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Dexmedetomidine and ketamine simultaneous administration in tigers (*Panthera tigris*): pharmacokinetics and clinical effects

Federica Di Cesare,[1] Petra Cagnardi ,[2] Roberto Villa,[3] Vanessa Rabbogliatti,[2] Lorena Lucatello,[4] Francesca Capolongo,[4] Daniela Gioeni,[2] Michele Capasso,[5] William Magnone,[6] Giuliano Ravasio[2]

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[1]Scienze Veterinarie per la Salute, la Produzione Animale e la Sicurezza Alimentare, Università degli Studi di Milano, Milano, Lombardia, Italy
[2]Medicina veterinaria, Università degli Studi di Milano, Milano, Lombardia, Italy
[3]Scienze veterinarie per la salute, la produzione animale e la sicurezza alimentare, Università degli Studi di Milano, Milano, Lombardia, Italy
[4]Biomedicina Comparata ed Alimentazione, Università degli Studi di Padova, Legnaro (PD), Veneto, Italy
[5]Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli Federico II, Napoli, Campania, Italy
[6]Parco Natura Viva, Verona, Italy

Correspondence to
Professor Petra Cagnardi; petra.cagnardi@unimi.it

ABSTRACT

Background The study determines the pharmacokinetic profiles of dexmedetomidine (DEX), ketamine (KET) and its active metabolite, norketamine (NORKET), after simultaneous administration. Moreover, the study evaluates the sedative effects of this protocol, its influence on the main physiological variables and the occurrence of adverse effects.

Methods Eighteen captive tigers were initially administered with a mixture of DEX (10 µg/kg) and KET (2 mg/kg) by remote intramuscular injection. In case of individual and specific needs, the protocol was modified and tigers could receive general anaesthesia, propofol or additional doses of DEX and KET.

Results Based on the immobilisation protocol, nine animals were assigned to the standard protocol group and the other nine to the non-standard protocol group. Higher area under the first moment curve ($AUMC_{0\text{-last}}$) and longer mean residence time ($MRT_{0\text{-last}}$) ($P<0.05$) were observed in the non-standard protocol group for DEX, KET and NORKET, and higher area under the concentration-time curve from administration to the last measurable concentration ($AUC_{0\text{-last}}$) only for KET. The KET metabolisation rate was similar ($P=0.296$) between groups. No differences between groups were detected in terms of stages of sedation and recoveries. All physiological variables remained within normality ranges during the whole observation period. During the hospitalisation period, no severe adverse reactions and signs of resedation were observed.

Conclusion The simultaneous administration of 10 µg/kg of DEX and 2 mg/kg of KET can be considered an effective protocol for chemical immobilisation of captive tigers, along with dosage adjusments or when other drugs are needed.

INTRODUCTION

Remote delivery of injectable drugs for chemical immobilisation is frequently required in captive large felids to perform physical examination, biological samples collection, drug administration, diagnostics and minor surgical procedures. Since it is often difficult to assess animal health status before performing chemical restraints, it is necessary that the compounds used are safe and have predictable clinical effects.[1]

Different drug combinations are used to immobilise captive tigers (*Panthera tigris*), all of them include α2 agonists, which nowadays are considered essential components of anaesthetic protocols.[2–6] In this species, α2 agonists contribute to producing dose-dependent, reliable immobilisation.[5] In addition to the well-known sedative effects, α2 agonists are reported to induce cardiovascular changes, including bradycardia, ventricular arrhythmias, hypertension and hypotension, including in tigers.[2 3 7] Moreover, these agents can be completely antagonised in order to avoid possible prolonged recoveries and resedation, as far as to promptly eliminate α2 agonists' cardiovascular effects during life-threatening situations (ie, bradyarrhythmias, cardiocirculatory arrest).[8]

Dexmedetomidine (DEX) is the most potent and highly selective α2 adrenoceptor agonist with sympatholytic, sedative, amnestic and analgesic properties, and has been described as a useful and safe drug in many clinical applications including sedation in non-cooperative subjects.[8 9] DEX decreases sympathetic stimulation and attenuates noradrenaline release, lowering brain excitatory neurotransmitters, and shows neuroprotective properties due to its cerebrovascular and cerebral metabolic effects.[10–12]

Ketamine (KET) is a dissociative anaesthetic N-methyl-D-aspartate (NMDA) receptor antagonist frequently employed in veterinary medicine which blocks the binding of excitatory neurotransmitters, glutamate and glycine, at the NMDA receptor, preventing conduction of ions (Na^+, K^+ and Ca^{2+}).[13] In tigers, KET is reported to cause hypersalivation, muscle rigidity, ataxia and seizures.[14]

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In the literature, the synergistic sedative and anaesthetic effects of KET and DEX in combination mediated by the α2 adrenergic receptors are well recognised.[15] Moreover, DEX is reported to regulate the NMDA receptor activity in the spinal dorsal horn by inhibiting tyrosine phosphorylation of NMDA receptor 2B subunit, modulating KET's clinical effects.[16] According to zoo and wildlife medicine, the most recent and employed protocol for tiger immobilisation is the use of KET in combination with an α2 agonist, such as medetomidine or DEX.[5 17–19] However, despite an easily recognisable clinical synergistic effect, little is still reported about the drugs' pharmacokinetic interactions when used in combination after simultaneous administration.[20–23]

To the authors' knowledge, data concerning pharmacokinetic studies in tigers are lacking, with only two manuscripts published,[24 25] and no data are reported on DEX and KET pharmacokinetics and on the simultaneous use of DEX and KET for chemical restraint. Thus, the primary aims of the study were to determine in captive tigers the pharmacokinetic profiles of DEX, KET and its active metabolite, norketamine (NORKET), following simultaneous intramuscular administration, and to explore variations in DEX and KET disposition and KET metabolisation rate when different immobilisation protocols were adopted. Furthermore, the secondary aims were to evaluate the onset, duration and recovery of the sedative effects of simultaneous administration of DEX and KET, its influence on the main physiological variables and the possible occurrence of adverse effects, and when modifications in the chemical immobilisation protocol were necessary.

