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Effects of flumazenil on fishers *Martes pennanti* restrained with tiletamine-zolazepam

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As part of a project to restore the fisher *Martes pennanti* in Pennsylvania, USA, we evaluated flumazenil (0.02 mg/kg) for partial reversal of tiletamine-zolazepam (10.0-11.0 mg/kg; i.e. antagonizing the effects of zolazepam) by monitoring immobilization intervals and physiologic responses of fishers (N = 4). Flumazenil reduced mean down time and alert time, but did not reduce mean recovery time. Trends in respiratory rate, body temperature, pulse rate and arterial oxygen saturation expressed by immobilized fishers were not altered by flumazenil within eight minutes post-injection. Flumazenil did not enhance practical utility of tiletamine-zolazepam at 10.0-11.0 mg/kg because fishers that received flumazenil exhibited residual tiletamine effects such as prolonged recovery and profound ataxia.

Key words: chemical restraint, fisher, flumazenil, *Martes pennanti*, tiletamine-zolazepam

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In 1994, efforts were initiated to restore extirpated fisher *Martes pennanti* populations in Pennsylvania, USA (Serfass, Brooks, Tzilkowski & Mitcheltree, unpubl. report). As part of the project, fishers underwent a captive management program that included chemical restraint (Mitcheltree, Serfass, Whary, Tzilkowski, Brooks & Peper 1997). The dissociative anesthetic tiletamine and the tranquilizer zolazepam, combined at a 1:1 ratio by

weight, has been used frequently to restrain carnivores (e.g. Boever, Holden & Kane 1977, Lariviere & Messier 1996), including fishers (Petrini 1992, Mitcheltree, Serfass, Tzilkowski, Peper, Whary & Brooks 1999, Dzialak & Serfass 2002).

Previous research on restraining fishers with tiletamine-zolazepam demonstrated that at doses of < 5.0 mg/kg, immobilization typically was shallow, and recovery

was rapid (Dzialak & Serfass 2002). However, at a dose of 10.0-11.0 mg/kg, tiletamine-zolazepam provided prolonged recovery in fishers and likely would enable invasive procedures such as tooth removal or surgical repair of injury (Gray, Bush & Beck 1974, Mitcheltree et al. 1999, Dzialak & Serfass 2002). Moderating the effects of tiletamine-zolazepam at this dose range would enhance its utility in field settings, where short recovery typically is desirable, by reducing prolonged recovery or modulating drug-affected physiologic responses. However, efforts to antagonize tiletamine-zolazepam (e.g. Hatch, Clark, Jernigan & Tracy 1988) are rarely reported because the tiletamine component has no known antagonist. Nonetheless, flumazenil may moderate tiletamine-zolazepam because: 1) flumazenil competitively excludes the specific binding of benzodiazepines (e.g. zolazepam) at the receptor level (Amrein, Leishman & Bentzinger 1987, Lheureux & Askenasi 1989); and 2) for several species, plasma half-life of tiletamine is shorter than plasma half-life of zolazepam, indicating that recovery time would be reduced by antagonizing zolazepam (Lin, Thurmon, Benson & Tranquilli 1993). Our objective was to evaluate and describe the effectiveness of flumazenil for reducing recovery time of fishers restrained with tiletamine-zolazepam at 10.0-11.0 mg/kg, and to characterize physiologic effects of administering flumazenil to fishers restrained with tiletamine-zolazepam.

Material and methods

Acquisition of wild fishers and their management in captivity at Frostburg State University, USA, during the course of our study, including housing, diet and ambient conditions, was as described by Mitcheltree et al. (1997). We restrained fishers (N = 4) with tiletamine-zolazepam (Telazol®, Fort Dodge Laboratories, Fort Dodge, Iowa, USA) on two separate events in March-April 1998. We derived doses by weighing each fisher while it was in a den box of known weight before drug administration. However, fishers often were physically active in the den box during weighing. To determine the degree to which this activity affected our estimation of weight, we reweighed each fisher after drugging. We present dose ranges for tiletamine-zolazepam to account for potential differences in pre- and post-drugging weight determination. We administered drugs intramuscularly in the femoral region by the methods of Dzialak, Serfass & Blankenship (2001). On the first restraint event, we administered tiletamine-zolazepam to fishers at 10.0-11.0 mg/kg. Eight days after the first restraint

event, we administered tiletamine-zolazepam to the same fishers at 10.0-11.0 mg/kg, followed in approximately 40 minutes with an intramuscular injection of flumazenil (Romazicon®, Roche Laboratories, Nutley, New Jersey, USA) at 0.02 mg/kg. Procedures performed on immobilized fishers during the course of the reintroduction project included veterinary evaluation and treatment, radio-collaring, and collection of morphological and demographic data (see Mitcheltree et al. 1997 for an extensive treatment of captive management protocols).

