ARTICLE ARTIKEL

TOLAZOLINE AS AN ANTAGONIST IN FREE-LIVING LIONS IMMOBILISED WITH A KETAMINE-XYLAZINE COMBINATION

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ABSTRACT: T.C. van Wyk.; Berry H.H. 1986 **Tolazoline as an antagonist in free-living lions immobilised with a ketamine-xylazine combination.** *Journal of the South African Veterinary Association* (1986) 57 No. 4, 221-224 (En) Directorate of Nature Conservation and Recreational Resorts, Private Bag 13306, 9000 Windhoek, South West Africa/Namibia.

A combination of ketamine at 8,0 mg/kg and xylazine at 3,2 mg/kg was found effective in immobilising lions *(Panthera leo)* for 4 hours. Ataxia and immobilisation were rapidly induced, with stable respiratory rate, heart rate and body temperature recorded. Tolazoline effectively antagonised xylazine via intravenous or intramuscular injection, resulting in a return to mobility within approximately 20 and 60 minutes, respectively. Tolazoline also elevated the respiratory rate. No mortalities occurred during 97 immobilisations of 76 lions.

Key words: Tolazoline antagonist, ketamine-xylazine combination, lion, wildlife.

INTRODUCTION

The major advantage of using ketamine hydrochloride (Ketalar, Parke-Davis) as opposed to phencyclidine hydrochloride (Sernylan, Parke-Davis) for the immobilisation of lions *Panthera leo* L. is the significant time difference in the post-anaesthetic recovery stage[4]. Using dosage rates of 7,8 – 15,1 mg/kg ketamine ($\bar{x}$ = 11,5 mg/kg) Smuts et al.[4] recorded the time to full recovery as 3 – 6 h ($\bar{x}$ = 4h12), whereas phencyclidine at a dosage rate of 1,4 – 2,4 mg/kg ($\bar{x}$ = 1,7 mg/kg) resulted in a time of 8 – 15 h ($\bar{x}$ = 12h) before the lions were completely recovered. Phencyclidine has further disadvantages, namely that the long period required for recovery makes the animal susceptible to hyperthermia[1], the occurrence of violent, epileptiform convulsions[1,2] and the restrictions placed on its prescription and handling due to its Schedule 8 rating by the South African Medical, Dental and Drugs Control Amendment Act, 1974. Ketamine's main disadvantage as an anaesthetic for free-ranging lions has been the relatively large volume required, necessitating projectile darts of 10 ml capacity[2] or applying a divided dosage in two separately fired darts[5]. Consequently, as part of a study on aspects of lion ecology at Etosha National Park, we also investigated the possibility of utilising the apparent synergistic effect of ketamine in combination with xylazine hydrochloride (Rompun, Bayer) to reduce the dosage rate of ketamine. Wiesner[6] recommended a concentration of 100 mg/ml ketamine in combination with 125 mg/ml xylazine. For immobilising captive, adult lion Wiesner[6] administered this combination at a dosage rate of 3 ml mixture plus 1 ml of ketamine. Furthermore, because the effects of xylazine include pronounced muscle relaxation with concomitant respiratory depression and a marked lack of co-ordination upon recovery, especially with high dosage rates, the use of tolazoline hydrochloride (Weimer Pharmaceuticals, Rastatt) as an antagonist to xylazine was also investigated during the present study. The antagonistic effect of tolazoline was reported by Zingoni et al.[7] who administered it to xylazine-sedated domestic sheep.

Tolazoline (2-benzyl-2-imidazoline hydrochloride) acts as an alpha-adrenoreceptor blocking agent which also has a direct dilator action on the peripheral blood vessels, especially the arterioles and capillaries[3]. By intramuscular injection tolazoline produces maximum effects after 30 – 60 minutes and is rapidly excreted largely unchanged via the urine. Minor side effects in humans include pilo-erection and flushing, while major side effects may include severe tachycardia, cardiac arrhythmia, vomiting and diarrhoea. Large doses may result in orthostatic hypotension. Tolazoline is light-sensitive and unstable at high ambient temperatures and should be stored under dark, cool conditions.

MATERIALS AND METHODS

Ketamine in powder form was made up to a standard concentration of 250 mg/ml by using isotonic saline and agitating and heating the solution to 45°C. Since this solute formed crystals when stored below 25°C, reheating and agitation were necessary shortly before preparing the combination with xylazine. Xylazine in powder form was obtained in bottled units of 500 mg. An experimental supply of tolazoline solution at a concentration of 20 mg/ml in sterile water (pH 3-4) was donated by Weimer Pharmaceuticals, Rastatt, West Germany.