MATERIALS AND METHODS

Animals

Eighteen captive tigers (seven males and 11 females), aged 2–18 years old and weighing 81–154 kg (mean±sd weight of 127.4±20.1 kg), scheduled for periodic physical examination or diagnostic procedures at the Veterinary Teaching Hospital of the University of Milan (Lodi, Italy) were enrolled in the study after obtaining owners' written consent. Tigers were fasted for 24 hours before immobilisation but had free access to water up to two hours before the procedure. During this period, tigers were kept in mobile dedicated cages to facilitate weighing operations and remote drug administration.

Study design

All tigers were initially administered with a combination of DEX hydrochloride (HCl) (Dexdomitor 0.5 mg/ml; Vetoquinol Italia, Italy) at 10 µg/kg and KET hydrochloride (HCl) (Ketavet 100 mg/ml; MSD Animal Health Srl, Italy) at 2 mg/kg, given by remote intramuscular injection into the hindquarter muscles through two consecutive blowpipe darts. The total volume of DEX and KET was calculated for each animal and mixed together in a single syringe. Then, the volume was equally divided into

Figure 1 Attainment of sternal recumbency in one tiger.

the two darts and finally administered in rapid sequence to each tiger. Darts were always blown by the same expert anaesthesiologist. Treatments were carried out in the morning. Once the animals have reached a satisfactory level of sedation, first verified by attaining sternal (figure 1) and then lateral recumbency (figure 2) with or without ear twitch reflexes (assessed from outside the cage by a wooden stick gently rubbed inside the auricle), two 20 G, 32-mm long intravenous catheters (Surflo IV Catheter; Terumo Italia, Italy) were inserted into the distal cephalic veins in order to administer other anaesthetic drugs or to perform bloodwork and drug quantification (figure 3). For the pharmacokinetic study, blood samples were taken from the peripheral venous catheter, and sampling times were measured starting from the time of the first dart (expressed in minutes and reported in table 1).

In some cases, to attain the level of immobilisation needed to safely and correctly perform clinical

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Figure 2 Attainment of lateral recumbency in one tiger.

procedures, the original immobilisation protocol was varied as follows: (A) Tigers scheduled for diagnostic procedures requiring general anaesthesia were additionally administered with intravenous titrate-to-effect propofol (Proposure; Boehringer Ingelheim Animal Health, Italy) to achieve orotracheal intubation and maintained with isoflurane (Isoflo; Zoetis, Italy) in 100 per cent oxygen. These animals were also administered with an intramuscular injection of butorphanol (Dolorex; MSD Animal Health, Italy) at 0.1 mg/kg on intubation. (B) Tigers showing no signs of sedation after 15 minutes post darting (ie, permanence of standing position and full responsiveness to environmental stimuli) were readministered with 10 µg/kg of DEX and 2 mg/kg of KET by remote intramuscular injection. (C) Tigers attaining lateral recumbency but showing ear twitch reflex were administered with intravenous titrate-to-effect propofol in order to abolish the reflex and ensure deeper sedation.

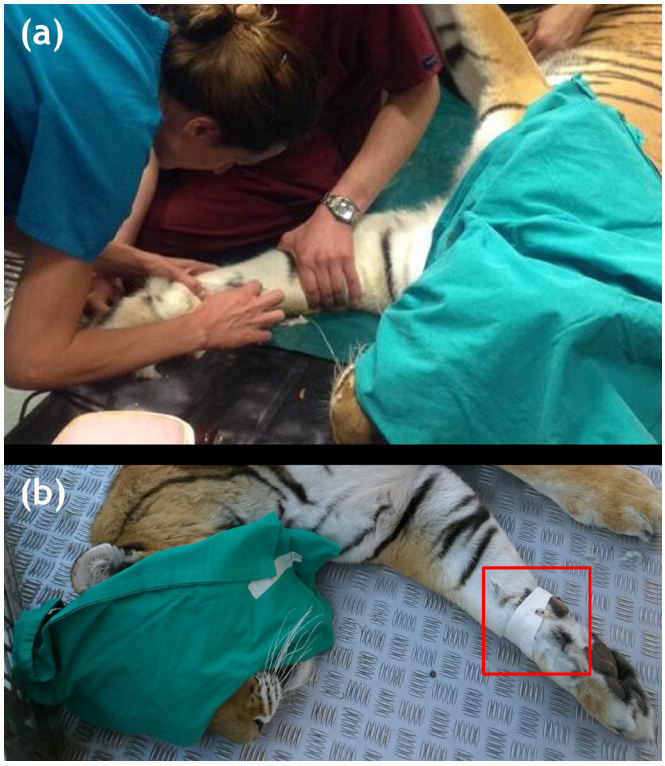

Figure 3 (a) Search and identification of the left distal cephalic vein in one tiger and (b) intravenous catheter positioned and secured in the right distal cephalic vein (red box).

Thus, based on immobilisation protocols, animals were assigned to the standard protocol (SP) group, receiving only the original remote intramuscular simultaneous administration of DEX at 10 µg/kg and KET at 2 mg/kg, or to the non-standard protocol (NSP) group, receiving the modified protocol of drug administration as previously described.

