, Fort Collins, Colorado, USA). Subsequently we used either carfentanil alone or in combination with xylazine hydrochloride (Rompun, Haver, Mississauga, Ontario, Canada).

We designated injection sites as good or poor depending on whether they overlaid the heavy muscle masses of the hind limb, shoulder, or hump (good), or other areas such as the flank, abdomen or lower limb (poor). Data on times to recumbency comparing good and bad injection sites were compared by Student's *t*-test for samples assuming unequal variances (Microsoft Excel version 4.0, Microsoft Canada Inc., Mississauga, Ontario, Canada). We used *t*-tests for samples assuming equal variances to compare data on immobilization between males and females, and adults and juveniles. Where there were no significant differences, data were pooled and descriptive statistics were used to provide results.

If recumbent animals showed tail thrashing when approached, or vigorous head movements, or if they had rolled into lateral recumbency and were thrashing their legs, we used additional xylazine, either intravenously (5 to 20 mg) in the tail vein, or intramuscularly (30 to 50 mg).

Data recorded included sex and age class of each animal; injection site; total dose of agonistic drugs; time to recumbency (to the nearest minute); type, administration route and dose of additive drugs used for extra sedation; duration of handling procedure; girth measurements; type, administration route and dose of antagonistic drugs and time to standing. Other data and samples relating to reproductive and disease status also were collected (Haigh et al., 1991; Komers, 1992; Tessaro et al., 1993).

An estimate of total body weight was calculated separately for males and females using regressions of weight on chest girth for bison (Kelsall et al., 1978). The regression for adult males was: weight (kg) = 0.00185 × chest girth $(cm)^{2.3257}$. For females, juveniles and subadult males, the regression used was: weight (kg) = 0.00168 × chest girth $(cm)^{2.3257}$.

Doses of the immobilizing drugs relative to body mass were calculated for those animals that received a single injection and became recumbent. The relationship between dose of carfentanil used and body mass was analyzed using a statistical procedure that obtained least squares estimates of the parameters in a nonlinear regression model (Statgraphics, NONLIN procedure, Manugistics Inc., 2115 East Jefferson St., Rockville, Maryland, USA). The procedure used an iterative search algorithm to determine estimates that minimized the residual sum of squares. The analysis did not provide probability estimates for the estimated coefficients because the usual texts associated with linear models did not apply. The size of the approximate standard errors relative to the estimated coefficients provided a gauge for evaluating the significance of the estimates.

As opioid antagonists, we used a combination of naloxone (Sigma Chemicals, St. Louis, Missouri, USA) intravenously and diprenorphine (M5050, Cyanamid of Canada, Willowdale, Ontario) intramuscularly, or naltrexone (Sigma Chemicals) intramuscularly. For nine animals, a dose of 100 to 200 mg of naloxone was combined with 4 or 5 mg of M5050. All others were treated with naltrexone. Three animals received 25 mg total dose, which was either 6:1 ($n = 2$) or 7:1 ($n = 1$) relative to their carfentanil doses. All other naltrexone doses exceeded a 67:1 ratio, with only four being less than 100:1. Time to standing after antagonist administration was recorded to the nearest minute.

RESULTS

We captured 155 animals over the 6-yr period of the study. There were no significant differences between net (μg/kg) carfentanil doses between males and females, or adults and juveniles. The mean (±SE) carfentanil dose for 139 animals on which weight calculations were made was 7.0 (±0.1) μg/kg. There also were no differences in xylazine doses used between male and female adults or between male and female juveniles or 6-mo-old and 1 to 2-mo-old calves, but differences between pooled doses for adults and juveniles were significant ($P < 0.0001$). The mean (±SE) pooled doses of xylazine for adults and juveniles were 63.7 ± 2.8 μg/kg ($n = 88$) and 118.4 ± 4.9 μg/kg ($n = 30$) respectively.

The relationship between the dosage of carfentanil used and estimated body mass was strongly curvilinear. We selected the rectangular hyperbolic function to describe this relationship. The equation takes the form $y = ax/(b + x)$, where y is the dosage of carfentanil in mg, x is the weight of the bison in kg, a represents the asymptotic dosage of carfentanil, and b is the bison weight at half the asymptotic

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TABLE 1. Estimated body weights of specific age and sex classes, and doses of carfentanil and xylazine used for immobilization of wood bison (*Bison bison athabascae*) in the Mackenzie Bison Sanctuary, 1986 and 1991.

