SHORT COMMUNICATIONS

Journal of Wildlife Diseases, 46(1), 2010, pp. 246–250

Use of Hyaluronidase to Improve Chemical Immobilization of Free-ranging Polar Bears (*Ursus maritimus*)

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ABSTRACT: We assessed the efficacy and safety of hyaluronidase to improve chemical immobilization of free-ranging polar bears (*Ursus maritimus*) captured from helicopter by remote drug delivery along the Ontario coast line of northwestern James Bay and southern Hudson Bay during September 2005 and October 2007. We used a single blind study design in which one person prepared and loaded all darts without the shooter knowing whether hyaluronidase (150 IU per dart) or sterile water was added to the immobilizing drug mixture of xylazine and zolazepam-tiletamine (XZT). We found that we often required more than one dart to immobilize bears in the control group (XZT+sterile water; >1 dart for 15 of 28 captures) versus the treatment group (XZT+hyaluronidase; >1 dart for seven of 26 captures). As a consequence, treatment bears were generally immobilized with smaller XZT dosages (7.9 vs. 9.4 mg/kg; $P=0.08$) and shorter induction (10 vs. 15 min; $P=0.004$) than control bears. We found no differences in vital rates and serum biochemistry results between control and treatment bears. We did find, however, that induction times correlated directly with rectal temperature at ≤15 min after immobilization ($r=0.39$, $P=0.004$), which suggests that use of hyaluronidase also helped prevent development of high body temperature (hyperthermia) in polar bears. Overall we found hyaluronidase to be effective and safe for capture of polar bears. We recommend further study to determine whether effects of hyaluronidase are dose dependent and recommend that others involved with capture of seasonally fat species such as polar bears consider use of hyaluronidase to improve chemical immobilization.

Key words: Chemical immobilization, hyaluronidase, polar bear, *Ursus maritimus*, xylazine, XZT, zolazepam-tiletamine.

Quick immobilization of wild animals by remote drug delivery requires complete injection of an adequate dose of drug into skeletal muscle followed by its absorption into blood circulation and transport to sites of action in the central nervous system (Cattet et al., 2005). At some times of the year, in animals such as bears (Ursidae), a thick layer of fat between skin and underlying muscle (i.e., subcutaneous fat) can present an obstacle to ensuring all drug is injected consistently into muscle. When drug is injected into fat, its absorption into circulation is slow, causing delayed or failed immobilization. As time between injection and immobilization (i.e., induction) lengthens, a darted animal is more likely to injure itself or develop adverse physiologic states, such as hyperthermia. Use of longer dart needles for capturing fat animals can increase the likelihood of complete intramuscular injection, but may also cause injury because long needles are more likely to damage muscle and strike deeper tissues or bone (Cattet et al., 2006).

Use of hyaluronidase as a component of immobilizing drug mixtures has been promoted to accelerate drug absorption from muscle or fat and reduce induction times for several species of terrestrial mammals, including white-tailed deer (*Odocoileus virginianus;* Allen, 1970), moose (*Alces alces;* Haigh, 1979), muskoxen (*Ovibos moschatus;* Clausen et al., 1984), black rhinoceroses (*Diceros bicornis;* Kock, 1992), African elephants (*Loxodonta africana;* Kock et al., 1993), and giraffes (*Giraffa camilopardalis;* Bush et al., 2001), as well as several species of

marine mammals (Pinnipeds; Gales, 1989). Hyaluronidase is a naturally occurring enzyme generally extracted from bovine or ovine testes and used widely in human medicine (Girish and Kemparaju, 2007) as a diffusing substance to increase permeability of connective tissue through hydrolysis of hyaluronic acid, a polysaccharide found in the intracellular ground substance of connective tissue. Hyaluronidase has been used to reduce induction times for wildlife capture in North America, but published reports are few (e.g., Allen, 1970; Haigh, 1979). Although used more widely in African species, published results confirming efficacy and safety are still difficult to find. Here we report on use of hyaluronidase to improve chemical immobilization of free-ranging polar bears (*Ursus maritimus*) and provide results that demonstrate its efficacy and safety for this species.

We captured 68 wild polar bears along the Ontario coastline from Hook Point (54°50′N, 82°15′W) on northwestern James Bay to the Hudson Bay coast at the Ontario-Manitoba border (56°50′N, 89°00′W) in September 2005 and October 2007 as part of a larger research program conducted by the Ontario Ministry of Natural Resources (research goals are summarized by Obbard et al., 2006). We immobilized bears from a Bell Long Ranger helicopter by remote drug delivery (Palmer Cap-Chur Inc., Powder Springs, Georgia, USA) using a combination of xylazine and zolazepam-tiletamine (XZT) administered intramuscularly as xylazine (Cervizine 300®, Wildlife Pharmaceuticals, Inc., Fort Collins, Colorado, USA) at 2 mg/kg and Telazol® (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) at 3 mg/kg estimated body weight (Cattet et al. 2003). We added 1 ml (150 IU) of hyaluronidase (Amphadase®, Amphastar, Rancho Cucamonga, California, USA) to XZT for 34 bears (treatment group: 17 females, 17 males, all bears ≥4 yrs) and 1 ml of sterile water for injection (Abbott Animal Health, Montreal, Quebec, Canada) to XZT for 34 bears (control group: 17 females, 17 males, all bears ≥4 yrs). Both hyaluronidase and water mixed easily with XZT without formation of any precipitate or change in color of the drug solution. We used a single blind study design where one person (M.C.) prepared and loaded all darts without the shooter (M.O.) knowing if hyaluronidase had been added to the immobilizing drug mixture. We recorded times for pursuit (time from sighting bear to delivery of first dart), first drug effect (time from delivery of first dart to loss of coordination), and induction (time from delivery of first dart to immobilization). We also examined all darts removed from bears to ensure the full volume of drug was injected, and in cases where injection had either failed or was only partial (i.e., some volume of drug remaining in dart), the data from these animals (treatment [six females+two males], control [two females+two males]) were excluded from statistical analyses.

