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ORIGINAL RESEARCH

Assessing the safety and efficacy of an anaesthetic protocol combining tiletamine, zolazepam and xylazine when used in tigers

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Abstract

Background: Due to the limited availability of ketamine in Mexico, alternative anaesthetic protocols for the immobilisation of big cats such as tigers (*Panthera tigris* ssp.) need to be considered. Therefore, this study aimed to assess the safety and efficacy of an anaesthetic protocol combining tiletamine, zolazepam and xylazine when used in tigers.

Methods: Sixteen tigers (seven *P. tigris tigris* and nine *P. tigris altaica*) at two zoological institutions in Mexico were included in the study. Tigers were either darted or hand injected with a combination of tiletamine/zolazepam (1.5 mg/kg) and xylazine (0.5 mg/kg). Physiological variables, including heart rate (HR), respiratory rate (RR), body temperature, arterial haemoglobin oxygen saturation (SpO_2), systolic blood pressure (SAP), diastolic blood pressure (DAP) and mean arterial pressure, were measured and recorded. The tigers were divided into two groups according to their weight: light (≤40 kg) and heavy (≥100 kg). Wilcoxon rank tests were then used to assess the effects of anaesthesia on HR, RR, SpO_2, SAP and DAP.

Results: HR and weight varied significantly among the tigers included in the study, but no statistically significant differences in RR, body temperature, SpO_2, SAP or DAP were found. Time to recovery was shorter in tigers that received atipamezole than in those that did not.

Limitations: The weight, age and sex group sample sizes were unequal. As such, caution should be employed when attempting to draw conclusions from these group comparisons.

Conclusion: Despite the controversy associated with tiletamine/zolazepam use in tigers, no adverse effects were observed. Therefore, a combination of tiletamine, zolazepam and xylazine is a suitable alternative for tiger anaesthesia when ketamine is not available.

KEYWORDS
anaesthesia, exotics, zoo animals

INTRODUCTION

Tigers (*Panthera tigris* ssp.) are globally well represented in zoos, sanctuaries and private collections, and Mexico is no exception. Immobilisation protocols that include ketamine are well established and documented in tigers.[1] However, due to the minimal availability of ketamine in Mexico, alternative protocols for the safe immobilisation of tigers without ketamine needed to be considered.

Although neurological complications have previously been associated with the use of tiletamine/zolazepam (TZ) in tigers, recent reports have found that using this drug combination at appropriate doses and in appropriate combinations with other drugs resulted in successful anaesthetic events without adverse side effects.[2, 3] These anaesthetic events were reported to result in good-quality immobilisation and recovery without seizures, sudden or subsequent death, ataxia or other complications.[4–12]

Previous work by the authors of the present study reported 10 successful anaesthetic immobilisations with TZ and xylazine (X) in a single Amur tiger cub over 5 months with no adverse reactions.[13]

This combination provided a safe anaesthetic level, and physiological parameters remained constant during the events. Complete blood cell counts and serum chemistry values remained normal between each immobilisation.

To assess the efficacy and safety of TZ as an alternative protocol to ketamine, the study reported in this paper evaluated a TZ and X immobilisation protocol in multiple tigers at two zoos in Baja California, Mexico.

METHODS

Seven Bengal tigers (*P. tigris tigris*) and nine Amur tigers (*P. tigris altaica*) from two different zoos in Baja California, Mexico, were immobilised for routine physical examinations and clinical procedures. The sex ratio was 10 males to six females. Bodyweights ranged from 23.5 to 260.0 kg (mean = 137.42 kg), measured with an on-site digital scale or obtained from recent clinical records. Ages ranged from 4 months to 14 years (mean = 5.6 years).

We performed 23 immobilisation events with a combination of TZ (1.5 mg/kg, Zelazol, Zoetis) and X (0.5 mg/kg, Procin Equus - xylazine 10%, PiSA Agropecuaria) administered intramuscularly by dart or manual injection in a large cat squeeze cage. Once recumbent and safe to approach, an assigned observer recorded physiological parameters every 5 minutes, and the veterinary team and two technicians performed the instrumentation, physical examination and elective procedures. The parameters evaluated included heart rate (HR, measured with a stethoscope), respiratory rate (RR, measured by visual assessment of the movement of the chest and abdomen), body temperature (measured with a digital rectal thermometer), arterial haemoglobin oxygen saturation (SpO_2), and systolic blood pressure (SAP), diastolic blood pressure (DAP) and mean arterial blood pressure (MAP) measured with an indirect blood pressure oscillometer and oximetry unit (CMS5000-VET, Contec Medical Systems). An inflatable pressure cuff corresponding to approximately 40% of the cuff width to circumference ratio was placed on a foreleg just distal to the elbow joint.