During the immobilisation period, blood samples were collected every 5–10 minutes and transferred into tubes containing clot activator or into heparinised tubes for DEX and KET quantification, respectively. Then, samples were centrifuged and serum and plasma stored at −80°C pending analyses of DEX, KET and NORKET, respectively.

At the end of the procedures, the effects were reversed with atipamezole (Antisedan 5 mg/ml; Vetoquinol Italia, Italy) administered to tigers at a dose of 50 µg/kg intramuscularly and 50 µg/kg subcutaneously, for a total dose of 100 µg/kg.

To evaluate the sedative effects, the times elapsed between the different clinical stages of sedation and recovery were determined. These times were (1) between drug administration and sternal recumbency, (2) between drug administration and lateral recumbency, (3) between lateral recumbency and atipamezole administration, and (4) between atipamezole administration and standing position. Moreover, physiological variables such as heart rate (HR), respiratory rate (RR), mean non-invasive blood pressure (mNIBP), peripheral oxygen saturation

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Table 1 Animal details, anaesthetic protocols and sampling times in 18 captive tigers and divided into the standard protocol group and the non-standard protocol group

Sex	Age (years)	Weight (kg)	Clinical procedure	KET dose (mg/kg)	DEX dose (µg/kg)	Other drugs (mg/kg)	Sampling time (minutes)
Standard protocol							
M	8	138	Routine examination	2	10	–	28, 33, 38, 43, 48
F	9	113	Routine examination	2	10	–	27, 32, 47, 52, 58, 64, 69, 73, 78, 83
F	8	122	Routine examination	2	10	–	20, 35, 39, 55, 58, 63, 69
M	4	120	Routine examination	2	10	–	21, 22, 26, 31, 36, 41, 46, 50, 53
F	5	119	Routine examination	2	10	–	24, 30, 35, 40, 45, 49
M	17	152	Routine examination	2	10	–	22, 27, 32, 37, 42, 47, 52, 57
F	17	112	Routine examination	2	10	–	20, 25, 31, 35, 40, 45, 50, 56
F	17	143	Routine examination	2	10	–	18, 23, 28, 36, 40, 46, 51, 56
F	16	146	Routine examination	2	10	–	20, 25, 30, 35, 40, 46, 51, 56
Non-standard protocol							
F	2	81	Echocardiography and laryngoscopy	2	10	BTF 0.1 PPF 0.5 ISO to effect	21, 24, 30, 36, 41, 46, 57, 72, 88, 107
M	9	149	Routine examination	4	20	–	43, 48, 50, 58, 63, 68, 73, 78, 83, 88, 94, 99
F	9	116	Routine examination	4	20	–	74, 79, 84, 89, 94, 99, 104
M	5	135	Routine examination	2	10	PPF 0.4	33, 38, 50, 65, 75, 80
M	5	154	Routine examination	2	10	PPF 0.6	22, 27, 32, 37, 42, 47
F	5	106	Routine examination	2	10	PPF 0.8	19, 24, 29, 34, 39, 44
M	17	152	CT	2	10	BTF 0.1 PPF 1 ISO to effect	20, 37, 42, 47, 57, 66, 72, 77, 82, 87, 98
F	3	127	Routine examination	4	20	–	25, 30, 35, 45, 50, 55, 60
F	18	109	CT	2	10	BTF 0.1 PPF 0.5 ISO to effect	24, 32, 41, 50, 60, 72, 82, 92

BTF, butorphanol; DEX, dexmedetomidine; F, female; ISO, isoflurane; ; KET, ketamine; M, male; PPF, propofol.

(SpO_2) and rectal temperature (RT) were recorded every 10 minutes using a multiparameter monitor (Vista 120 S; Draeger Medical Italia SpA, Italy), from intravenous catheter insertion to atipamezole administration. In tigers intubated and maintained under general anaesthesia for diagnostic purposes, SpO_2 was measured with 60 per cent fraction of inspired O2 (FiO_2), while for all other tigers SpO_2 was measured under ambient air conditions (FiO_2=20 per cent).

Finally, during the hospitalisation period at the Veterinary Teaching Hospital, all tigers were accurately observed for potential incidence of slight (eg, nausea and emesis) and severe adverse effects, with particular attention to prolonged recoveries (>60 minutes), neurological disorders (eg, seizures, coma) or cardiocirculatory disorders (eg, severe hypotension or severe bradycardia/bradyarrhythmias).

DEX, KET and NORKET analysis and method validation

For drug quantification, DEX was extracted from tiger serum and analysed according to a validated Liquid Chromatography- Mass Spectrometry (LC-MS/MS) method,[26] while KET and NORKET were extracted from tiger plasma and analysed according to a validated High Performance Liquid Chromatography-Ultraviolet (HPLC-UV) method.[27] Both methods were employed with slight modifications and were subject to intralaboratory validation in compliance with the recommendations defined by the European Community[28] and with international guidelines.[29] Validation data for DEX, KET and NORKET are reported in table 2. Since no blank tiger serum or plasma (without sedatives or anaesthetics) was available, the calibration curves were prepared in cat blank serum or plasma with six spiked solutions obtained by diluting the original stock solution of DEX HCl, KET

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Table 2 Intralaboratory validation of analytical methods for DEX, KET and NORKET in cat serum or plasma samples, respectively

Parameter (units)	DEX	KET	NORKET
LOQ (ng/ml or µg/ml)	0.025	0.01	0.01
LOD (ng/ml or µg/ml)	0.005	0.00027	0.00031
Trueness (%)	95.6–104.7	93.4–102.1	91.8–100.7
Intraday repeatability (CV%)	4.0–6.6	6.9–12.8	7.1–14.2
Recovery (%)	87±8	84±6	84±8

LOQ and LOD are expressed as ng/ml (DEX) and µg/ml (KET and NORKET).
Trueness and intraday repeatability reported as range values.
Recovery reported as mean±sd.
CV%, coefficient of variation; DEX, dexmedetomidine; KET, ketamine; LOD, limit of detection; LOQ, limit of quantification; NORKET, norketamine.

