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A ZOLETIL®-ROMPUN® MIXTURE AS AN ALTERNATIVE TO THE USE OF OPIOIDS FOR IMMOBILIZATION OF FERAL RED DEER

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ABSTRACT: Sixty chemical immobilizations of red deer (*Cervus elaphus hippelaphus*) have been carried out during an etho-ecological study from August 1994 to December 1996 in a 35 ha pen in the district of Nitra (Slovak Republic). Our objective was to determine the efficacy and standard dosages of Zoletil® and Rompun® for the immobilization of adult red deer in feral conditions as an alternative to the use of the highly toxic opioids. We therefore compared an Immobilon®-Rompun® combination (ImRo) with a 1:1 mixture of Zoletil® and Rompun® (ZoRo) as an injectable solution. Use of both combinations led to the immobilization of >92% of deer with an injection volume <3 ml. Mean (SD) dose to achieve immobilization was 35 (14) µg/kg ethorphone + 0.14 (0.056) mg/kg acepromazine + 0.36 (0.14) mg/kg xylazine compared to 1.2 (0.8) mg/kg tiletamine + 1.2 (0.8) mg/kg zolazepam + 2.3 (1.6) mg/kg xylazine. This corresponds to a volume of 1.8 (0.7) ml/100 kg body mass (BM) for ImRo (range = 1.0 to 4.6) and to 2.3 (1.6) ml/100 kg BM for ZoRo (range = 0.7 to 4.0), respectively. Heart rate, respiratory rate and oxyhaemoglobin saturation values did not differ significantly between the two groups during immobilization. Three deer (5%) died during immobilization, but fatalities could not be directly associated with the drug effect. Mean (SD) time from darting to complete immobilization was 5.5 (4.2) min for ImRo and 7.5 (6.1) min for ZoRo, respectively. Differences were not statistically significant. Anesthesia with both combinations of immobilizing agents could be reversed within 2 min using sarmazenile-yohimbine for ZoRo and diprenorphine-yohimbine for ImXy immobilizations, respectively. We conclude that the 1:1 combination of Zoletil® and xylazine is a valuable alternative to the use of opioids for the immobilization of adult red deer including feral adult animals.

Key words: *Cervus elaphus hippelaphus*, ethorphone, immobilization, red deer, sarmazenile, tiletamine, xylazine, yohimbine, zolazepam.

INTRODUCTION

Red deer (*Cervus elaphus hippelaphus*) have first been chemically immobilized with nicotine salicylate and gallamine by Schloeth et al. (1960) and Boch et al. (1961), but success was limited. Since then, numerous narcotic agents have been tried worldwide in deer, but not all drugs or combinations tested were successful. Opioids are known to be very reliable for the immobilization of wildlife, and substances such as etorphine, fentanyl or carfentanyl are often used (Nielsen, 1999) despite their potential toxicity to humans (Haigh and Haigh, 1980). Opioids require a special permit for acquisition and use and are therefore not easily available in many countries. As an alternative, xylazine

as well as medetomidine, pure or in combination with ketamine, are commonly used for the immobilization of deer in Europe (Jalanka and Roeken, 1990; Janovsky, 1996). However, the use of alpha-2 adrenoreceptor agonists in even relatively high doses is often unsatisfactory, particularly in free-ranging deer or in deer kept in large paddocks (Pond and O'Gara, 1996; Haigh and Hudson, 1993).

Recently, mixtures of xylazine (Tranquivet®) and tiletamine-zolazepam (Telazol®) were used to immobilize Rocky Mountain elk (*Cervus elaphus nelsoni*, Millspaugh et al., 1995) and white-tailed deer (*Odocoileus virginianus*, Kilpatrick and Spohr, 1999). In elk, the mean (SD) dosage used [2.6 (0.6) mg/kg Telazol® + 0.3 (0.1) mg/kg of xylazine] provided an

effective level of anesthesia for trapped animals. Tiletamine-zolazepam is an injectable anesthetic combination which provides rapid and smooth induction of anesthesia (Lin, 1996). A combination of Zoletil® with xylazine induces better muscle relaxation and longer lasting anesthesia with smaller volumes compared to the use of Zoletil® only (Thurmon et al., 1989).