All tigers had their eyes covered with a towel to reduce visual stimulation. The parameters used to evaluate anaesthetic efficacy included injection time (the time when the dart or manual injection was performed intramuscularly), time to first signs of sedation (ataxia, light sedation, relaxed eyelids) and decubitus time (entirely lateral or sternal). Once the depth of immobilisation was deemed safe for staff to approach, the tiger was removed from the immobilisation area and transported to an examination table. An initial measurement of the depth of anaesthesia was made via response to manipulation and mandibular relaxation. Anaesthetic depth was assessed using standard veterinary anaesthesia scoring tables (stage I: light; stage II: moderate; stage III: deep—planes 1, 2 and 3; and stage IV: very deep).

Ten study tigers were endotracheally intubated to provide supplemental 100% oxygen (2–5 L/min), while six were fitted with a nasal cannula to supplement 100% oxygen (5–7 L/min). Five tigers received supplementation with isoflurane at 1–2.5% for prolonged maintenance of anaesthesia, and nine tigers received an intramuscular injection of atipamezole (0.015 mg/kg; Antisedan, Zoetis) at the end of the procedure. The tigers remained instrumented and monitored until a point when arousal became unsafe for staff members.

During the procedures, all the tigers received Hartmann solution intravenously, at a rate of 12 mL/kg/h, through an 18–22 gauge catheter placed in the external saphenous vein. Blood samples for complete blood count and serum biochemistry analysis were obtained from the medial saphenous vein.

Institutional directors and animal curators approved the procedural protocols, and considered that procedures met the humane care stipulations of their institutional guidelines.

Statistical analysis

Given the age and weight differences among the tigers, we divided them into two groups: light (<40 kg, $n = 4$) and heavy (>100 kg, $n = 12$). Tigers were ranked and numbered by weight, from lightest to heaviest. When a tiger was operated on and weighed more than once, the first number assigned was kept, and the corresponding weight rank was assigned. We assessed the effects of anaesthesia and weight on HR, RR, SpO_2, SAP and DAP using the Mann–Whitney rank test as implemented in R as the Wilcoxon test.[14] Two tigers were immobilised multiple times, violating the need for independence in parametric statistics. Given the small sample size, a normal distribution or homogeneity of variance of the weights and responses measured was not attained. Therefore, we used the non-parametric two-sample median test, accounting for ranks equal to the median rank when necessary.[15] This test compares rank sums of two groups using a chi-squared test, where the null hypothesis states that the medians of the two groups are not statistically different.[15] We used R 4.4.2 GUI 1.81 Big Sur ARM build (8462) (R Core Team 2021) to do most calculations, except for the ranks, which were done by hand.

RESULTS

HR and weight varied significantly different among the cohort of tigers examined, but no significant differences in RR, body temperature, SpO_2, SAP or DAP were found (Table 1).

The first sedative effects (ataxia, drooping eyelids, lowered head) were observed between 2 and 10 minutes (mean = 5.4 minutes) after injection. A profound immobilisation (recumbency in sternal or lateral position, not responsive to manipulation with

TABLE 1 Differences in weight and physiological variable values among the tigers included in the study

Subject number	Weight (kg)	Heart rate (beats/min)	Respiratory rate (breaths/min)	Temperature (°C)	Oxygen saturation level (%)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
1	18	98.8	45.8	38.3	95.0	NA	NA
2	25.4	93.7	18.4	37.8	96.9	149.1	86.2
3	28.6	93.2	15.3	37.9	97.2	157.7	92.9
4	30.5	95.2	26.5	38.0	96.4	153.4	89.6
5	106	73.3	15.7	37.5	91.6	187.0	137.9
5	110	77.2	8.4	38.4	94.9	156.1	96.1
5	113	86.0	21.7	37.9	94.3	161.8	101.6
5	120	85.0	20.2	37.9	94.4	174.5	109.8
6	107	76.4	21.1	37.3	84.3	228.2	145.0
7	112	71.6	9.0	38.5	99.0	202.4	129.2
8	130	66.9	20.2	37.7	98.0	249.8	193.3
9	145	83.4	20.2	37.9	94.7	182.0	118.2
10	150	93.7	18.4	37.8	96.9	149.1	86.2
11	160	77.5	17.0	38.8	96.0	194.0	141.8
12	180	79.7	17.4	38.0	94.7	NA	NA
12	180	80.5	18.0	38.0	94.6	NA	NA
12	180	63.6	27.6	39.4	97.5	129.0	85.3
13	230	74.6	22.3	36.9	94.2	199.0	136.6
14	250	90.3	14.5	37.5	96.0	NA	NA
15	260	83.0	10.0	37.5	95.5	170.0	112.9
16	260	86.0	11.7	41.4	79.3	159.0	93.4
Wilcoxen's W	0	66.5	475.0	39.0	50.5	6.5	5.5
p-Value	0.003	0.004	0.244	0.725	0.152	0.078	0.059