HCl or NORKET HCl to achieve concentrations ranging from 0.025 to 10 ng/ml for DEX and from 0.01 to 100 µg/ml for KET and NORKET. DEX (>99 per cent pure) was purchased from Tocris (Milan, Italy), and tolazoline (>99 per cent pure) was purchased from Sigma-Aldrich (Milan, Italy) and used as internal standard for DEX quantification. KET (>99 per cent pure) and NORKET (>99 per cent pure) were purchased from LGC Standards Srl (Milan, Italy). All salts and solvents were of LC-MS grade (Sigma-Aldrich, Milan, Italy; or Carlo Erba Reagenti, Milan, Italy). There was a linear relationship (r^2 >0.98) between the concentrations of the drugs and the area of the peak over the investigated range. The intraday repeatability was measured as a coefficient of variation (per cent) from six replicates of three concentrations, whereas trueness (per cent) was measured as the closeness to the concentration added on the same replicates. The results fell within the accepted ranges for precision and trueness (table 2). For DEX, a limit of quantification (LOQ) value of 0.025 ng/ml and a limit of detection (LOD) value of 0.005 ng/ml were observed. For KET, LOQ and LOD values were 0.01 µg/ml and 0.00027 µg/ml, respectively. For NORKET, LOQ and LOD values were 0.01 µg/ml and 0.00031 µg/ml, respectively. The specificity of the methods was demonstrated by the absence of interference in 20 blank cat serum or plasma samples at the DEX, KET and NORKET retention times.

Pharmacokinetic analysis

Pharmacokinetic parameters were determined from serum/plasma concentration–time data using the Phoenix WinNonLin V.8.0 software (Pharsight Corporation, USA), which allows compartmental and non-compartmental analyses of the experimental data. Visual inspection of the curve, residual analysis and minimum Akaike's information criterion estimates[30] were done to choose the model best fitting the data. All data points were weighted by the inverse square of the fitted value. The dispositions of DEX, KET and NORKET following remote intramuscular administration in tigers were described by standard non-compartmental analysis. The elimination half-life ($t_{1/2\lambda z}$) was calculated as $\ln 2/\lambda z$. The area under the concentration-time curve from administration to the last measurable concentration ($AUC_{0\text{-last}}$) and the area under the first moment curve ($AUMC_{0\text{-last}}$) were calculated using the trapezoidal method. The mean residence time ($MRT_{0\text{-last}}$) was determined using the following equation[31]:

$$MRT_{0\text{-last}}=AUMC_{0\text{-last}}/AUC_{0\text{-last}}.$$

The peak concentrations, C_{max}, and the time to peak, T_{max}, were obtained by visual inspection from the experimentally observed data. Pharmacokinetic parameters were reported as mean and sd.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics V.26.0 (SPSS, Chicago, USA). The normality of data distribution was assessed by a Shapiro-Wilk test at the α=0.05 level. Mean values of the principal kinetic parameters obtained after DEX, KET and NORKET analyses, together with the KET metabolisation rate (expressed as the ratio between NORKET and KET AUCs), and mean values of the different times elapsed for the clinical stages of sedation and recovery were compared between groups using unpaired *t* test and Mann-Whitney U test for normal and non-normal data, respectively.

For statistical analysis of the physiological variables (HR, RR, mNIBP, SpO_2 and RT), data from the one-hour monitoring (60 minutes) during chemical immobilisation were used. In particular, this monitoring period was represented by an evaluation of seven time points, that is, T0, T10, T20, T30, T40, T50 and T60. A general linear model for repeated measures was applied to these data in order to compare differences between groups related to the chemical immobilisation protocol. Furthermore, the same statistical approach with Bonferroni's post-hoc adjustment for pairwise comparisons was used to assess time-related differences within each group. Differences with P<0.05 were considered significant.

RESULTS

All tigers successfully completed the study without any type of complications and with no risks to the veterinarians and other operators.

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Table 3 Mean±sd of non-compartmental parameters for DEX, KET and NORKET in 18 captive tigers following intramuscular administration of DEX and KET in the SP and the NSP group

Pharmacokinetic parameters	Unit	SP	NSP
DEX			
$t_{1/2\lambda z}$	Minutes	45.72±25.36	43.46±20.82
T_{max}	Minutes	24.00±4.97	33.22±17.53
C_{max}	ng/ml	6.68±2.11	5.66±1.65
$AUC_{0\text{-last}}$	Minutes*ng/ml	229.77±74.81	275.94±109.64
$AUMC_{0\text{-last}}$	Minutes*minutes*ng/ml	7610.31±2354.98*	13671.91±7444.86*
$MRT_{0\text{-last}}$	Minutes	33.37±3.14*	46.61±16.28*
KET			
$t_{1/2\lambda z}$	Minutes	91.12±57.75	54.00±25.76
T_{max}	Minutes	30.00±5.68	45.00±29.61
C_{max}	µg/ml	0.65±0.17	0.67±0.18
$AUC_{0\text{-last}}$	Minutes*µg/ml	24.92±5.81*	36.24±14.24*
$AUMC_{0\text{-last}}$	Minutes*minutes*µg/ml	889.46±278.08*	1971.47±1108.28*
$MRT_{0\text{-last}}$	Minutes	35.36±3.84*	49.92±17.01*
NORKET			
T_{max}	Minutes	54.67±6.71	72.33±24.29
C_{max}	µg/ml	0.23±0.08	0.26±0.07
$AUC_{0\text{-last}}$	Minutes*µg/ml	7.12±4.15	11.81±5.20
$AUMC_{0\text{-last}}$	Minutes*minutes*µg/ml	295.76±225.92*	722.32±445.38*
$MRT_{0\text{-last}}$	Minutes	39.13±5.46*	54.89±17.53*

*P<0.05.
$AUC_{0\text{-last}}$, AUC from 0 to the last concentration; $AUMC_{0\text{-last}}$, area under the first moment curve from 0 to the last concentration; C_{max}, maximum concentration; DEX, dexmedetomidine; KET, ketamine; $MRT_{0\text{-last}}$, mean residence time from 0 to the last concentration; NSP, non-standard protocol; SP, standard protocol; T_{max}, time to maximum concentration; $t_{1/2\lambda z}$, elimination half-life.