	Mean body weight (kg ± SE)	Mean carfentanil (μg/kg ± SE)	Number sampled	Mean xylazine (μg/kg ± SE)	Number sampled
1 to 2 months, both sexes	95.1 ± 4.0	6.5 ± 0.3	18	108.5 ± 4.8	18
6 mo-old, both sexes	195.4 ± 6.2	7.5 ± 0.3	12	133.3 ± 8.5	12
Adult females	485.5 ± 10.9	7.8 ± 0.4	21	54.3 ± 9.1	17[a]
Juvenile males (1 to 3 yr)	495.7 ± 19.9	8.8 ± 0.3	25	80.9 ± 6.7	23[a]
Adult males >3 yr	768.0 ± 12.4	6.0 ± 0.1	63	53.7 ± 1.7	48[a]

[a] Remaining animals did not receive xylazine in the dart.

dosage. The analysis provided the following regression model.

Carfentanil dosage (mg) = (9.007 x wt in kg)/756.260 ± wt in kg) (R^2 = 0.889, SE_a = 0.843, SE_b = 129.732).

The large standard error of coefficient b reflects the wide range in body mass of bison successfully immobilized with dosages of between 4 and 5 mg carfentanil. These doses were used for bison ranging in weight from approximately 400 to 1,000 kg. Carfentanil doses ranged from 6.0 to 8.8 μg/kg and xylazine doses ranged from 54 to 133 μg/kg (Table 1).

For 107 animals receiving one dart in good sites, the mean (±SE) time to recumbency was 6.5 ± 0.4 min, with a range of 2 to 32 min. For another 36 animals injected in poor locations the mean (±SE) time to recumbency was 11.9 (±1.2) min. Based on a *t*-test these groups were significantly different ($P < 0.001$). The coefficient of variation for animals injected in good sites was 48.2 compared to 62.2 for animals injected in poor sites.

Thirty-four animals required additional sedation with xylazine after becoming recumbent. Eleven of 16 males captured with no xylazine in the dart received 12 to 20 mg intravenously (IV). Two further males were given 30 and 35 mg, respectively; in both cases a second dose of 15 or 20 mg was added to the initial dose of 15 mg after no clinical effect had been seen within 10 min. Ten of 71 males captured with the combination of carfentanil and xylazine received additional doses of xylazine. Eight of these 10 males were given 30 to 50 mg intramuscularly (IM), and the other two 5 to 10 mg IV. Only one female of four that had had no xylazine in the dart required additional doses. Another eight females that had received xylazine initially required an extra IV dose, of 5 to 20 mg.

In 1986 the first eight bison captured were treated with a mixture of naloxone and M5050 as antagonists. Six female and two male bison were treated with 100 or 200 mg of naloxone respectively, together with 4 or 5 mg of M5050. Two females survived for >1 yr without additional treatment, another was seen stumbling and disorientated two days after capture and was treated with a dose of 10 mg M5050 by dart. She recovered completely and was resighted on several occasions until 1988. Three females died within a short time after capture. Of the two male animals, one was relocated 48 days after capture and appeared to be thriving; the other, which had not been collared, was not relocated.

The next three female bison captured were treated with 10 mg naltrexone IV and 15 mg subcutaneously (SC). All three rose to their feet within 5 min and left the capture site at a run. Two of the three thus treated were found dead within 6 days of capture. The other was seen within 4 hr after capture lying in sternal recumbency in a deep snow bank. It was approached on foot after it had failed to respond to

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buzzing by helicopter. A dose of 10 mg M5050 was administered by hand, and the animal rose 10 min later. It was sighted on numerous occasions for two subsequent years.

The mean (±SE) dose of naltrexone for the remaining 144 animals, expressed in terms of ratio of antagonist to agonist, was 129.3:1 (±1.7). These doses ranged from 375 to 600 mg in adult females and 525 to 825 mg in adult males. Six month-old calves received 188 mg and 1 to 2 mo-old calves received 75 mg. Mean (±SE) time to standing after naltrexone administration for 141 animals was 6.2 (±0.2) minutes. The time to standing after other antagonist regimes was 6.6 (±0.1) min.

Opioid recycling was seen in two animals that had been treated with more than 25 mg naltrexone. One was a female that had been immobilized with two darts, each containing 4.5 mg carfentanil only, and both of which had struck it in the flank; 22 min after immobilization it was treated with 520 mg of naltrexone and it rose to its feet after 3 min. It was seen 22 hr later lying in lateral recumbency, with obvious signs of having thrashed about for some time. It was approached on foot, and a further 450 mg of naltrexone was administered IM. After 7 min it was assisted to its feet and moved off. It was seen 3 mo later with a calf at foot. The net dose of naltrexone for initial antagonism used on this animal was 58 mg per mg of carfentanil.

Another case of possible recycling was seen in a bull that had been captured with a standard dose of carfentanil and xylazine (4.5 and 30 mg respectively) prior to branding. Fourteen hours after it was treated with antagonist at a dose ratio of 150:1 naltrexone : carfentanil and seen to move off normally it was seen showing clinical signs that may have been associated either with recycling or a reaction to the branding. After it showed some signs of aggressive behaviour towards the humans, it was darted with a further dose of carfentanil and xylazine. After it fell into recumbency it was treated with a dose of naltrexone at 200:1 naltrexone : carfentanil. It recovered in 4 min and was seen again on several occasions over the next few months.