Abbreviation: NA, not applicable/or not obtained.

an object, loss of stimulation of the ear hair reflex or absence of movements) was observed between 4 and 15 minutes (mean = 10.2 minutes) after injection. There were no significant differences in time to first signs of sedation ($\chi^2 = 0.156$; $p = 0.93$) or time to deep sedation ($\chi^2 = 0.867$; $p = 0.65$) between lighter and heavier tigers. The total immobilisation time ranged from 43 to 155 minutes (mean = 75.6 minutes), and was dependent on the procedures being performed.

Early signs of recovery included ear movement and blinking, progressing to the ability to rise to sternal recumbency with progressive head raising. The total recovery time was highly variable, ranging from 3 to 313 minutes (mean = 57.9 minutes). Tigers receiving no atipamezole had a recovery time of 3–313 minutes (mean = 87.7 minutes), while those receiving atipamezole generally had shorter recoveries (mean = 25 minutes, range 4–73 minutes). Table 2 summarises the parameters measured for all tigers.

Raw data on the animals' physiological parameters are available in the Supporting Information. Younger tigers (patients 1–4) had the fastest inductions, with first signs observed between 2 and 4 minutes after injection, stage 3 anaesthetic depth achieved between 4 and 12 minutes after injection and recovery times using atipamezole ranging from 6 to 14 minutes. During their procedures, tigers 6, 9, 12, 13, 15 and 16 received oxygen supplementation through a nasal cannula at at rate of 5–7 L/min. All other tigers were intubated and supplemented with oxygen at a rate of 1.5–7 L/min.

Due to its chronic rhinitis, tiger 8 required isoflurane supplementation since nasal endoscopy and nasal lavage had to be performed several times. Tigers 6, 9 and 12 were neutered, with a local block of 2% lidocaine (Piscaina, PiSA Farmacéutica) administered in each testis. These procedures were well tolerated, and no signs of premature awakening were observed.

Tigers undergoing surgical or painful procedures received a dose of meloxicam (0.2 mg/kg IM; Melocaxyl, PiSA Agropecuaria) before completion of the procedure, and for three additional days after the procedure. Tiger 6 demonstrated elevated body temperature and suboptimal SpO_2 during the anaesthetic event. This tiger was agitated and reluctant to enter the compression cage. He paced vigorously before darting, which was likely a cause for the increased temperature, RR and decreased oxygen saturation early in the procedure. Once a more relaxed stage was attained, the depth and frequency of breathing and oxygen

TABLE 2 Individual description and physiological responses to immobilisation with tiletamine/zolazepam (1.5 mg/kg) plus xylazine (0.5 mg/kg) in tigers

Subject number	Sex	Subspecies	Age (years)	Events	Procedure	Isoflurane (yes/no)	Weight (kg)	Heart rate (beats/min)	Respiratory rate (breaths/min)	Temperature (°C)	Oxygen saturation level (%)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Mean arterial blood pressure (mmHg)
1	F	*Altaica*	0.4	1	p	N	25.4	93.67	18.44	37.8	96.9	149	86	107
2	M	*Altaica*	0.4	1	p	N	28.6	93.18	15.27	37.9	97.2	158	93	115
3	M	*Altaica*	0.4	1	p	N	30.5	84.40	18.00	38.4	96.4	191	127	149
4	F	*Tigris*	0.5	2	x	N	18.0	98.75	45.75	38.3	95.0	*	*	*
5	F	*Altaica*	3	1	p	Y	107.0	76.40	21.09	37.3	84.3	228	145	173
6	M	*Altaica*	4	1	p	N	260.0	86.00	11.67	41.4	79.3	159	93	115
7	M	*Altaica*	4	3	p	N	180.0	75.15	15.53	38.6	94.5	129	85	100
8	F	*Tigris*	4	4	n	Y	112.3	76.50	12.70	37.9	94.2	179	127	144
9	M	*Altaica*	5	1	u	N	260.0	83.00	10.00	37.5	95.5	170	113	132
10	F	*Tigris*	6	1	p	N	112.0	71.60	9.00	38.5	99.0	202	129	154
11	M	*Tigris*	7	1	p	Y	145.0	66.10	13.30	39.4	96.3	213	144	167
12	M	*Altaica*	8	1	u	N	250.0	90.25	14.50	37.5	96.0	*	*	*
13	F	*Altaica*	10	1	p	N	130.0	66.89	20.22	37.7	98.0	250	193	212
14	M	*tigris*(l)	11	2	e	Y	160.0	77.50	17.00	38.8	96.0	194	142	159
15	M	*tigris*(l)	13	1	P	N	150.0	93.67	18.44	37.8	96.9	149	86	107
16	M	*tigris*(l)	14	1	P	N	230.0	74.62	22.31	36.9	94.2	199	137	157

