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Tissue Residue Levels after Immobilization of Rocky Mountain Elk (*Cervus elaphus nelsoni*) using a Combination of Nalbuphine, Medetomidine, and Azaperone Antagonized with Naltrexone, Atipamezole, and Tolazoline

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ABSTRACT: Previous studies demonstrated that nalbuphine, medetomidine, and azaperone (NalMed-A) can effectively immobilize adult elk (*Cervus elaphus nelsoni*), and be antagonized using naltrexone and atipamezole, with or without tolazoline. To assess duration of tissue residues for this immobilization package, we immobilized 14 captive adult elk with NalMed-A, then euthanized animals and collected tissues 0, 3, 6, 14, 21, or 28 d later. Except for two animals euthanized immediately, all elk were recovered using naltrexone, atipamezole, and tolazoline. Tissue residues (≥ 0.01 parts per million) for the tranquilizers nalbuphine, medetomidine, and azaperone were detected in liver and muscle tissue samples from elk euthanized within 40 min postinjection (PI) and one animal that died 12–24 h PI, but not in tissues from any of the animals euthanized at 3, 6, 14, 21, or 28 d PI. Tissue residues for the antagonists naltrexone, atipamezole, and tolazoline were detected in liver and muscle of the animal that died 12–24 h PI. Only naltrexone was detected in liver from the two elk euthanized at day 3, and no antagonist residues were detected thereafter.

Key words: Atipamezole, azaperone, *Cervus elaphus nelsoni*, medetomidine, nalbuphine, naltrexone, tissue residue, tolazoline.

Previous studies demonstrated that nalbuphine, medetomidine, and azaperone (NalMed-A) can be combined to effectively immobilize adult elk (*Cervus elaphus nelsoni*; Wolfe et al. 2014). NalMed-A is a low-volume and reversible drug combination that shows promise for immobilizing a variety of free-ranging wildlife including elk, deer (*Odocoileus* spp.), black bears (*Ursus americanus*), bighorn sheep (*Ovis canadensis*), and bison (*Bison bison*; Wolfe et al. 2014, 2016, 2017).

Nalbuphine HCl is a synthetic opioid agonist–antagonist with potent analgesic properties and a reportedly short elimination half-life in dogs (Pao et al. 2000). Medetomidine is a potent alpha-2 adrenoreceptor agonist with good sedative and analgesic properties. Azaperone, a neuroleptic in the butyrophenone class of tranquilizers, is metabolized by the liver and rapidly eliminated (Booth 1982; Crowell-Davis and Murray 2006). Azaperone was not detected in pigs even when euthanized at 6 h postinjection (PI; Mestorino et al. 2013) and the Food Animal Residue Avoidance Databank recommends 8 d PI for a withdrawal time (Haskell et al. 2003). Tissue residues for components of a similar immobilization combination (butorphanol, azaperone, and medetomidine) and the antagonists atipamezole and naltrexone were not detected 11 or 21 d PI in white-tailed deer (*O. virginianus*; Cook et al. 2016). Immobilization with NalMed-A is antagonized with a combination of naltrexone, an opioid antagonist, and atipamezole, a highly selective alpha-2 antagonist, with or without the addition of tolazoline (Wolfe et al. 2014). Tolazoline is an alpha-adrenergic antagonist and is approved for use in cattle in New Zealand with a recommended withdrawal of 8 d (Haskell et al. 2003).

Few drugs are approved by the US Food and Drug Administration for use in wildlife (including those that are hunted and therefore considered food animals) and most usage is considered “extra label” and falls under the US Animal Medicinal Drug Use Clarification Act (AMDUCA, Title 21, Code of Federal

Regulations, Part 530). Under AMDUCA, a practitioner should “take appropriate measures to assure that assigned time frames for withdrawal are met and no illegal drug residues occur in any food producing animal subjected to extra-label treatment” (US Food and Drug Administration 2014). Withdrawal times often have not been established for analgesics and tranquilizers (Papich 1996). Understanding the duration of drug residue is not only important for regulatory compliance, but also helps in providing assurances that harvested game animals are safe for consumption.

We used 14 captive adult female elk 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 approximately 1,519 m) to evaluate tissue residues from immobilization with NalMed-A (Wildlife Pharmaceuticals, Fort Collins, Colorado, USA) and antagonism with naltrexone, atipamezole (Wildlife Pharmaceuticals), and tolazoline (Lloyd Inc., Shenandoah, Iowa, USA). Animals were slated for euthanasia as part of another study and we used them opportunistically to assess tissue residues. Subject elk were housed in outdoor enclosures and fed a standard diet consisting of grass hay and alfalfa mix. Animals were not fasted before handling. The study plan was approved by the Colorado State University Animal Care and Use Committee (Protocol # 17-7151A).