We monitored four immobilization intervals following injection of tiletamine-zolazepam: induction time, down time, alert time and recovery time. Induction time was the time from injection until fishers no longer responded to stimuli (a researcher's voice or movements). Down time was the time from loss of responsiveness to stimuli, until fishers regained responsiveness to stimuli. Alert time was the time from injection until fishers regained responsiveness to stimuli. Recovery time was the time from injection until fishers regained mobility and coordination (Boever et al. 1977, Mitcheltree et al. 1999). Anesthesia intervals were obtained by direct observation of immobilized fishers and recorded in minutes.

We recorded physiologic responses of fishers given tiletamine-zolazepam and flumazenil to determine if flumazenil at this dose affected physiologic trends expressed by fishers during immobilization. We obtained respiration rate by direct observation of thoracic excursions and recorded data in breaths/minute. We used a pulse oximeter (SDI Vet/Ox™, SDI Sensor Devices, Waukesha, Wisconsin, USA) to obtain pulse rate (bpm) and arterial oxygen saturation (SpO₂, in %), and a vital signs monitor (DINAMAP™, Critikon, Tampa, Florida, USA) to obtain rectal temperature (°C). The oximeter sensor was placed on the tongue. We recorded physiologic data immediately after induction (i.e. 0 minutes post induction), and every four minutes thereafter for 20 minutes. Additionally, we recorded physiologic data at two 4-minute intervals post flumazenil injection. Using SAS® (SAS Institute Inc., Cary, North Carolina, USA), we performed paired-sample t-tests to evaluate drug effects on anesthesia intervals, and to evaluate differences in mean tiletamine-zolazepam dose. To characterize physiologic effects of flumazenil, we examined mean physiologic values as a function of time post injection, and identified extreme observations (i.e. outlying mean values) using simple linear regression. We arcsine transformed percentage data (SpO₂). We logarithmic transformed data on rectal temperature, respiratory rate, and pulse rate because trends in these physiologic parameters generally were curvilinear and exhibited

constant coefficients of variation (Neter & Wasserman 1974). Using SAS[®], we performed tests for regression linearity and for identifying outlying observations (Cook's D). At each time interval at which physiologic data were recorded, Cook's D values >2 standard deviations from mean Cook's D values among all intervals were considered to be indicative of extreme observations. Differences were considered to be significant if $P \leq 0.05$. Data are presented as $\bar{x} \pm SE$ unless specified otherwise.

Results

All fishers were immobilized effectively with tiletamine-zolazepam. As suspected, pre-drugging estimation of weight differed slightly from post-drugging weight (0.46 ± 0.19 kg). Nonetheless, the dose of tiletamine-zolazepam did not differ significantly between the two restraint events (10.2 ± 0.45 mg/kg and 10.4 ± 0.52 mg/kg, respectively; $t_{0.05,3} = 1.31$, $P = 0.28$). As expected, induction time did not differ between the first and second restraint events (4.0 ± 0.7 and 5.6 ± 1.3 minutes, respectively; $t_{0.05,3} = -1.58$, $P = 0.21$). However, fishers given tiletamine-zolazepam and flumazenil exhibited shorter down time (195.9 ± 24.0 minutes) than fishers given tiletamine-zolazepam alone (236.3 ± 24.7 minutes; $t_{0.05,3} = 6.45$, $P = 0.01$), and shorter alert time (201.5 ± 24.3 minutes) than fishers given tiletamine-zolazepam alone (240.3 ± 25.3 minutes; $t_{0.05,3} = 5.10$, $P = 0.01$). Recovery time did not differ between fishers given tiletamine-zolazepam and flumazenil (379.8 ± 13.5 minutes) and fishers given only tiletamine-zolazepam (357.8 ± 30.9 minutes; $t_{0.05,3} = -0.67$, $P = 0.55$). Administration of flumazenil did not alter existing physiologic trends discernibly within eight minutes post injection (Table 1). Upon transformation of the dependent variable (physiologic values), regressions for all physiologic parameters were linear ($F_{1,6} \geq 268.5$, $P < 0.01$). Cook's D values that exceeded two standard deviations from mean Cook's D values among time intervals were associated with

