Comparative study between S-(+)-ketamine–midazolam and fentanyl–droperidol in black-tufted marmosets (*Callithrix penicillata*)

Estudo comparativo entre S-(+)cetamina-midazolam e fentanil-droperidol em saguis-de-tufo-preto (Callithrix penicillata)

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ABSTRACT

Sedative and antinociceptive effects of two anesthetic protocols in black-tufted marmosets were compared in this study. Twenty-six marmosets underwent chemical immobilization for physical examination, blood sampling, tattooing, and microchipping. Animals were randomly treated with S-(+)-ketamine (10 mg/kg) and midazolam (1 mg/kg) (KM) or fentanyl (12.5 µg/kg) and droperidol (625 µg/kg) (FD) given by intramuscular injection. Heart and respiratory rates were recorded. Sedation, antinociception, muscle relaxation, posture, auditory, and visual responses were evaluated using a scoring system. Sedation in KM was achieved faster ($p < 0.001$) and lasted for a shorter period of time ($p = 0.0009$). KM was similar to FD in its cardiorespiratory effects, auditory and visual responses. Both protocols promoted adequate sedation to allow manipulation. Animals in KM assumed lateral recumbency while animals in FD maintained a quadrupedal posture during evaluation. FD produced less intense sedation and muscle relaxation but a higher degree of antinociception compared to KM and is suitable for procedures that require analgesia in black-tufted marmosets.

Keywords: Anesthesia. Antinociception. Opioid. Primate. Sedation.

RESUMO

O presente estudo comparou os efeitos cardiorrespiratórios, sedativos e antinociceptivos de dois protocolos anestésicos em saguis-de-tufo-preto (*Callithrix penicillata*). Vinte e seis saguis foram submetidos à contenção química para exame físico, coleta de sangue, tatuagem de identificação e microchip. Os animais foram tratados aleatoriamente com a associação de S-(+)-cetamina (10 mg/kg) e midazolam (1 mg/kg) (KM) ou fentanil (12,5 µg/kg) e droperidol (625 µg/kg) (FD), administrados por injeção intramuscular. Foram avaliadas frequência cardíaca, frequência respiratória, sedação, antinocicepção, relaxamento muscular, postura e resposta ao estímulo auditivo e visual. A sedação em KM foi alcançada mais rapidamente ($p < 0,001$) e teve um tempo hábil mais curto ($p = 0,0009$). KM foi semelhante a FD nos efeitos cardiorrespiratórios, respostas auditivas e visuais. Os dois protocolos promoveram sedação adequada para manipulação. Os animais do grupo KM permaneceram em decúbito lateral durante a avaliação, enquanto os animais em FD mantiveram postura quadrupedal. FD resultou em sedação e relaxamento muscular de menor intensidade, porém com maior escore de antinocicepção em comparação com KM, sendo adequada para procedimentos que requerem analgesia em saguis-de-tufo-preto.

Palavras-chave: Anestesia. Antinocicepção. Opioide. Primata. Sedação.

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Introduction

The black-tufted marmoset (*Callithrix penicillata* É. Geoffroy, 1812) is a small neotropical primate naturally found in the Brazilian savanna and Caatinga (Vale et al., 2020). The species can reach 20 cm and weigh between 0.3-0.5 kg (Boere et al., 2005; Fuzessy et al., 2014). Marmosets present exploratory behavior that, along with urbanization and habitat fragmentation, favors their contact with human communities, and facilitates the illegal pet trade (Secco et al., 2018). Marmosets are commonly admitted to wildlife rehabilitation centers and often require chemical immobilization. Efficient sedation with a fast onset of action and few adverse effects is desirable, since the animal's actual health status is usually unknown.

Primates are agitated and often aggressive during restraint, making intramuscular administration a convenient route for drug delivery. Intravenous administration is hardly accessible in fully conscious animals and implies the need for fast-acting anesthetics that cause little damage to the surrounding tissues in case of extravascular administration. Ketamine has been widely administered for minor procedures in primates, alone or in combination with other anesthetic agents (Bakker et al., 2013; Furtado et al., 2010; Selmi et al., 2004a, 2004b; Theriault et al., 2008). Low doses of ketamine (1-5 mg/kg) have been related to muscle spasms, head movements, licking reflex, and salivation (Bakker et al., 2013; Shiigi & Casey, 1999, 2001) while higher doses (6-15 mg/kg) provide adequate immobilization, with excessive salivation and head movements (Theriault et al., 2008). Ketamine is commercially available as S-(+)-ketamine or the racemic mixture of two enantiomers. S-(+)-ketamine has a greater affinity for the N-methyl-D-aspartate receptor, a lower cardiodepressant effect, and promotes recovery without psychedelic effects when compared to the racemic form (Fisher et al., 2000; Lauretti, 2000; Molojavyi et al., 2001; Müllenheim et al., 2001). Dissociative agents are often associated with midazolam in primates to promote light anesthesia with muscle relaxation aiming to reduce stress and facilitate physical restraint (Capriglione et al., 2013; Furtado et al., 2010; Raposo et al., 2015; Votava et al., 2011).