Our objective was to assess the suitability of a mixture based on tiletamine-zolazepam and xylazine for the immobilization of adult red deer as an alternative to the highly toxic opioids and to the sometimes unreliable xylazine-ketamine or medetomidine-ketamine mixtures. We therefore tested a 1:1 mixture of Zoletil® and Rompun® to immobilize adult red deer under feral conditions and compared this mixture to anesthesia with an Immobilon®-xylazine combination. Criteria for the evaluation of chemical captures were the effectiveness of drug combinations to quickly immobilize adult animals with a dart volume <3 ml, the duration of immobilization, the side effects and the possibility of antagonization.

MATERIALS AND METHODS

We conducted the study in a foothill area near the village of Velčice, Slovak Republic (48°18'N/18°09'E, 280 m elevation), in a facility of the Institute of Animal Production of the University of Nitra (Slovak Republic). Use of animals in this study followed the Slovak Republic Animal Welfare Act (No. 115/1995, §24, §28, and §31, Protocol no. 672/96-500). Animals were kept in a 35 ha pen with a >2.0 m high fence. The enclosure is covered to 35% by forest, mainly black locust (*Robinia pseudoacacia*) and blackthorn (*Prunus spinosa*). The age of the experimental animals ranged from 2- to 10-yr-old and the body mass between 90 and 240 kg. From October to March the animals were fed daily with hay and mixed grain. Water was supplied ad libitum.

An ethoecological project involved repetitive immobilizations of individuals and subcutaneous implantation of heart rate transmitters in some cases (Weigerstorfer, 1996). Animals were darted at the muscles of the upper hind quarter from a distance between 20 and 35 m. We used dartguns (Daninject IM, Boerkop, Denmark; Paxarms MK24, Timaru, New Zealand; Telin-

ject G.U.T. 50, Roemerberg, Germany), 3 ml darts (Daninject; Paxarms) as well as 1.5 × 38 mm (Daninject) and 12 gauge 38 mm 1 barb standard needles (Paxarms), respectively. We defined (1) time from injection to laying down with raised head (t_1), (2) time from injection to complete immobilization with head down (t_2), and (3) recovery time as the duration from the antagonist drugs application until the deer were standing (t_3). Duration of immobilization was defined as time from darting to antagonist administration or repeated dosing. Following immobilization, the animals were weighed with a transportable scale (Rhewa-Waagenfabrik, Mettmann, Germany). An ophthalmic ointment (Oleovit-Augensalbe, Laevosan, Linz, Austria) was applied to the deer's eyes to prevent corneal drying. A blindfold was putted over the eyes to help calm the animals. We placed immobilized deer in lateral recumbency on the right side for surgery and to prevent blowing. Heart and respiration rates, as well as relative oxyhaemoglobin saturation values (SpO₂) were recorded every 10 min after deer were initially restrained. Rectal temperature was checked once after the first contact using a rectal digital thermometer (Geraberger Thermometerwerk GmbH, Gschwenda, Germany) with a measuring range of 32.00–42.99 °C. Heart rate was measured by cardiac auscultation and respiratory rate by direct observation. SpO₂ was monitored using a portable pulse oximeter (Pulsox-8, Minolta, Schaffhausen, Switzerland), with the sensor located on the tongue or on the eyelid.

Blood samples from the jugular vein were collected in Li-heparin tubes (Sarstedt, Wiener Neudorf, Austria) as well as in NaF tubes (Sarstedt) every 10 min from the first contact with the immobilized animal until antagonization time. Blood was centrifuged and plasma stored at -20 °C until analysis. We used standard tests (urease/Berthelot-reaction, Boehringer, Mannheim, Germany, and GOD-Perid-method, Boehringer) for detection of blood urea and blood glucose, respectively. To reverse capture drugs, specific antagonists were administered intravenously to the animals. After immobilization, all deer were monitored visually until their departure from the capture site. All animals that died in association with the use of the drugs were necropsied following standardized protocols (Roffe et al., 1996).