Five ml ketamine at 250 mg/ml were injected into a bottle containing 500 mg of xylazine powder and the mixture shaken briefly. This resulted in a concentration of 250 mg/ml ketamine and 100 mg/ml xylazine. Crystallisation of this mixture was less frequent than with the ketamine in solution alone. When crystals formed, only moderate heating (35°C) and agitation were necessary to dissolve them. Due to the light-sensitivity of ketamine, the mixture was always stored in a dark, cool place.

Ketamine-xylazine was administered in 3–5 ml reusable aluminium dart syringes with barbed NC 2 needles and propelled by a 32-gauge rifle fitted with a ,22 adaptor (Palmer Chemical and Equipment Co., Georgia, U.S.A.). "Stun-load" 0,22 blank cartridges

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(Abattoir Engineering Supplies, Ravenmoor, R.S.A.) were used to fire the darts at distances of 20 – 30 m. For distances less than 20 m a Palmer CO_2 gas Cap-chur Rifle was preferable, because of the reduced impact of the dart on the lion. Based on an estimated body mass of 150 kg for an adult lioness and 200 kg for an adult lion, darts of 3 and 4 ml, respectively, were used, resulting in a calculated initial dosage rate of 5 mg/kg ketamine and 2 mg/kg xylazine. This was intended to render the lion sufficiently immobile so that a booster injection of 1,5 ml for females and 2 ml for males could be given by syringe. Thereby the total dosage rate was theoretically set at 7,5 mg/kg ketamine and 3 mg/kg xylazine. Each immobilised lion was weighed to the nearest kilogram to calculate the actual dosage rate. At a later stage this procedure was modified by firing a complete dose in 4 ml darts for adult females and 5 ml darts for adult males. Boosting by hand syringe was only done when it was evident that the animal was insufficiently immobilised. Lions were considered immobile when they became sternally or laterally recumbent after darting and could no longer raise their head. The body mass of large cubs (12 – 24 months) and sub-adults (2 – 4 years) was first estimated and the volume of the drug reduced accordingly. The embedded dart was removed as soon as the lion was tractable to check if complete injection of the drug had occurred. To facilitate rapid absorption of the drug combination, the dart was fired into a body area with a rich blood supply such as the shoulder muscle or the buttocks. Lions were approached to within darting range by vehicle during daytime. If they were not in a suitable position or were found lying in dense vegetation, the gutted carcase of a springbok (*Antidorcas marsupialis*) was dragged as bait around their immediate vicinity. Alternatively, to attract lions, a larger carcase such as gemsbok (*Oryx gazella*), kudu (*Tragelaphus strepsiceros*), or zebra (*Equus burchelli*) was gutted and dragged in the vicinity of a pride's favoured waterhole and then chained to a tree.

Captured lions were routinely given an injection of 15 – 20 ml of long-acting penicillin (Compropen, Glaxo-Allenbury). Dart wounds or any naturally occurring skin injuries were treated with an antibiotic aerosol (Liquamycin, Pfizer). Vitamins and amino-acids concentrate (Stress-Vitamin, Weimer Pharmaceuticals) were injected at a dosage rate of 15 – 20 ml per animal. Possible eye damage through dryness or foreign particles was prevented by liberal applications of antibiotic ophthalmic ointment (Terramycin, Pfizer). To protect the eyes from sunlight a cloth blindfold was applied immediately after the lion was immobile. To prevent hyperthermia, immobilised lions were shaded and when their rectal temperature increased to 40°C they were cooled with water spray, using a pressurised cylinder to obtain finely-dispersed droplets. Critical body functions, namely respiration, heart beat and temperature were monitored every 30 minutes until the lion showed signs of recovery. Recovery from immobilisation was considered to have started when a lion was able to move into a sternal position and raise its head. Under field conditions the emergence from ketamine anaesthesia was gauged by reflexes in the eyes, tongue and jaw muscles.

Because a dosage rate of tolazoline was unknown for lion, increasing doses from 0,5 – 5,0 mg/kg were given by intramuscular and then intravenous route to ascertain the effect. The preferred intravenous route was via the jugular vein.

Statistical evaluations were done, using Student's t-test for 2 means and applying a null hypothesis of no difference between mean recovery times of different treatments.

RESULTS

The effects of a combination of ketamine and xylazine on free-living lions were summarised in Table 1. The effects of tolazoline on lions immobilised with ketamine-xylazine combination are presented in Table 2.