From the global sample of tigers (N=18), nine subjects were chemically immobilised with an intramuscular simultaneous administration of DEX (10 µg/kg) and KET (2 mg/kg) and were consequently assigned to the SP group (n=9). To the NSP group were assigned (1) three tigers (tigers 1, 7 and 9) that required general anaesthesia for diagnostic procedures and consequently received intravenous propofol (0.5, 1 and 0.5 mg/kg, respectively), isoflurane and butorphanol, as described for protocol A; (2) three tigers (tigers 2, 3 and 8) that received a second remote intramuscular administration of DEX (10 µg/kg) and KET (2 mg/kg) since they were showing no signs of sedation within 15 minutes from the first dart, as described for protocol B; and (3) three tigers (tigers 4, 5 and 6) that were administered intravenous propofol (0.4, 0.6 and 0.8 mg/kg, respectively) to ensure deeper sedation since they were showing ear twitch reflex, as described for protocol C (table 1).

During this study, sample collection for drug and metabolite quantification was limited to the period of the animals' safe manipulation, which lasted variably among tigers in a range of 18–83 and 19–107 minutes for the SP and the NSP group, respectively (table 1)

The results of pharmacokinetic parameters for DEX, KET and NORKET, respectively, in the serum and plasma of the 18 tigers are reported in table 3. The ratio between NORKET and KET $AUC_{0\text{-last}}$ (ie, the KET metabolisation rate) was 0.28±0.11 and 0.32±0.05 for the SP and the NSP group, respectively, with no significant difference between protocols (P=0.296).

Concerning the clinical outcome, all administrations performed by remote intramuscular drug delivery were uneventful and the level of immobilisation obtained was satisfactory and sufficient to complete the respective procedures. For each tiger, times elapsed in attaining the different clinical stages of sedation and recovery are reported in table 4. No statistically significant differences between groups were detected in any of the stages of sedation nor recovery.

All the monitored physiological variables (HR, RR, mNIBP, SpO_2 and RT) remained within normality ranges for the species throughout the whole period when tigers were safely approachable.

In particular, the mean values recorded for HR were 79±7 beats per minute (min 59; max 107) and 83±7 beats per minute (min 60; max 110) for the SP and the NSP group, respectively. The mean values for RR were 16±4 breaths per minute (min 9; max 32) and 19±5 breaths per minute (min 8; max 33), while the mean values for mNIBP were 97±10 mmHg (min 79; max 117) and

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Table 4 Mean and sd of time (minutes) elapsed between administration (admin) and different clinical stages of sedation and recovery in 18 captive tigers following intramuscular administration of dexmedetomidine and ketamine in the standard protocol and in the non-standard protocol group

Tiger	Admin to sternal recumbency	Admin to lateral recumbency	Admin to reversal	Lateral recumbency to reversal	Reversal to standing position
Standard protocol					
1	10	20	52	30	31
2	7	10	88	78	14
3	4	7	92	85	18
4	10	14	54	40	50
5	4	6	50	44	27
6	2	7	59	52	50
7	4	9	58	49	17
8	4	9	59	50	29
9	8	9	62	53	12
Mean	6	10	64	53	28
sd	3	4	14	17	14
Non-standard protocol					
1	6	9	123	114	10
2	31	37	103	66	17
3	61	63	109	46	32
4	14	27	85	58	26
5	12	16	53	37	11
6	3	8	51	43	35
7	8	9	103	94	15
8	16	18	65	49	10
9	9	10	101	91	31
Mean	18	22	88	66	19
sd	17	17	25	25	9

*P<0.05.

95±11 mmHg (min 68; max 121), for the SP and the NSP group, respectively. Regarding SpO_2, a mean value of 93±2 per cent (min 87; max 99) was registered in the SP group, while it was 92±2 per cent (min 84; max 99) in the NSP group. Finally, the mean values recorded for RT were 38.3°C±0.4°C (min 36.9; max 38.8) and 38.2°C±0.4°C (min 36.1; max 39.2) for the SP and the NSP group, respectively.

Concerning the influence of the different chemical immobilisation protocols between groups, no statistically significant differences were detected in any of the physiological variables evaluated (HR, P=0.651; RR, P=0.494; mNIBP, P=0.409; SpO_2, P=0.791; RT, P=0.435).

Statistical results from pairwise comparisons between the different time point evaluations (T0, T10, T20, T30, T40, T50 and T60) were obtained for all the physiological variables evaluated.