Zoletil® (Virbac, Carros, France) is a 1:1 combination of tiletamine and zolazepam. Tiletamine is a dissociative anesthetic with a similar pharmacological activity to ketamine (Lin et al., 1993), but is more potent (Short et al., 1989). Zolazepam is a benzodiazepine agonist and similar in pharmacological activity to diaz-

epam (Loescher, 1999). Xylazine is an α -2 adrenoreceptor agonist which is widely used in veterinary anesthesia. For this study, 500 mg Zoletil® (supplied in a sterile vial as a lyophilized powder containing 250 mg of tiletamine and 250 mg of zolazepam) and 500 mg xylazine (Rompun® TS, Bayer, Leverkusen, Germany, supplied in a sterile vial as a lyophilized powder containing 500 mg of xylazine) were diluted in 5 ml sterile water. This mixture (ZoRo) is simple to mix and contains 43 mg tiletamine, 43 mg zolazepam, and 86 mg xylazine per ml.

Large Animal Immobilon® (C-Vet Ltd., Bury St. Edmunds, UK) is a mixture of 2.45 mg etorphine + 10 mg acepromazine per ml. Etorphine is a highly potent opioid agonist. It is widely used alone, or preferably, combined with a sedative or tranquilizer for the chemical immobilization of nondomestic ruminants (Nielsen 1999). To each ml of Large Animal Immobilon®, we added 25 mg xylazine as a 10% solution (ImRo). For reversal of immobilizations with ImRo, animals received diprenorphine (Large Animal Revivon®, C-Vet Ltd.) together with yohimbine (Adler Apotheke, Wels, Austria). To antagonize ZoRo immobilizations we injected yohimbine (Adler Apotheke). In 15 out of 24 cases we added sarmazenile (Sarmasol®, Graeub, Bern, Switzerland) for reversal of zolazepam in ZoRo-immobilizations. Sarmazenile is a benzodiazepine antagonist.

Differences in induction time periods, heart rate, respiratory rate, body temperature, and parameters of blood serum were tested. For repeatedly measured parameters (heart rate, respiratory rate, SpO₂, and parameters of blood serum chemistry), we considered the average of all values. Because some of the animals were immobilized more than once, the two groups of measurements can not be viewed as independent samples from different populations. We therefore carried out a blocked comparison, and the blocks corresponded to the individuals. We performed stratified (blocked) Wilcoxon tests (Van Elteren, 1960; Anonymous, 1995). This procedure compares ImRo to ZoRo in respect of its effect on the same individual, and confounding of treatment and block effects is avoided. As a consequence, only data from animals that were immobilized at least once with each of the two drug combinations could be used. The test statistic equals the sum of the individual Wilcoxon rank sums for the blocks. Exact two-sided *p*-values were calculated using the StatXact software package. Criterion for detection of statistically significant differences was $P \leq 0.05$.

RESULTS

From August 1994 to December 1996, 60 red deer were successfully captured. Thirty two animals (11 males, 21 females) were immobilized with ImRo and 28 animals (nine males, 19 females) with ZoRo. Mean (SD) dose to achieve an effective level of immobilization was 35 (14) μ g/kg ethorphine + 0.14 (0.056) mg/kg acepromazine + 0.36 (0.14) mg/kg xylazine compared to 1.2 (0.8) mg/kg tiletamine + 1.2 (0.8) mg/kg zolazepam + 2.3 (1.6) mg/kg xylazine. This corresponds to a volume of 1.8 (0.7) ml/100 kg body mass (BM) for ImRo (range = 1.0 to 4.6) and to 2.3 (1.6) ml/100 kg BM for ZoRo (range = 0.7 to 4.0), respectively. Both drug combinations were efficient for immobilization of >92% of the animals with a volume <3 ml. Two deer in each group required redosing to achieve adequate immobilization. There was no statistical difference between the 2 drug combinations in the reliability of immobilization with one dart. All trials produced a smooth and uneventful induction of immobilization, and mean induction time for both combinations until complete immobilization was rapid (Table 1). Using ZoRo, t_1 was significantly longer ($P = 0.041$) than in immobilizations with ImRo. In contrast, no significant difference was observed for t_2 . Mean (range) duration of immobilization using ImRo ($n = 18$) was 58 (20–143) min and 65 (20–110) min for ZoRo ($n = 7$) respectively. However, differences in duration were not statistically significant.

Heart rate slightly decreased during immobilizations with both drug combinations and leveled off at 40 to 60 beats per min after 60 min after injection (p.i.). With both drug combinations, course of respiratory rate (10 to 20 breaths per min) remained nearly constant during immobilization. There was no significant difference in heart rate and respiratory rate during immobilization between ImRo and ZoRo immobilizations. SpO₂ values increased after a short decrease at the beginning of

TABLE 1. Induction times in min (mean \pm SD (range)) for immobilization and antagonization of red deer, 1994–1996.