Twelve elk were moved from their enclosure to a manual squeeze chute where they were weighed and given 2.0 mL of NalMed-A (equivalent to 80 mg of nalbuphine, 20 mg of azaperone, and 20 mg of medetomidine) by intramuscular hand injection in the hind quarter. Two additional elk were darted in their enclosure (and subsequently not weighed) with 2.0 mL of NalMed-A with a dart rifle (Daninject ApS, Børkop, Denmark) and 2-mL type U darts (Pneu-Dart, Williamsport, Pennsylvania, USA). To assure that we could detect tissue residues the first two elk were euthanized at 40 min PI via a captive bolt applied to the back of the skull. The

remaining 12 elk were antagonized with 600 mg of tolazoline, 100 mg of atipamezole, and 50 mg of naltrexone given intramuscular and held in their enclosures until scheduled for euthanasia (Table 1). One elk died 12–24 h PI and necropsy revealed chronic peritonitis and pneumonia. Although the death appeared unrelated to immobilization, the stress from handling most likely exacerbated the progression of pre-existing disease. Two of these elk were subsequently immobilized with 400–500 mg of Telazol® (Zoetis, Parsippany, New Jersey, USA) delivered via projectile syringe on day 6 PI and were euthanized with a captive bolt. Because of difficulty darting these animals in the paddock, the remaining elk were euthanized via gunshot to the head on 3, 14, 21, or 28 d PI. Necropsy was performed within an hour of death and tissues, including fresh liver and semimembranosus or semitendinosus muscle for use in drug residue analyses, were collected in sterile plastic bags (Whirl-Pak®, Nasco, Fort Atkinson, Wisconsin, USA) and stored at –70 C until submitted for laboratory analysis. Tissue residues were analyzed by liquid chromatography–tandem mass spectrometry (Texas A&M Veterinary Medical Diagnostic Laboratory, Texas A&M University, College Station, Texas, USA) after isolation by solid-phase extraction. The limit of detection for the assay was 0.01 ppm. Results were not quantified and consequently, positive tests only indicated the presence of ≥ 0.01 ppm of the drug in that tissue sample. Tissue residue limits for these drugs have not been established in the US.

Tissue residues (≥ 0.01 ppm) for the tranquilizers nalbuphine, medetomidine, and azaperone were detected in liver and muscle of the two elk that were euthanized within 40 min PI and the animal that died 12–24 h PI (Table 1). However, nalbuphine, medetomidine, and azaperone residues were not detected in animals euthanized 3, 6, 14, 21, or 28 d PI (Table 1). Tissue residues for the antagonists tolazoline, atipamezole, and naltrexone were detected in liver and muscle of the animal that died 12–24 h PI and only naltrexone was detected in liver from the two elk that were euthanized at day 3 (Table 1).

TABLE 1. Detection of tissue residues (≥ 0.01 ppm) in elk (*Cervus elaphus nelsoni*) liver and muscle tissue after injection of a combination of nalbuphine, medetomidine, and azaperone (NalMed-A) and the antagonists tolazoline, atipamezole, and naltrexone. Each elk was given an intramuscular hand injection with 2 mL of NalMed-A (80 mg of nalbuphine, 20 mg of medetomidine, 20 mg of azaperone) for initial immobilization. The first two elk euthanized did not receive antagonists; all other elk received 500 mg of tolazoline, 100 mg of atipamezole, and 25 mg of naltrexone intramuscular via hand injection.

Time interval between immobilization and euthanasia	n	Cause of death	Detection of drugs in liver and muscle by liquid chromatography–tandem mass spectrometry ^a																						
			Nalbuphine		Medetomidine		Azaperone		Tolazoline		Atipamezole		Naltrexone		Zolazepam		Tiletamine								
			Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle	Liver	Muscle							
40 min	2	Captive bolt	Yes	Yes	Yes	Yes	Yes	nd	na	na	na	na	na	na	na	na	na	na	na						
12–24 h	1	Captive bolt	Yes	Yes	Yes	Yes	nd	Yes	na	na	na	na	na	na	na	na	na	na	na	na					
		Sepsis	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes				
3 d	2	Gunshot	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd				
		Gunshot	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd			
6 d	2	Captive bolt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd			
		Captive bolt	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		
14 d	2	Gunshot	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd		
		Gunshot	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
21 d	3	Gunshot	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	
		Gunshot	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
28 d	2	Gunshot	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
		Gunshot	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

^a Yes = tiletamine-zolazepam was administered to four animals to facilitate euthanasia, where two of those animals were not adequately sedated and were euthanized at a later time with gunshot; nd = not detected (<0.01 ppm); na = not applicable because the drug was not administered for all instances.

No antagonist residues were detected in animals euthanized 6, 14, 21, or 28 d PI (Table 1).

The apparent absence of tissue residues for NalMed-A components at ≥ 3 d or antagonists in elk at ≥ 6 d PI suggested rapid clearance after immobilization. Our findings should be useful in developing drug withdrawal guidelines for using this immobilization combination in elk and other wildlife species.

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