In particular, for HR all the comparisons between time points were statistically significant (P<0.05), with the only exception being T10 versus T20 (P=0.553). RR differed significantly among time point evaluations, with the exception of T10 versus T20 (P=0.161), T20 versus T30 (P=0.220), T30 versus T40 (P=0.067), and T40 versus T50 (P=0.179). Pairwise comparison for mNIBP differed significantly between all time points evaluated (P<0.05). For SpO_2 time point comparison, statistically significant differences were detected (P<0.05), with the exception of T0 versus T10 (P=1) and versus T30 (P=0.114), of T10 versus T20 (P=0.377) and versus T30 (P=0.071), of T20 versus T30 (P=1) and versus T40 (P=0.383), of T30 versus T40 (P=1), and of T40 versus T50 (P=1). Regarding RT, all the comparisons between time points were statistically significant (P<0.05), with the only exception being T0 versus T10 (P=0.077). The mean values of HR, RR, mNIBP, SpO_2 and RT over time for both the SP and the NSP group are reported in figure 4.

During the hospitalisation period, no severe adverse reactions (ie, seizures, dysphoria, hyperthermia, respiratory depression, hypotension, bradycardia, bradyarrhythmias) were recorded. One tiger (tiger 4) in the SP group showed slight signs of nausea (ie, ptyalism and lip licking) a few minutes after drug administration, while

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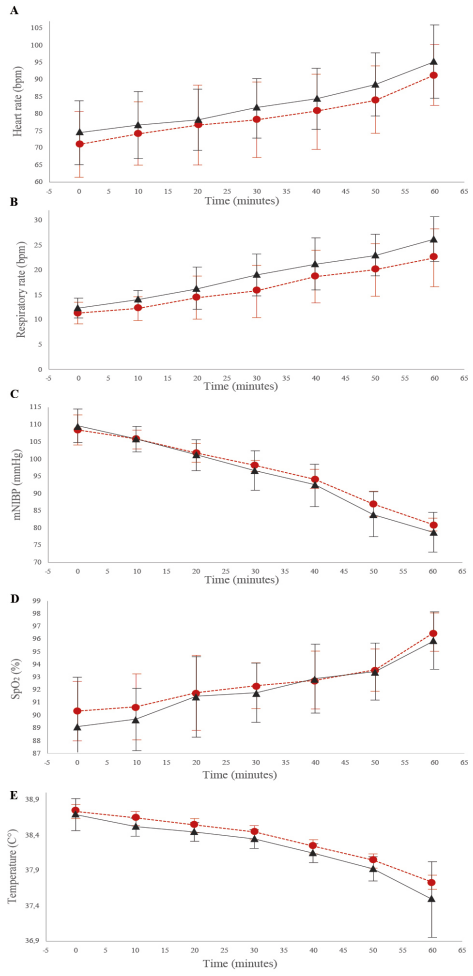

Figure 4 Mean±sd values over time for (a) heart rate (bpm, beats per minute), (b) respiratory rate (bpm, breaths per minute), (c) mean non-invasive blood pressure (mNIBP), (d) peripheral oxygen saturation (SpO_2) and (e) rectal temperature of captive tigers following remote intramuscular simultaneous administration of dexmedetomidine and ketamine in the standard protocol group (red circle; n=9) and in the non-standard protocol group (black triangle; n=9).

two tigers (tigers 1 and 9) in the NSP group vomited before attaining lateral recumbency. Throughout the time the animals were immobilised, no signs of eventual sudden arousal (eg, spontaneous eyelid movement, nystagmus and pedalling) were observed in either the SP or the NSP group.

All tigers recovered uneventfully and were able to stand and walk with no ataxia or hyperkinesia inside their enclosures within one hour after atipamezole administration and without any signs of resedation at six hours of observation. In the SP group, the mean time elapsed between reversal with atipamezole and attaining a standing position was longer than in the NSP group (28±14 minutes and 19±9 minutes, respectively), but two tigers in the SP group were awake and conscious in sternal recumbency for 20 minutes before attaining a standing position. Finally, the animals from both the SP and the NSP group resumed full normal activities, including feeding behaviours, within six hours after recovery.

DISCUSSION

To the authors' knowledge, this is the first study where DEX and KET are used in combination for simultaneous intramuscular administration in *P tigris*. Moreover, this is the first study that evaluates the pharmacokinetics of DEX, KET and NORKET in captive tigers and the clinical effects of this immobilisation protocol.

Chemical immobilisation is a paramount tool in zoo and wildlife medicine since it allows potentially harmful animals, such as tigers, to be handled safely when medical or management procedures are required; however, the employed drugs should have well-known pharmacokinetic properties and be safe and with predictable clinical effects.[1 7]

In the present study, chemical immobilisation of tigers was performed by an intramuscular remote injection of DEX at 10 µg/kg and KET at 2 mg/kg. DEX was selected for combined administration with KET due to its sedative, amnestic and analgesic properties. It was hypothesised, in fact, that DEX–KET mixture would allow reduction in the dose of inductor agent (KET) required to achieve adequate immobilisation, reducing the incidence of severe adverse reactions, according to what has been affirmed by other authors.[4 18] Other studies reported the use of α2 agonists such as xylazine[3 4 32] or medetomidine[2 17 19] combined with KET for chemical restraint of captive tigers; nevertheless, in this study DEX was selected due to its synergistic sedative and anaesthetic effects expressed after simultaneous administration with KET.[15] Moreover, due to their well-recognised synergism of action, it was decided to lower the doses of DEX and KET, as reported in another study where DEX and KET were used in combination for chemical restraint of tigers, but not simultaneously.[5]

In zoo medicine, assuming that the main goal with tigers and other potential harmful animals is to reach a satisfying level of sedation or anaesthesia to safely perform the required clinical procedures, it is not uncommon to use chemical immobilisation protocols adapted to a single animal.[14] For this reason, the study was designed assigning each tiger to the SP or the NSP group according to the chemical immobilisation protocol used.