Lions receiving ketamine-xylazine in a single dose, followed by tolazoline, remained immobile for a significantly shorter period than lions not receiving tolazoline ($t=7,61$; $P<0,001$). Similarly, consecutive doses of ketamine-xylazine, followed by tolazoline, resulted in significantly reduced immobilisation periods compared to when tolazoline was not administered ($t = 5,99$; $P<0,001$). When tolazoline was administered intravenously following a single dose of ketamine-

Table 1. Effects of ketamine and xylazine in combination on free-living lions in Etosha during 1981–84

Parameter measured	Drug combination			
	Ketamine-xylazine in single dose (n = 20)		Ketamine-xylazine in two or more consecutive doses (n = 34)	
	$\bar{x}$	±S D	$\bar{x}$	± S D
Body mass (kg)	127	21	161	33
Total ketamine (mg)	1 006	143	1 318	263
Total xylazine (mg)	403	57	519	92
Dosage ketamine (mg/kg)	8,0	1,2	8,3	1,1
Dosage xylazine (mg/kg)	3,2	0,5	3,3	0,4
Time to ataxia (min)	3'06"	47"	6'02"	2'08"
Time to immobilisation (min)	4'33"	1'26"	9'08"	5'47"
Clinical after 60 min				
Respiratory rate/min	16	3	15	3
Heart rate/min	56	11	54	17
Rectal temperature (C°)	38,4	0,9	38,9	1,2
Total immobilisation time (hrs/min)	4h33	1h13	4h23	1h15

Table 2. Effects of tolazoline on ketamine-xylazine immobilised lions in Etosha during 1981–84

Parameter measured	Drug combinations			
	Ketamine-xylazine in single dose followed by tolazoline (n = 18)		Ketamine-xylazine in two or more consecutive doses, followed by tolazoline (n = 24)	
	$\bar{x}$	± S D	$\bar{x}$	± S D
Body mass (kg)	136	16	161	51
Total ketamine (mg)	1 038	83	1 305	319
Total xylazine (mg)	415	33	552	128
Dosage ketamine (mg/kg)	7,7	0,9	8,5	2,2
Dosage xylazine (mg/kg)	3,1	0,4	3,4	0,9
Time to ataxia (min)	3'14"	36	3'39"	1'42"
Time to immobility (min)	5'39"	1'32"	7'20"	3'57"
Clinical after 60 min				
Respiratory rate/min	16	4	15	3
Heart rate/min	54	11	53	7
Rectal temperature (°C)	38,5	1,3	38,6	1,0
Tolazoline given				
Intramuscular (mg)	379	190	428	182
Intravenous (mg)	430	209	639	284
Dosage (mg/kg)	4,0	0,6	3,7	0,9
Respiratory rate after 15 min	18	6	17	3
Respiratory rate after 30 min	21	11	23	12
Respiratory rate after 45 min	41	24	29	28
Respiratory rate after 60 min	67	53	69	44
Time to mobility (min)				
via intramuscular route	74	40	50	23
via intravenous route	20	15	22	16
Total immobilisation time (hrs/min)	2h27	45 min	2h19	38 min

xylazine, lions recovered their mobility in significantly less time than when tolazoline was given intramuscularly ($t = 3,27$; $P < 0,02$). Likewise, intravenous administration of tolazoline following consecutive doses of ketamine-xylazine, reduced the immobilisation period compared to when tolazoline was given intramuscularly ($t = 3,01$; $P < 0,02$).

The ketamine-xylazine combination retained its potency after a storage period of 4 months.

DISCUSSION

The results show that a dosage level of approximately 8,0 mg/kg ketamine in combination with 3,2 mg/kg xylazine provides an effective immobilisation of both sexes of lion for up to 4 hours. It is preferable to administer this combination in a single dose, which is usually possible with all lionesses, but sometimes, in the case of heavy males weighing over 200 kg, a booster injection of additional drugs may be necessary. Ataxia, which was characterised by an hypnotic stare and uncoordinated movement, was noticeable within 3–6 minutes and complete immobilisation took place within 4 – 9 minutes.

Clinical measurements reflected an even respiratory and heart rate, whilst body temperature was not unduly elevated. Prevention of hyperthermia in lions immobilised with ketamine-xylazine is a critical consideration when ambient temperatures are high, if it is considered that panting rates of 120 – 150 per minute were recorded in resting lions subjected to Etosha's summer daytime shade temperatures of 40°C. Tables 1 & 2 show a respiration rate of 15 – 16 per minute after 60 minutes of immobilisation, which represents a decrease of nearly 90% over panting rate.