mean pulse rate, respiratory rate, and arterial oxygen saturation at 0 minutes post induction (see Table 1). Mean \pm SD Cook's D among time intervals, and Cook's D at 0 minutes post induction for mean pulse rate, respiratory rate and arterial oxygen saturation were 0.26 ± 0.38 and 1.1 , 0.33 ± 0.67 and 1.9 , and 0.34 ± 0.56 and 1.1 , respectively. However, at no other time interval did Cook's D exceed two standard deviations from the mean for any physiologic parameters evaluated.

Discussion

In our study, constraints associated with conservation objectives of fisher restoration compromised our intention of a repeated measures experimental design (Dzialak et al. 2001) and resulted in low sample size (e.g. Arnemo, Moe & Soli 1994, Walzer & Huber 2002). Consequently, analytical and inferential power was low. Nonetheless, inferences regarding the utility of flumazenil for reversing effects of tiletamine-zolazepam in fishers can be made.

Clinical effects of flumazenil on benzodiazepine-induced neuroendocrine, cardiovascular, and behavioural responses have been evaluated extensively using laboratory animals (e.g. dogs, cats and rats; Lemke, Tranquilli, Thurmon, Benson & Olsen 1996, Saldivar, Gomez, Martinez & Arias 2000). Hatch et al. (1988) and Bednarski, Muir & Tracy (1989) evaluated flumazenil for reversing tiletamine-zolazepam in dogs and cats. However, neither the metabolic disposition of tiletamine-zolazepam, nor the safety and efficacy of flumazenil had been evaluated previously for fishers. In our study, similar induction times between restraint events provided evidence of a generally uniform response to tiletamine-zolazepam among fishers before administration of flumazenil. Fishers that received flumazenil became alert and responsive to stimuli sooner than fishers that did not receive flumazenil. However, antagonizing the benzodiazepine component enabled expression of residual dissociative effects including prolonged recovery, profound ataxia, diminished pupillary reflex and repeated,

Table 1. Rectal temperature (in °C), respiratory rate (breaths/minute), pulse rate (bpm), and arterial oxygen saturation (SpO₂ in %) of fishers (N = 4) that received tiletamine-zolazepam and flumazenil at Frostburg State University, Frostburg, Maryland, USA, in 1998. Data were recorded every four minutes upon induction for 20 minutes, as well as four and eight minutes post flumazenil injection.

Physiologic response	Post induction period										Post flumazenil injection					
	0 min		4 min		8 min		12 min		16 min		20 min		4 min		8 min	
	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE	\bar{x}	SE
Body temperature	39.3	0.1	39.0	0.1	38.8	0.2	38.6	0.2	38.5	0.2	38.2	0.2	37.6	0.2	37.5	0.2
Respiratory rate	85.0 ^a	13.2	34.5	4.1	25.5	2.1	22.5	2.1	23.0	1.0	21.5	2.2	19.0	1.8	20.0	1.9
Pulse rate	264.0 ^a	12.3	224.0	20.2	204.0	12.7	184.0	12.4	170.0	10.8	162.0	11.7	170.0	15.8	162.0	12.7
SpO ₂	65.0 ^a	5.9	73.0	6.2	76.0	7.6	82.8	4.2	88.0	1.8	92.8	1.7	97.3	1.9	97.5	0.7

^a Associated Cook's D was indicative of outlying data.