Time period (min)	ImRo ^a		ZoRo ^b	
	<i>n</i>		<i>n</i>	
t ₁ ^c	12	4.0 \pm 2.35* (2–10)	8	8.5 \pm 6.13* (3–18)
t ₂ ^d	14	5.5 \pm 4.23 (2–19)	10	7.5 \pm 6.05 (3–20)
t ₃ ^e	21	1.0 \pm 1.14 (1–3)	17	1.0 \pm 23.85 (1–10)

^a Immobilon® + 25 mg xylazine per ml Immobilon®.^b 86 mg Zoletil® + 86 mg xylazine per ml.^c t₁ = time from injection to laying down with raised head.^d t₂ = time from injection to complete immobilization with head down.^e t₃ = time from the application of antagonists until standing.* Values are significantly different ($P = 0.041$, Stratified Wilcoxon tests).

anesthesia using both drug combinations and leveled off at 80–95%. Mean (range) body temperature at the first contact with the immobilized deer was 38.5 C (37.5–39.1) for ImRo ($n = 6$) and 38.6 C (37.8–39.8) for ZoRo ($n = 9$), which signifies no significant difference between the two drug combinations. This data set excludes values of three deer that died during immobilization. Starting at a level of 3 to 9 mmol/l 20 min p.i., plasma glucose rose until 70 min p.i. in immobilizations with both drug combinations. With ImRo, plasma glucose then remained high (11 to 14 mmol/l), whereas in animals immobilized with ZoRo the glucose level fell to values comparable to the first 30 to 40 min p.i. (8 to 10 mmol/l). Serum urea values during immobilization with both drug groups amounted to 7 to 11 mmol/l. Statistically significant differences in serum glucose and serum urea levels between ImRo and ZoRo were not detected.

To antagonize the effects of ImRo, animals received a mean (SD) of 84 (30) μ g/kg diprenorphine together with 0.23 (0.01) mg/kg yohimbine. To antagonize ZoRo immobilizations we injected 0.45 (0.13) mg/kg yohimbine ($n = 23$). In 15 cases, we added 36 (17) μ g/kg sarmazenile to achieve antagonism against zolazepam. Antagonization was rapid (Table 1) and complete in both drug groups, and animals did not show significant ataxia or disorientation when leaving the observation site.

The use of sarmazenile did not influence the antagonization time significantly. Mean (median) t₃ values using sarmazenile were 2 (1) min ($n = 14$) compared to 2 (2) min using yohimbine alone ($n = 3$).

In most cases, no side effects such as regurgitation, excessive salivation, or cyanosis were observed during immobilization using ImRo or ZoRo. Excitations were not observed, neither during induction time nor during immobilization and recovery. Seven hinds have been immobilized during different stages of pregnancy, mostly during the second third. All of them gave birth to a healthy calf. In one case, a pregnant female immobilized with ZoRo on 11 May produced a viable, full term calf one day later. Three deer (5%) died during immobilization. Two of these animals were immobilized with ImRo and one with ZoRo. In two cases (1 ImRo, 1 ZoRo) the individuals captured were highly stressed. Rectal temperature in these two animals at the first measurement during immobilization was >42 C and both died on the effects of hyperthermia. The level of blood urea of one animal was increased (21.8 mmol/l). Pathologic findings were an enlarged zona fasciculata of the adrenal cortex in all three cases.

DISCUSSION

In this study, the 1:1 combination of til-etamine-zolazepam (Zoletil®) and xylazine (Rompun®) was effective for the capture

of adult male and female red deer in a large enclosure. Compared with immobilizations using etorphine alone (Coggins, 1975) or xylazine and ketamine (Golightly and Hofstra, 1989; Janovsky, 1996), induction time until complete immobilization was rapid. Reliability to immobilize deer with single injections, course of measured clinical parameters, and antagonization were comparable to the use of a combination of Immobilon® and Rompun®. Duration of immobilizations was about one hour with both drug combinations tested, which is sufficient to find the immobilized animals in dense forests and to perform short lasting manipulations.