No recycling was seen in any of the remaining 142 bison treated with naltrexone at doses ranging from 67 to 237 mg per mg of carfentanil.

DISCUSSION

When the Pneu-dart equipment first was tried on adults, short (13 mm) needles were used on three animals. Despite good hits, no clinical effects were seen after 30 to 60 min. The data on these animals were excluded. Longer (32 mm) needles subsequently were used successfully. Thirteen mm needles were used successfully on neonates and 6-mo-old calves. The use of R51163 was discontinued after we found that its mixture with carfentanil began to show white floccules after about 30 min in the syringe. Within 2 hr the mixture had the appearance of a gel.

Good injections generally led to more rapid induction, although occasional prolonged inductions may be explained by inadequate doses, partial dart failure, injection into fascia between muscle bundles, trauma from the darting site, or aberrant responses (Harthoorn, 1976). In our study the time to recumbency after dart injections into areas overlying heavy muscles was 55% of that for other areas of the body. This figure was similar to that for moose (*Alces alces*) and wapiti (*Cervus elaphus*) in which recumbency times were compared relative to injection sites (Haigh, 1979, 1991). Not only were the induction times shorter after good injections, but the coefficient of variation was lower, evidence that reliability was improved. Furthermore, three bison that had been injected over poorly muscled areas failed to become recumbent. They were injected with antidote by darting from the helicopter.

Recycling, also known as renarcotization, is a well recognized problem of opioid

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immobilized animals that is particularly seen when carfentanil is used in capture mixtures (Harthoorn, 1976; Haigh, 1990). Several regimes have been developed in attempts to overcome it. These include the use of simultaneous intravenous and subcutaneous injections, marked increases in antagonist dose relative to recommended levels and the admixture of two different antagonists (Franzmann et al., 1984; Kock and Berger, 1987; Franzmann and Lance, 1988; Allen, 1989). Naltrexone and nalmefene also have been tried as they are reputed to have longer duration of activity than other antagonists (Allen, 1989; Haigh, 1990). Recycling was a major problem early in the study reported here when low doses of naltrexone or mixtures of naloxone and M5050 were used.

Recycling was seen in only one of 26 plains bison in South Dakota (USA) treated with a mixture of carfentanil and xylazine and antagonized with naloxone (Kock and Berger, 1987). The doses of carfentanil in that study were less than half (on a μg/kg basis) of those used in our study. Xylazine doses for adults were similar. Naloxone doses in the South Dakota study were administered at rates approximately 100:1 naloxone : carfentanil and the authors of that study considered that "adequate reversal can be achieved with approximately 100–150 mg naloxone/1 mg carfentanil" (Kock and Berger, 1987).

In WBNP, bison had a high incidence of death soon after reversal of narcosis with naloxone alone (Peterson and Mercer, 1991). Fifty-eight bison were captured using doses of 5 to 6 mg carfentanil and 100 mg xylazine. Of these, 36 bison were radiocollared with mortality transmitters. Eight animals received doses ≤100 mg naloxone (83 to 100 mg) and six were found dead within 15 days. These doses were approximate ratios of naloxone : carfentanil of 16:1 to 20:1. Twenty-eight animals received higher naloxone doses (125 to 300 mg). Six (21%) of these also were found dead within the same period (Peterson and Mercer, 1991). Although it is impossible to be certain that these deaths were related to recycling, it is likely given the direct observations in our study and previous reports (Allen, 1989; Haigh, 1990).

It is likely that the relatively lower doses of carfentanil, combined with the higher doses of naloxone, accounted for the difference in recycling incidence between the South Dakota and WBNP studies. It would appear that the attempted prevention of recycling in our study by the addition of M5050 administered IM to the IV naloxone dose had little or no positive effect. The mortality rate was similar to that in WBNP where only naloxone was used. Similarly, we found that 25 mg of naltrexone, used at ratios of 6 or 7:1 to carfentanil, was inadequate.

Although the pharmacokinetics of neither naloxone nor naltrexone have been reported in any ruminant, it is apparent that naloxone and naltrexone differ in duration of effect in a variety of species (Allen, 1989; Haigh, 1990). Naloxone is subject to first-pass elimination in the liver. In those animals that have been studied it has a half-life of about 60 min (Viguera, 1986). Naltrexone on the other hand has an active metabolite, 6-β-hydroxy naltrexone, that is known to prolong its activity in some species (Gonzalez and Brogden, 1988). In humans the half-life of an oral dose is approximately 10 hr (Gonzalez and Brogden, 1988) but in dogs the half-life does not differ from that of naloxone (Pace et al., 1979). Based upon our own results we recommend that non-eventful recovery of bison immobilized with 4 to 10 μg/kg carfentanil can be achieved with a dose of 125 mg naltrexone : 1 mg carfentanil.

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