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Chemical Immobilization of Crested Porcupines with Tiletamine HCl and Zolazepam HCl (Zoletil®) under Field Conditions

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ABSTRACT: The combination of tiletamine HCl and zolazepam HCl has been used on many species of wild mammals. Short induction time, low dosage, satisfactory safety margins, relatively constant immobilization time, and smooth recovery are benefits reported. This combination (Zoletil® 100) was used during a study on behavioural ecology of the crested porcupine (*Hystrix cristata*) in a Mediterranean coastal area (Maremma Regional Park, Tuscany, Italy). We used this mixture 42 times on 31 individuals. Mean adult dose was (±SE) 7.24±0.37 mg/kg (74.0±3.0 mg/individual). Average adult induction time was 5.3 min (±1.1) and average adult immobilization time was 22.6 min (±6.0). One adult male porcupine died after chemical restraints. The use of tiletamine-zolazepam seems adequate for chemical immobilization of crested porcupines under field conditions, mainly because of its short induction time, small volume to be injected and wide safety margin.

Key words: Chemical immobilization, chemical restraint, *Hystrix cristata,* rodents.

Chemical immobilization is often necessary in wildlife studies when capturing and handling medium-large mammals (Animal Care and Use Committee, 1998). An ideal sedative should meet several requirements of safety and effectiveness including a high therapeutic index (lethal dose/effective dose), low effective volume, short induction time, constant immobilization time, and the existence of an antidote (Pond and O'Gara, 1996).

Several authors reported the use of mixtures of xylazine hydrochloride (20 mg/ml) and ketamine hydrochloride (100 mg/ml) for chemical immobilization of *Hystrix* spp. (Filibeck et al., 1981; Alkon and Saltz, 1984; Van Aarde, 1985; Pigozzi, 1987; Sonnino, 1998). This mixture has some undesirable effects such as occasional bradycardia and respiratory depression (Pond and O'Gara, 1996). Pharmacologic characteristics of the xylazine-ketamine mixture have been reported elsewhere (Ramdsen et al., 1976; Kreeger et al., 1990a, b; Pond and O'Gara, 1996).

Zoletil® (Virbac, Carros, France) is a non-opioid, non-barbiturate, injectable anesthetic consisting of an equal mixture (weight) of tiletamine HCl and zolazepam HCl (250 mg each). Reconstitution with 5 ml of sterile water results in a 100 mg/ml solution (Zoletil® 100). Use of tiletamine-zolazepam mixture on wildlife has been reported to be safe, with a short induction time, good muscle relaxation, smooth recovery with few convulsions, and with minimal effect on respiration, although emesis and excessive salivation may occur (Kreeger et al., 1990a; Ballard et al., 1991; Poole et al., 1993; Hale et al., 1994; Vila and Castrovejo, 1994; Larivière and Messier, 1996; Pond and O'Gara, 1996). Its wide safety margin is especially useful if the animal body weight can be only roughly estimated (Larivière and Messier, 1996) as under field conditions. A mixture of tiletamine and zolazepam (TZ) and xylazine is used to immobilize deer under field conditions (Kilpatrick and Spohr, 1999; R. Fico, pers. comm.).

Our objective was to assess the effectiveness of TZ on crested porcupines, under field conditions, during a long-term study on their behavioral ecology. The crested porcupine is a quill-covered, nocturnal rodent (males: 11 kg; females: 13 kg; unpubl. data), that spends the day in underground burrows in thickly wooded areas of central and north Africa, as well as from south to central Italy. Although the crested porcupine is considered a "protected" species by Italian law, it can locally cause damage to gardens and cultivations.

Our study site was located in the Maremma Regional Park (42°39′N, 11°05′E, county of Grosseto, Tuscany, Italy). In this Mediterranean coastal area the vegetation is characterized by dense scrubwood, pinewood, sparse pastures, cultivated and abandoned olive-yards, maize, and sunflower (Arrigoni, 1988). Mean monthly temperature was 15.0 C, and mean monthly rainfall was 57.6 mm. Altitude ranged from sea level to 417 m above sea level.

The study took place between May 1998 and July 1999. Animals were trapped using 14 double entrance box traps (150×40×55 cm) positioned along the main trails used by porcupines. Traps were baited daily, and activated for at least 7 nights/month, to be checked at dawn.

Animals were given intramuscular injections in the lumbar region using an air-compressed syringe (Telinject®, Germany), administered by a blow-pipe 1.5 m long. We used a TZ (250 mg tiletamine + 250 mg zolazepam=500 mg total, made up to 5 ml=100 mg/ml) to assess its efficiency under field conditions.