Immobilization of deer in feral conditions using single remote injections of ZoRo was possible in >92% of the cases, including capture of adult males. This result is comparable with the effectiveness of ImRo, which is known to be very reliable for the immobilization of deer regardless of sex, age and living conditions (Haigh and Hudson, 1993; Nielsen, 1999). In contrast, capture of adult feral red deer with a standard mixture of xylazine and ketamine as described in Wiesner (1998) requires significantly higher volumes, and induction times are two to three times longer (Janovsky, 1996). Our tiletamine-zolazepam dosage is comparable to the one used by Millspaugh et al. (1995) in trapped elk, but we injected 7.7 times more xylazine. This higher xylazine dose may be necessary for reliably capturing free-ranging deer. Furthermore, a 1:1 combination of Rompun® and Telazol® or Zoletil® is more practicable to mix. The major advantage of the tested ZoRo-mixture compared to ImRo is the strong risk reduction of drug accidents related to human contact with capture drugs. In fact, neither xylazine (Carruthers et al., 1979) nor Zoletil® (Lin et al., 1993) is lethal to humans in small dosages and all components have wide safety margins in animals.

In immobilized animals of both groups, heart rate was slightly reduced, while respiratory rate and body temperature were

slightly increased compared to normal values (60–70 beats per min 8–12 breaths per min, and 38.3 C, respectively, Pond and O’Gara, 1996). Serum urea values were normal to slightly increased compared to values in unsedated red deer (8.56 mmol/l; Wilson and Pauli, 1983). Levels of serum glucose increased during immobilization with both drug combinations. Values >9 mmol/l observed 40 to 80 min p.i. in both drug groups were considerably higher than normal values in red deer (6.9 mmol/l, Wilson and Pauli, 1983) or elk immobilized with succinylcholinchloride (4.4 mmol/l, Pedersen and Pedersen, 1975). Levels of serum glucose are interpreted as indicators for stress by Baronetzky-Mercier (1995). However, xylazine as an alpha-2 adrenoreceptor agonist inhibits insulin secretion which is mediated by alpha-2 receptors in pancreatic beta cells. This results in hypoinsulinemia with subsequent hyperglycemia (Lin, 1996). Therefore, high plasma glucose levels do not indicate stress when alpha-2 adrenoreceptor agonists such as xylazine are used for deer immobilizations.

In this study, the fatalities could not be directly associated with the drug effect. Capture related (hunting) as well as environmental circumstances (ambient temperature) may have been the key factors for death in three cases. In effect, the deer which died during immobilizations have been hunted for >1 hr in fence corridors immediately before immobilization. Additionally, ambient temperature was high and the hide was insulated, and two deer developed lethal hyperthermia (>42 C). Hyperthermia may additionally have been increased by the effects of immobilization drugs known to affect thermoregulation (Burroughs, 1993). The level of blood urea of one animal which died (21.8 mmol/l) was more than twice the normal value (8.6 mmol/l, Wilson and Pauli, 1983), indicating renal insufficiency and/or dehydration. A mortality rate of 5% is high compared to fatalities found when animals in small enclosures or zoos are captured (Wiesner,

1998). Mortality rates reported for the capture of free ranging ungulates ranged from 0% to 26% (Bergerud et al., 1964; Nielson and Shaw, 1967; Houston, 1969; Jolicoeur and Beaumont, 1986; Perez et al., 1997; Kilpatrick and Spohr, 1999), most of which being around 5% as in our study. In free-ranging moose (*Alces alces*) captured using succinylcholine chloride, carfentanil/xylazine, and xylazine, Delvaux et al. (1999) reported mortality rates of 7%, 6%, and 0%, respectively. Although the use of carfentanil/xylazine was associated with a comparatively high mortality (6%), these authors considered this combination as the best one for effectively immobilizing free-ranging moose. Capture of free-ranging deer is associated with a much higher effort to dart animals than in zoo conditions, and therefore effectiveness may have a high priority in some cases.

We conclude that the mixture of tiletamine-zolazepam and xylazine is a useful alternative to the use of opioids for the immobilization of feral red deer. We therefore recommend the use of 2.3 ml/100 kg BM of a 1:1 mixture of Zoletil® and Rompun® as a 172 mg/ml injectable solution to immobilize feral adult red deer. This corresponds to 1.2 mg/kg tiletamine + 1.2 mg/kg zolazepam + 2.3 mg/kg xylazine. However, chemical capture of excited animals should be strictly avoided regardless of the anesthetics used to reduce mortality when deer are immobilized.

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