Note: Symbol '*' denotes 'not recorded'. Subspecies includes 'l'—leucistic. 'Events' are denoted as the number of immobilisations. 'Procedure' includes e: endodontic, n: nasal endoscopy, u: neutering, p: physical examination, x: x-rays.
Abbreviations: F, female; M, male.

saturation became normalised. This tiger recovered without incident. No tigers experienced intraoperative or postoperative complications.

DISCUSSION

The statistical differences in HR between individual tigers can be attributed to their size rather than anaesthetic effects. The large differences in weight between the lighter and heavier tigers are therefore a limitation of the study. Weight can also significantly impact drug metabolism due to the distribution of drugs in body fat, which may be a confounding factor in the analysis.

The recovery times were longer after each procedure on tigers that were immobilised repeatedly. However, overall, there were no reported complications with the use of TZ in these tigers. This and previously published studies do not seem to support the accepted dogma that the use of tiletamine and zolazepam is more harmful to tigers than other members of the *Panthera* genus.[16] Indeed, the use of TZ with other alpha 2 agonists, such as detomidine, provides an excellent induction with good anaesthesia depth and a decreased recovery time when administering atipamezole.[8] Compared with protocols using X, those using detomidine demonstrated similar induction times, first signs of sedation, depth of anesthesia, SpO_2 and MAP.[8] A recent report in which TZ plus dexmedetomidine was used with and without buprenorphine also appeared to be safe, with physiological values comparable to those observed in the present study.[17]

The TZX protocol (1.5 mg/kg + 0.5 mg/kg) has been used repeatedly in an Amur tiger cub with good efficacy and no adverse effects.[13] Similarly, the current study demonstrated that the TZX protocol was safe and effectively produced deep anasthesia in multiple individuals undergoing physical examination, blood draws, catheter placement and minor surgical procedures such as castration. No seizures were observed during the procedures, or during the postanaesthetic period. Furthermore, no adverse signs have been observed in the immobilised tigers in the three years that elapsed between the procedures being performed and the writing of this report.

This study adds to a growing body of scientific evidence suggesting that the use of TZ in tigers is not as risky as previously supposed.[2, 3] Single case reports and published protocols with smaller sample sizes than the present study ($n = 6$ and 9) have shown that TZ doses for tigers can be reduced if used in combination with an alpha 2 agonist. In these recently reported cases, adverse effects have been absent.[8, 13, 17, 18] Further studies with larger sample sizes and comparable weight, sex and age groups are recommended, as are studies of comparable groups using different alpha 2 agonists. However, finding institutions with large numbers of tigers and normally distributed populations is challenging.

In conclusion, the current study has demonstrated that the TZX protocol is a safe and suitable alternative to protocols that require ketamine when ketamine is scarce.

AUTHOR CONTRIBUTIONS

Creator of the anaesthetic combination tested, data collection, performed procedures and writer: Julio A. Mercado. *Data analyses, statistics and co-writer*: Horacio de la Cueva. *Assisted in the development of the anaesthetic protocol and reviewed the manuscript to improve the English*: Thomas G. Curro. *Data collection and performed procedures*: Alejandro Román. *Reviewer of manuscript and consultant of data collection*: Paulina Haro. All contributors have agreed to the place and order of their participation in the manuscript and have reviewed it.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest. No AI-assisted technologies were used to generate this manuscript.

FUNDING INFORMATION

The authors received no specific funding for this work.

DATA AVAILABILITY STATEMENT

The data that underlie this study are available in the Supporting Information.

ETHICS STATEMENT

The animal welfare committee of the Instituto de Investigaciones en Ciencias Veterinarias, Universidad Autonoma de Baja California approved this study (approval register number AWC-201-5-2019-2).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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