First injection doses of TZ ranged from 30 to 80 mg/individual depending on estimated weight and age. Injection time and dose were recorded along with induction time, first handling time, immobilization time, and time to first recovery. Induction was measured as latency between injection to the time a porcupine lowered its head and could be handled without any resistance; immobilization time was time from induction to the time an animal first showed signs of awakening (head rising). First recovery time (first signs of recovery) was measured from induction to the first upright posture (standing).

Handling procedures consisted of sexing, weighing, taking standard body measurements, assessing tooth wear, and ear-tagging. Adult porcupines were equipped with VHF radio-collars (150–151 MHz, Televilt® Ltd, Sweden, and Biotrack® Ltd, UK) and relocated the first day after release as well as twice a week for at least 6 mo. During handling, we also recorded ambient air temperature (C), rectal temperature (C), heart rate (beats/15 sec), and respiratory rate (breaths/15 sec) at first handling and at first awakening. Following these procedures, porcupines were placed in a trap covered with vegetation and released about 2–3 hr later.

The Mann-Whitney *U*-test was performed to verify differences between sexes, and the Wilcoxon Signed Ranks test was used to compare paired measurements of physiologic parameters (rectal temperature, heart and respiration rate) at first handling and first awakening (Siegel and Castellan, 1988). For both tests, exact significance levels were computed by means of permutation procedures (Mehta and Patel, 1996; Good, 2000). Data were then submitted to Spearman's correlation analysis to test for association between dose injected, physiologic parameters, and times of induction, immobilization, and recovery. Multiple comparisons were taken into account by correcting the α level using the Dunn-Šidák significance level correction method: $\alpha' = 1-(1-\alpha)^{1/k}$, where k is the number of comparisons (Sokal and Rohlf, 1995).

Statistical analyses were performed using SPSS 9.01 (Statistical Package for Social Sciences, SPSS Inc., Chicago, Illinois, USA). Means are reported with standard error (±SE). Given small samples, data referring to young animals, to replicates, and to cases with additional doses during manipulation, were excluded from analyses.

Fifty-three porcupines were trapped 116 times (24 adult males, 20 adult females, five young males, one young female, three cubs), and in 42 cases (36%) we used TZ on 31 animals (14 adult males, 14 adult females, and three young males). One (2%) adult male died following chemical immobilization. One adult female aborted two male fetuses when left in the trap to recover from handling.

Multiple injections (two to four) were necessary to gain immobilization in 3/42 cases (7%; two males and one female). Upon initial arousal, in 7/42 cases (17%;

four adult males and one adult female) an additional dose (0.34 mg/kg±0.08), was given to maintain immobilization during manipulation. If a trapped individual had been previously chemically immobilized, a second procedure was conducted if more than 6 mo had elapsed. Statistical analyses were performed only on recordings taken during the first chemical restraint event and when no multiple injections were given, so that the sample was 23 individuals (10 adult males and 13 adult females). Adult males and females differed significantly in weight (9.63±0.29 kg, n=10, and 11.00±0.39 kg, n=13).

Pooled mean TZ dose injected was 7.24±0.37 mg/kg, 3.85–10.0 (n=23; 74.0±3.0 mg/individual). The mean dose for males was 7.67±0.59 mg/kg (n=10; 73.0±5.0 mg/individual). The mean dose for females was 6.92±0.48 mg/kg (n=13; 75.0±4.0 mg/individual).

Differences between sexes for physiologic parameters were not statistically significant except for the greater rectal temperature of females (U=19.5, W=64.5, P=0.012; Table 1). Physiologic parameters did not vary by dose (mg/kg) or by induction time. Neither excessive salivation during immobilization, nor any case of excitement or increased motor activity after recovery were recorded. The mixture also provided good muscle relaxation.

Mean induction time was 5.3±1.1 min, while mean immobilization time was 22.6±2.3 min, and mean recovery time was 28.7±3.2 min (Table 2). The Spearman's correlation analysis showed a significant association (with k=3, $\alpha_{0.05}$=0.017, $\alpha_{0.01}$=0.0033, $\alpha_{0.001}$=0.00033) of dose and recovery time (r=0.93, n=6, P=0.008).

Our data also suggest that TZ provides safe immobilization under field conditions, as we observed few deaths following chemical restraint (2%), although our small sample size and lack of precise diagnoses require us to limit our conclusions. Death could be due to other factors associated with capture (e.g., capture myopathy), and pharmacologic effects can be hard to detect in the field. Pigozzi (1987) reported 12% of porcupines (n=17) died following xylazine HCl–ketamine HCl in-

TABLE 1. Mean, standard error (SEM), minimum, maximum, and number of cases for physiologic parameters at first handling (1st) and first awakening (2nd) in adult porcupines immobilized by Zoletil® 100 (TZ). Mann-Whitney's *U*, Wilcoxon's W, and exact *P* value (2-tailed) for the Mann-Whitney test between sexes are reported for each temporal measurement.