Specifically, all enrolled subjects that showed signs of sedation within 15 minutes from remote intramuscular administration of the original protocol (DEX 10 µg/kg and KET 2 mg/kg), that did not require general anaesthesia and that once had attained lateral recumbency did not show any sign of arousal were assigned to the SP group. In all other cases, tigers were assigned to the NSP group. In particular, animals requiring general anaesthesia were administered intramuscular butorphanol for pain relief during diagnostic procedures. In tigers requiring a second remote intramuscular administration, likely because the first administration was not successful as drugs were not correctly injected by the blow dart, an equal dose of DEX (10 µg/kg) and KET (2 mg/kg) was used. In tigers successfully attaining lateral recumbency but with signs of slight sedation, it was possible to insert

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an intravenous catheter and thus propofol was administered to ensure a deeper level of sedation, to safely complete the clinical procedures.

Pharmacokinetic analysis was conducted with DEX, KET and NORKET concentration data collected during a very short sampling time period (maximum of 107 minutes). Nonetheless, this situation is similar to the only other study concerning pharmacokinetics of anaesthetic drugs in tigers.[24] In fact, due to the harmful behaviours of these animals, it is considered normal in this species to perform blood sampling only when they are chemically immobilised.

Significant differences (P<0.05) between groups in $AUMC_{0\text{-last}}$ and $MRT_{0\text{-last}}$ were observed for DEX. Surprisingly, T_{max} (24.00±4.97 minutes in the SP group versus 33.22±17.53 minutes in the NSP group), C_{max} (6.68±2.11 ng/ml in the SP group versus 5.66±1.65 ng/ml in the NSP group), $t_{1/2\lambda z}$ (45.72±25.36 minutes in the SP group versus 43.46±20.82 minutes in the NSP group) and $AUC_{0\text{-last}}$ (229.77±74.81 minutes*ng/ml in the SP group versus 275.94±109.64 minutes*ng/ml in the NSP group) did not show differences between groups, although especially for $AUC_{0\text{-last}}$ the sd showed a high interindividual variability, which could have hindered the detection of significant differences.

KET showed a statistically significant difference between groups with regard to $AUC_{0\text{-last}}$ (24.92±5.81 minutes*µg/ml in the SP group versus 36.24±14.24 minutes*µg/ml in the NSP group), $AUMC_{0\text{-last}}$ (889.46±278.08 minutes*minutes*µg/ml in the SP group versus 1971.47±1108.28 minutes*minutes*µg/ml in the NSP group) and $MRT_{0\text{-last}}$ (35.36±3.84 minutes in the SP group versus 49.92±17.01 minutes in the NSP group). This result was expected; however, as for DEX, observing the high sd for mean T_{max} in the NSP group (30.00±5.68 minutes in the SP group and 45.00±29.61 minutes in the NSP group), it was not possible to exclude that the wide data distribution hindered the possibility of detecting the difference once again. Moreover, it is possible that with the simultaneous administration of the two drugs, DEX might have influenced the T_{max} values of KET, as also reported by other authors,[21–23] in particular due to the peripheral vasoconstriction and consequent delayed drug absorption produced by DEX's interaction with the precapillary sphincter α2B receptors of the peripheral vascular beds.[20] However, a study performed by other authors highlighted longer (not significant) T_{max} values in cats after intramuscular administration of KET plus xylazine versus KET alone; however, in that study, the α2 agonist was administered 15 minutes before KET, and not simultaneously.[33] In the present clinical study, it was not possible to better explore DEX and KET interactions by adding a control group of tigers administered with either DEX or KET alone, since tigers were chemically restrained for medical reasons. Moreover, the use of KET alone is strongly contraindicated in this species due to serious side effects, such as onset of seizures.[14 34] On the other hand, the use of DEX alone has also been considered unsafe due to the possible occurrence of sudden arousals from sedation (*sedation rupture*).

NORKET concentrations increased throughout the observation period, as metabolite production lasted longer than the rather short sampling period. In both groups, the main pharmacokinetic parameters, that is, T_{max}, C_{max}, $AUC_{0\text{-last}}$, $AUMC_{0\text{-last}}$ and $MRT_{0\text{-last}}$, were successfully estimated by the software, with the exception of elimination half-life ($t_{1/2\lambda z}$), due to the lack in sampling during the true elimination phase of the drugs. In this study, the ratio between NORKET and KET $AUC_{0\text{-last}}$ (ie, KET metabolisation rate) showed no significant difference between groups, suggesting that all animals were able to metabolise KET at the same rate regardless of the chemical immobilisation protocol. Despite the small sample size, this information is particularly interesting since tigers in the NSP group were chemically immobilised with different variations (eg, with doubled DEX and KET doses, additional administration of butorphanol, propofol and isoflurane) from the original simultaneous DEX–KET combination (10 µg/kg and 2 mg/kg, respectively). In addition, some animals (tigers 1, 7 and 9) in this group underwent general anaesthesia, which modified their cardiovascular function,[35] nonetheless leaving KET metabolisation rate unchanged.

Regarding the times elapsed in attaining different clinical stages of sedation and recovery, the statistical evaluation between the SP and the NSP group showed no significant differences.

Considering all the animals enrolled, three out of 18 tigers (17 per cent) in the NSP group did not achieve any sign of sedation after the first administration; nevertheless, they achieved complete immobilisation with the second DEX–KET administration. In these animals, lateral recumbency was attained 39±23 minutes after the second darting. It has been hypothesised that, in these animals, the second DEX–KET administration was necessary probably because the first dose was not successfully injected. Finally, 15 out of 18 tigers (83 per cent) were effectively immobilised with the DEX–KET combination at the first attempt of administration, since the placement of venous catheters could only be achieved with successful immobilisation. Lateral recumbency was attained in 11±6 minutes, a time consistent with that reported by other authors for medetomidine and KET combination (8.7±2.9 minutes).[2] Thus, these findings suggest that, when properly administered, the DEX–KET combination would allow successful immobilisations.