We recorded pulse and respiratory rates, rectal temperature, and hemoglobin oxygen saturation (Nellcor NPB-40 pulse oximeter, Nellcor, Pleasanton, California, USA) at onset of handling and every 15 min thereafter during a 45-min handling period. Following placement of a mental nerve block using bupivicaine (Marcaine®, Sanofi, Markham, Ontario, Canada) at a dose of 10–15 mg, we extracted a premolar tooth to estimate age by counting cementum annuli (Calvert and Ramsay, 1998). We recorded standard morphometric measurements and weighed bears in a sling suspended beneath a load cell (Norac Ltd., Saskatoon, Saskatchewan, Canada). We collected blood from the jugular vein into sterile tubes for separation by centrifugation within 8 hr of collection. We froze extracted serum at −20 C for biochemistry analysis (Roche Hitachi 912 Chemistry Analyzer, Roche Diagnostics, Laval, Quebec, Canada). In 2005, we also measured blood lactate concentrations for 27 bears

TABLE 1. Mean drug dosage and capture time[a] required for chemical immobilization of 32 polar bears along the northern Ontario coastline (2005 and 2007). Results[b] are calculated from captures that required one dart only.

Group[c] (*n*)	XZT dosage (mg/kg)	Pursuit (min)	First effect (min)	Induction (min)
Control (13)	7.1±2.27 (3.8–12.5)	2±1.2 (1–5)	4±2.0 (1–8)	6±3.4 (1–12)
Treatment (19)	7.1±1.49 (3.4–9.5)	3±1.7 (1–8)	3±1.2 (2–5)	6±2.3 (3–10)

[a] Capture time is defined as duration of time required for pursuit (from sighting bear to delivery of first dart), first effect (from delivery of first dart to loss of coordination), and induction (from delivery of first dart to immobilization).

[b] Results presented as mean±standard deviation with minimum and maximum values in parentheses. Results were compared between groups using an independent samples *t*-test with statistical significance (*) assigned at $P \leq 0.05$.

[c] Control=xylazine and zolazepam-tiletamine (XZT)+sterile water, treatment=XZT+hyaluronidase

on site using a portable lactate analyzer (Roche Accutrend® Lactate Analyser System, Roche Diagnostics). At the conclusion of handling, we administered atipamezole (Antisedan®, Novartis Animal Health Canada Inc., Mississauga, Ontario, Canada) at 0.20 mg/kg intramuscularly to reverse effects of xylazine.

All capture and handling procedures were approved annually by the Animal Care Committee of the Ontario Ministry of Natural Resources and followed guidelines of the American Society of Mammalogists' Animal Care and Use Committee (Gannon et al., 2007) and Canadian Council on Animal Care (2003).

We found no differences ($P>0.05$) in XZT dosages or capture times between control (XZT+water) and treatment (XZT+hyaluronidase) bears when we restricted comparisons to captures that required only one dart (Table 1). From this, we presume use of hyaluronidase provided no benefit when a single dose of XZT injected into muscle was adequate to induce immobilization. However, when we considered all captures irrespective of number of darts required per capture, we found on average that XZT dosages tended to be lower, and first effect and induction times were shorter, for bears in the treatment group (Table 2; dosage: $t=1.8$, $P=0.08$; first effect: $t=3.32$, $P=0.002$; induction: $t=3.0$, $P=0.004$). This finding was explained by the fact that we often required more than one dart to immobilize bears in the control group (median=2; frequencies: one dart for 13 captures, two darts for 13 captures, three darts for two captures), whereas one dart was usually sufficient to immobilize bears in the treatment group (median=1; frequencies: one dart for 19 captures, two darts for seven captures). We interpret these results to indicate addition of hyaluronidase to XZT at 150 IU per dart was generally effective to reduce the amount of drug needed and hasten induction for capture of free-ranging polar bears. We point out, however, the amount we added per dart in this study was considerably less than amounts reported by other sources, which vary from 2,000 IU for African elephants (Osofsky, 1997) to 7,500 IU for giraffes (Bush et al., 2001), but similar to amounts cited in earlier reports (for example, Haigh, 1979; Trillmich, 1983; Clausen et al., 1984). At the time we conducted this study, we were constrained to using a small amount by lack of availability of hyaluronidase in Canada, limiting us to its importation under government approval as Amphadase, and by the need to keep dart volumes to a minimum. However, it is conceivable the efficacy of XZT+hyaluronidase could be improved by using hyaluronidase in higher amounts. This possibility warrants further investigation.