unsuccessful efforts to regain mobility (Bednarski et al. 1989, Nielsen 1999). We observed no resedation following flumazenil, and efforts to regain mobility persisted throughout recovery until fishers were ambulatory. Whereas fishers that did not receive flumazenil exhibited calm recovery from tiletamine-zolazepam immobilization, the alert condition in fishers that received flumazenil was a potentially injurious situation because in attempting to regain coordinated mobility, alert fishers forcibly struck interiors of recovery cages. This condition also would be unsafe in field settings because an uncoordinated, partially mobile fisher may attract and be susceptible to predators, or be unable to thermoregulate efficiently. In contrast to our results, Walzer & Huber (2002) reported that in cheetahs *Acinonyx jubatus* immobilized with tiletamine-zolazepam at 4.2 mg/kg, flumazenil at approximately 0.03 mg/kg shortened the recovery time considerably. Similarly, Spelman, Summer, Karesh & Stoskopf (1997) administered flumazenil at 0.08 mg/kg to North American river otters *Lontra canadensis* immobilized with tiletamine-zolazepam at 4.0 mg/kg and reported that flumazenil effectively reduced the recovery time. Both studies reported that flumazenil reduced ataxia during recovery (Spelman et al. 1997, Walzer & Huber 2002). Disparate results between our study and the studies of Spelman et al. (1997) and Walzer & Huber (2002) provide evidence of considerable interspecific variation in responses to tiletamine-zolazepam and flumazenil among carnivores. In our study, residual tiletamine effects expressed by fishers suggest that plasma half-life of tiletamine is similar to, or longer than plasma half-life of zolazepam. This is the case in dogs where plasma half-life of tiletamine and zolazepam are 1.2 and 1.0 hours, respectively (Lin et al. 1993). However, in cats, and possibly other felids (Walzer & Huber 2002), plasma half-life of tiletamine and zolazepam are 2.5 and 4.5 hours, respectively (Lin et al. 1993). An alternate consideration in the differences observed among these results may be associated with fishers in our study having received a much greater dose of tiletamine-zolazepam than cheetahs and otters restrained by Walzer & Huber (2002) and Spelman et al. (1997), respectively. This would imply that in carnivores, tiletamine at high doses could act to impair its metabolism, thereby extending its plasma half-life. However, this implication is not supported by the literature, nor is there evidence of metabolic inhibition in studies reporting use of other cyclohexamines (e.g. ketamine) for immobilization of wildlife. Nonetheless, further research examining multiple dose regimens of tiletamine-zolazepam and flumazenil in fishers may be appropriate.

Our analysis of physiologic response trends and out-

lying observations suggested that the transient, heightened cardiopulmonary responses exhibited by fishers shortly after induction could be considered extreme compared to physiologic responses at subsequent time intervals during immobilization. Initial cardiopulmonary responses likely reflected physical exertion by fishers during drugging (Mitcheltree et al. 1997, Dzialak et al. 2001), so these observations have implications mainly for pre-drugging protocols instead of the effects of flumazenil. However, hypoxemia among fishers (i.e. SpO₂ values < 80.0%; Spelman et al. 1997, Tremper & Barker 1989) for ≥ 8 minutes post induction is cause for concern and deserves comment. In previous research chemically restraining fishers using the same drug administration protocols, no instances of hypoxemia were observed (Dzialak et al. 2001, Dzialak, Serfass, Shumway, Hegde & Blankenship 2002). Persistent low SpO₂ values observed in our study suggest that researchers considering restraining fishers with tiletamine-zolazepam (or any chemical restraint with known respiratory depressive actions) should minimize pre-drugging physical exertion by fishers and be prepared to monitor the SpO₂ closely. Generally, body temperature, pulse rate and respiratory rate of fishers during the 20-minute period before administration of flumazenil were consistent with known pharmacologic effects of tiletamine-zolazepam (Boever et al. 1977). Our analysis suggested that during eight minutes post flumazenil injection, deviations from existing physiologic trends were negligible, thus, flumazenil at this dose did not modulate physiologic response of fishers restrained with tiletamine-zolazepam. Similarly, Hatch et al. (1988) and Bednarski et al. (1989) reported that administration of flumazenil to chemically restrained dogs and cats did not alter physiologic response trends or induce undesirable side effects. Unfortunately, Spelman et al. (1997) and Walzer & Huber (2002) did not report on the physiologic effects of flumazenil. Given the variability in responses to tiletamine-zolazepam among species, future research on benzodiazepine antagonism in wildlife should include physiologic evaluation.

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