Physiologic parameters	Sex	Mean	SEM	Min.	Max.	*N*	U	W	*P*
1st rectal temp. (C)	M	36.43	0.19	35.50	37.20	9	19.5	64.5	0.012
	F	37.29	0.22	35.60	38.50	12			
	Pooled	36.92	0.18	35.50	38.50	21			
1st heart rate (pulse/min)	M	126.00	5.01	104.00	152.00	9	35.5	113.5	0.198
	F	111.08	6.95	56.00	141.00	12			
	Pooled	117.48	4.71	56.00	152.00	21			
1st breath rate (breaths/min)	M	20.57	3.82	10.00	40.00	7	32.0	87.0	0.803
	F	18.80	2.15	12.00	36.00	10			
	Pooled	19.53	1.96	10.00	40.00	17			
2nd rectal temp. (C)	M	36.32	0.26	35.60	37.10	5	11.0	26.0	0.221
	F	37.11	0.38	35.30	38.60	8			
	Pooled	36.81	0.27	35.30	38.60	13			
2nd heart rate (pulse/min)	M	120.86	7.81	92.00	140.00	7	38.0	104.0	0.983
	F	122.55	7.04	80.00	168.00	11			
	Pooled	121.89	5.12	80.00	168.00	18			
2nd breath rate (breaths/min)	M	25.33	3.53	20.00	32.00	3	7.5	35.5	0.542
	F	23.14	2.58	16.00	36.00	7			
	Pooled	23.80	2.01	16.00	36.00	10			

TABLE 2. Mean, standard error (SEM), minimum, maximum, and number of cases for time of induction, immobilization and recovery in adult porcupines immobilization by Zoletil® 100 (TZ). Mann-Whitney's *U*, Wilcoxon's W, and exact *P* value (2-tailed) for the Mann-Whitney test between sexes are reported for each temporal measurement.

Times	Sex	Mean	SEM	Min.	Max.	*N*	U	W	*P*
Induction time (min)	M	5.6	2.0	2.0	23.0	10	54.0	145.0	0.502
	F	5.1	1.4	2.0	20.0	13			
	Pooled	5.3	1.1	2.0	23.0	23			
Immobilization time (min)	M	19.7	3.4	6.0	32.0	7	32.5	60.5	0.442
	F	24.3	3.1	12.0	45.0	12			
	Pooled	22.6	2.3	6.0	45.0	19			
Recovery time (min)	M	31.5	1.5	30.0	33.0	2	2.0	12.0	0.467
	F	27.3	4.9	18.0	41.0	4			
	Pooled	28.7	3.2	18.0	41.0	6			

jections, but he did not ascribe these casualties to chemical immobilization.

Additionally, TZ injections required a smaller volume, if compared to the procedure reported in Pigozzi (1987), thus reducing the risk of tissue damage because solutions are injected under pressure (Pond and O'Gara, 1996).

Induction times of TZ were considerably shorter than those reported by Pigozzi (1987), because he described induction time as time from the second injection (ketamine) and not from the first one with xylazine (10 min previously). Results by Poole et al. (1993) seem to confirm our observations.

Our results on independence of dose and rectal temperature, heart, and respiration rate confirm those reported by Hale et al. (1994) for North American porcupines (*Erethizon dorsatum*). Furthermore, Hale et al. (1994) found a negative correlation between induction time and heart rate in the first 15 min of manipulation, but we did not find any comparable evidence. Our results concerning dose dependent effects on latency partially confirmed those reported both for the North American porcupine (Hale et al., 1994) and striped skunk (*Mephitis mephitis*; Larivière and Messier, 1996); induction time was not correlated with TZ dose, and recovery latency was correlated, but immobilization time was not.

The miscarriage of two fetuses after injection with TZ could be related more to stress due to long handling procedures, weather conditions, time spent in the trap, or clinical status than to chemical restraint. Tiletamine and zolazepam are not reported to have any impact on pregnant females (Ballard et al., 1991; Larivière and Messier, 1996).

Tiletamine and zolazepam have proved to be an effective and safe immobilizing drug combination for crested porcupines. Short induction times, good safety margins, and small intra-muscular injection volumes are practical advantages of TZ for chemical immobilization in field conditions.

We suggest a dose of TZ ranging from 70–80 mg/individual (7–8 mg/kg) to gain an immobilization for 20–25 min, and a possible additional dose of 30–40 mg/individual (3–4 mg/kg) for adult crested porcupines.

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