When tolazoline was administered, a significant reduction in immobilisation time was achieved in all cases. It allowed a return to mobility, albeit uncoordinated, within 50–74 minutes following intramuscular injection and 20 – 22 minutes when infused intravenously. A dosage rate of 4,0 mg/kg was found to produce the best reaction. Increasing the dosage rate to 5,0 mg/kg resulted in muscular seizures in 2 cases. These may have been caused by the total removal of the sedative affects of xylazine, leaving the lion susceptible to typical ketamine excitation. Because lions which were returned to mobility by tolazoline at 4,0 mg/kg showed ataxia to varying degrees, it appears that some effects of xylazine and ketamine were still present. With our present experience this seems preferable to exposing the recovering lion to convulsions and resultant stress and hyperthermia. Tolazoline, in our opinion, should be administered at a sub-maximal dosage rate. When tolazoline is administered it is important to allow the anaesthetic effect of ketamine to wear off which is normally about 2 hours after administration.

Without tolazoline, immobilised lions required about 4,5 hours to become sternal and still displayed ataxia after 5 – 6 hours. In contrast, tolazoline-treated lions were able to walk more steadily 2,5 hours after being darted and joined up with their pride members soon afterwards. An additional advantage of tolazoline is that the lion can defend itself within 30 – 60 minutes of antidoting and need not be guarded for periods of up to 6 hours as was previously the case. Moreover, a tolazoline-aided return to mobility was accompanied by an elevated respiration rate which facilitated cooling. Table 2 shows mean respiration rates of 67 and 69 per minute, 60 minutes after administration of tolazoline, whereas lions which did not receive tolazoline had a

mean respiration rate of 17 per minute (n = 34) after the same time had elapsed (4 hours following immobilisation).

Although no problems were experienced during or after the intravenous infusion of tolazoline, we draw attention to the major side effects listed under the introduction of this paper, especially the possibility of sudden drop in blood pressure which may accompany the intravenous administration of a vasodilator. The intravenous injection of tolazoline was found to be particularly important when rapid recovery was required, for example, to return a lioness to small cubs, or to prevent hyperthermia on hot days. However, if such urgency was not present the intramuscular route was preferred, which reduced the likelihood of severe side effects. Furthermore, 4 out of 10 lions receiving intravenous infusion of tolazoline began reviving whilst it was being administered. During this investigation 97 immobilisations on 76 lions were successfully performed with no mortality or permanent disablement to any of them. No ill-effects of the immobilisations were detectable after 24 hours and no increase in shyness or aggressiveness were noted in any of the animals and even in individuals which were immobilised on 4 separate occasions. We can therefore report with confidence on the efficacy of a combination of ketamine-xylazine for immobilising free-living lions.

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ABSTRACT **SAMEVATTING**

SIMULIUM CONTROL IN A SMALL POLLUTED RIVER IN SOUTH AFRICA

The effects on *Simulium adersi* and *S. hargreavesi* larvae of 2 *Bacillus thuringiensis* var. *israelensis* products, the liquid formulation "Teknar" (Sandoz) and a powder formulation produced by the Ben Gurion University, Israel, were compared in the laboratory and in the Pienaars River. This river was heavily polluted with effluent from a nearby sewage works and contained 77 mg/ℓ chloride.

In the laboratory *S. adersi* and *S. hargreavesi* larvae showed 26; 48; 95 and 100 % mortality 6 hours after a 10-minute application of 0,8; 1,6; 3,2 and 16 ppm "Teknar" in rain water. The powder formulation applied at 0,2; 1,2; 2,0 and 30 ppm resulted in a 7; 17; 35 and 100 % mortality. In polluted river-water the mortality was 85 % with 16 ppm "Teknar" and 80 % with 30 ppm *B. thuringiensis* powder.

In the field trials "Teknar" at 1,6 ppm and *B. thuringiensis* powder at 3 ppm did not cause any larval mortality at flow rates of 3 060ℓ/min and 2 040ℓ/min, respectively. However, 24 hours after application of the powder formulation, numbers of *S. hargreavesi* decreased significantly (P = 0,05) 20 m below the application point. A further 24 hours later, after "Teknar" had been applied, the numbers of *S. adersi* decreased and those of Chirnomidae increased significantly. There was a significant increase in *S. hargreavesi* 200 m downstream after treatment with "Teknar".

(Car, M., 1984. Laboratory and field trials with two *Bacillus thuringiensis* var. *israelensis* products for *Simulium* (Diptera: Nematocera) control in a small polluted river in South Africa. *Onderstepoort Journal of Veterinary Research*, 51, 141-144 (1984).)