We found no differences ($P>0.05$) in vital rates and serum biochemistry results between control and treatment bears. However, we did find that capture times correlated directly with rectal temperature at ≤15 min after immobilization (Fig. 1;

TABLE 2. Mean drug dosage and capture time[a] required for chemical immobilization of 54 polar bears along the northern Ontario coastline (2005 and 2007). Results[b] are calculated from all captures irrespective of number of darts required per capture.

Group[c] (*n*)	XZT dosage (mg/kg)	Pursuit (min)	First effect (min)	Induction (min)
Control (28)	9.4±3.50 (3.8–19.2)	3±1.7 (1–7)	11±8.2 (1–31)*	15±10.2 (1–39)*
Treatment (26)	7.9±2.60 (3.4–15.4)	3±1.7 (1–8)	5±4.4 (2–15)*	10±5.5 (3–19)*

[a] Capture time is defined as duration of time required for pursuit (from sighting bear to delivery of first dart), first effect (from delivery of first dart to loss of coordination), and induction (from delivery of first dart to immobilization).

[b] Results presented as mean±standard deviation with minimum and maximum values in parentheses. Results were compared between groups using an independent samples *t*-test with statistical significance (*) assigned at $P \leq 0.05$.

[c] Control=xylazine and zolazepam-tiletamine (XZT)+sterile water, treatment=XZT+hyaluronidase.

Pearson correlation: first effect: $r=0.52$, $P<0.001$, $N=54$; induction: $r=0.39$, $P=0.004$, $n=54$). An obvious explanation for this association is that body temperature increased in proportion to the intensity and duration of physical exertion between pursuit and immobilization. However, stress may also have played a predominant role in affecting this response (Meyer et al., 2008). Irrespective of mechanism, because addition of hyaluronidase to XZT was effective at reducing capture times, as well as number of darts required per capture, we believe use of hyaluronidase also helped prevent development of high body temperature (hyperthermia) in polar bears.

In conclusion, we found use of hyaluronidase at a low dose (150 IU per dart) generally improved chemical immobilization of free-ranging polar bears by reducing the amount of drug required and hastening induction. We found no evidence to suggest hyaluronidase had negative effects on vital rates or serum biochemistry of polar bears. In fact, to the contrary, we suggest that use of hyaluronidase helped prevent development of high body temperature (hyperthermia) by reducing duration of induction. We recommend further study to compare the efficacy and safety of hyaluronidase at higher doses than used in this study to determine if effects are dose dependent. We also recommend that persons engaged in capture of seasonally fat species such as polar bears, or indeed any wildlife species where pursuit and capture places animals at risk of conditions such as hyperthermia or capture myopathy, consider use of hyaluronidase to improve chemical immobilization.

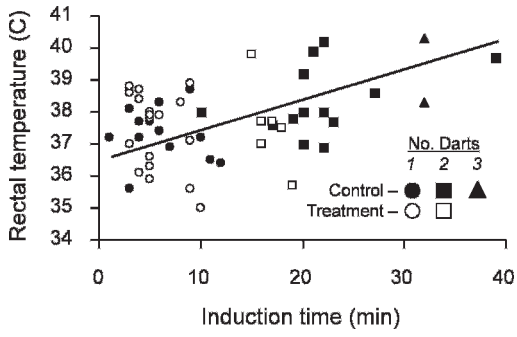

FIGURE 1. Rectal temperature of 54 polar bears at≤15 min after capture in relation to number of darts used per capture and induction time. Control (*n*=28): xylazine+zolazepam-tiletamine (XZT)+sterile water; treatment (*n*=26): XZT+hyaluronidase.

Funding for field work was provided by the Ontario Ministry of Natural Resources (OMNR) Wildlife Research and Development Section, Nunavut Department of Environment, Makivik Corporation, Ontario Parks, Safari Club International (Ontario Chapter), Safari Club International (Detroit Chapter), Les Brasseurs du Nord, La Fondation de la Faune du Québec, La Societé de la faune et des parcs du Québec, and OMNR's Climate Change Program: Project CC-05/06-036. We thank the following OMNR Aviation Services staff for support of capture operations: helicopter pilots G. Bain and

D. Holtby; Twin Otter pilots F. Aquino, C. Burella, and K. Crant; and aircraft maintenance engineers D. Allick and J.-M. Kelley. We thank C. Chenier, C. Greenwood, J. Hamilton, J. Inglis, K. Mills, T. Moody, and D. Potter for assistance in the field. M. Hunter provided radio coverage during field operations and assisted with logistics. N. Caulkett, M. Kerr, and S. Myers provided helpful advice on interpretation of serum biochemistry results.

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Received for publication 15 October 2008.