The time elapsed between lateral recumbency attainment and atipamezole administration, indicating the safe time for animal handling by the medical staff, lasted for approximately one hour and was comparable in the two groups (53±17 minutes and 66±25 minutes in the SP and the NSP group, respectively), resulting in a stable and effective immobilisation.

The time elapsed between atipamezole administration and attainment of standing position was longer in the SP group compared with the NSP group (28±14 minutes and

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19±9 minutes, respectively). Actually, the mean value in the SP group could be altered, since two tigers in this group (tigers 4 and 6) were awake and conscious in sternal recumbency for 20 minutes before attaining a standing position. However, as soon as they were stimulated, these animals got up and walked within their enclosures without signs of ataxia.

The reversal drug was injected half intramuscularly and half subcutaneously, different from what has been reported in tigers by Miller and others,[2] who administered the whole atipamezole dose intramuscularly. This decision was taken to avoid episodes of sudden arousal or excitement during the recovery phase, so as to prevent resedation in the six-hour follow-up during recovery time.[36]

Concerning physiological variables, the study showed no significant differences between the SP and the NSP group. This finding seems to confirm the non-influence of the concurrent drugs (ie, propofol, butorphanol and isoflurane) of the immobilisation protocol administered to the NSP group, and this is also supported by the disposition of DEX and KET, as well as the metabolisation rate of KET, which did not differ between groups. Therefore, it is possible to affirm that DEX–KET simultaneous administration seems not to influence the physiological variables considered (HR, RR, mNIBP, SpO_2 and RT), which remained within physiological ranges for the species.[3 5]

On the other hand, in both the SP and the NSP group, the influence of time on all physiological variables was determined. In particular, tigers presented a gradual increase in HR values from the start of chemical immobilisation to the end of the monitoring period. This was probably because DEX vago-mediated bradycardia, remarkable at the start of the procedure[37] and progressively attenuating. Similarly, RR showed a gradual increase over time for both groups, due to both DEX and KET metabolism or excretion over time resulting in concomitant lightening of sedation.[2] As regards mNIBP, there has been a gradual decrease over time. This was attributed to the initial α2B-mediated effect of DEX, which gradually fades from administration time to the end of monitoring.[37 38] Among all the physiological parameters in both the SP and the NSP group, SpO_2 showed the greatest variability over time. This observation is in agreement with other studies that indicate SpO_2 monitoring as a variable and consequently unreliable parameter.[39 40] Finally, RT presented a gradual decrease over time in both groups, explained by the normal lowering of this parameter during sedation and/or general anaesthesia.[38]

During the entire hospitalisation period, none of the tigers experienced any severe adverse reaction (ie, seizures, dysphoria, respiratory depression, arrhythmias). Only two animals in the NSP group showed slight side effects (ie, nausea and emesis), commonly related to both DEX and KET administration.[8] Conversely, Clark-Price and others,[5] in the only other study that performed chemical restraint in tigers using a combination of DEX and KET, reported many episodes of dysphoria and seizures. The most likely explanation is that in the study of Clark-Price and others[5] DEX and KET were not administered simultaneously. Specifically, KET was administered 15 minutes after DEX, and with such elapsed time in administration DEX is likely to have failed in modulating KET's clinical effects.[16] In fact, it could be possible that DEX was not completely able to exert its known action in lowering brain excitatory neurotransmitters and its neuroprotective properties.[10–12]

The study here reported had some limitations, mainly due to the execution during the clinical practice that has restricted the possibility to randomise the study design and thus have more homogenous groups of animals, that is, previously selected according to specific needs, as type of clinical procedures or diseases. A larger sample size might have helped in this case. Furthermore, as mentioned, due to either the clinical situation and the harmful behaviours of the species, the blood sampling period was time-restricted and considered too short to explore the real excretive profile of DEX, KET and NORKET. More accurate determinations could only be achieved with longer sampling time during the post-dosing period, which is difficult in awake large felids. Finally, since in the NSP group some animals underwent general anaesthesia for medical reasons or were administered with other drugs, the variability in the protocol used in each animal could have contributed to the increased variability in the groups, which may have hindered the determination of the significance of the pharmacokinetic parameters and the investigation of the influences of DEX and KET disposition.

CONCLUSIONS

Despite the short period of blood sampling, for a complete pharmacokinetic evaluation, a favourable kinetic profile of DEX, KET and NORKET in tigers was observed. Moreover, the additional administration of other drugs seems not to affect either the disposition of DEX and KET nor the KET metabolisation rate in this species. When properly administered, all animals achieved satisfactory immobilisation for all clinical procedures, with predictable influence on physiological variables, smooth sedation and good recovery, and with complete absence of life-threatening adverse reactions. Given the positive results with the simultaneous administration of 10 µg/kg of DEX and 2 mg/kg of KET, the authors suggest its application in chemical immobilisation of captive tigers, along with necessary modifications, such as dosage adjustments or administration of other drugs, based on animals' specific needs or clinical procedure requirements.

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Contributors FDC, VR, MC, WM, DG an GR were involved in the study design and sample collection. FDC and RV performed the method validation and sample

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quantification for ketamine and norketamine. LL and FC performed the method validation and sample quantification for dexmedetomidine. FDC and PC performed the data analysis and interpretation and drafted the manuscript. RV, GR and PC contributed to study design and supervised the whole work. All authors have read and approved the final version of the manuscript.

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Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information.

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ORCID iD

Petra Cagnardi http://orcid.org/0000-0001-6232-4850

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