Fentanyl is a μ-opioid receptor (MOR) agonist that promotes analgesia and has been shown to promote sedation in great apes (Hunter et al., 2004). Droperidol is used as an antiemetic, sedative, and antipsychotic. Droperidol may affect anesthesia via its antagonistic effect on dopamine D2 receptors and α1-adrenergic receptors (Araki et al., 2018). The fixed combination of droperidol and fentanyl, marketed as Innovar-Vet® (0.4 mg/ml fentanyl and 20 mg/ml droperidol), has been demonstrated to promote neuroleptanalgesia in primates (Field et al., 1966), but the use of this combination is rarely reported.

Due to a lack of knowledge on the sedative, antinociceptive, and muscle relaxation effects of fentanyl-droperidol, this study aimed to evaluate chemical restraint with ketamine-midazolam compared to fentanyl-droperidol in black-tufted marmosets.

Materials and Methods

Twenty-six black-tufted marmosets underwent chemical immobilization for physical examination, blood sampling, tattooing, and microchipping. The animals belonged to the Technical Division of Veterinary Medicine and Wildlife Management, Environment and Green Areas Secretary, São Paulo, Brazil, and were awaiting subsequent relocation.

Food was withheld overnight and water was withheld two hours before the experiment. Animals were housed in individual cages (150 × 150 × 150 cm) from where they were caught by hand and transferred to a cage for weighing and transportation. Adult and juvenile (socially independent, but still sexually immature) marmosets were included in the study. All procedures were performed in the morning.

Marmosets were randomly allocated into two groups: group KM (4 females and 9 males; 11 adults and 2 juveniles) received 10 mg/kg S(+)-ketamine (50 mg/ml; Ketamin (S+)® Cristália, São Paulo-SP, Brazil) and 1mg/kg midazolam (5 mg/ml; Dormire® Cristália, São Paulo-SP, Brazil) while group FD (6 females and 7 males; 8 adults and 5 juveniles) were treated with 12.5 μg/kg fentanyl (50 μg/ml; Fentanest® Cristália, São Paulo-SP, Brazil) and 625 μg/kg droperidol (2.5 mg/ml; Droperdal®, Cristália, São Paulo-SP, Brazil), given by intramuscular injection in the thigh while animals were

manually restrained. All animals were weighed before drug administration to ensure the correct dose for each drug.

After drug administration, onset time to anesthesia (the time from drug administration to a decrease of muscle tone associated with posture) and effective time (time elapsed from the onset of anesthesia until the animal presented resistance to handling) were recorded. Heart rate (HR) and rhythm (lead II ECG) were measured with a multiparametric monitor (InMax Vet Series, Instramed, Brazil). Animals breathed room air and the respiratory rate (RR) was measured by chest wall movements. HR and RR were recorded immediately following administration (T0), and the following 5 (T5), 10 (T10), 20 (T20), 30 (T30), 45 (T45), and 60 (T60) min. Manipulation of the animal for the procedures started at T10 with a physical examination. At T20, a modified scoring system (Selmi et al., 2003) was used to evaluate sedation, antinociception, muscle relaxation, auditory, visual, posture, and manipulation responses (Appendix 1). Response to manipulation consisted of putting the animal in a dorsal position and evaluating the attempt to return to the previous position. After the evaluation, tattooing and microchipping were performed. Finally, blood sampling was performed at T30. Following the procedure, the animals were continuously observed until normal ambulation.

Data analysis

Statistical analysis was performed using RStudio software (Version 0.99.903 – © 2009-2016 RStudio, Inc.). Normality and equal variances of the data were verified by the Shapiro-Wilk test and Bartlett's test, respectively. Repeated measures analysis of variance (ANOVA) was followed by a post-hoc Tukey test for comparison of the different observation times in the same group. For comparison between the experimental groups, a Student's t-test was used. Degree of sedation, muscle relaxation, antinociception, and postural, auditory, and visual responses was analyzed using the Mann-Whitney test. For all analyses, $p < 0.05$ was considered statistically significant.

Results and Discussion

No significant differences in gender ($p = 0.687$), age ($p = 0.377$), or weight ($p = 0.242$) were found between KM (BW 314.6 ± 61 g) and FD (BW 278.9 ± 87.7 g).

Sedative onset time (KM = 1.95 ± 0.56 min; FD = 6.5 ± 0.66 min; $p < 0.001$) and effective time (KM = 50.7 ± 16.1 min; FD = 79.2 ± 21.8 min; $p = 0.0009$) were shorter in KM. Ketamine promoted a short onset time as previously reported in marmosets (Bakker et al., 2013;

Furtado et al., 2010) and rhesus macaques (Bertrand et al., 2016). Effective time was significantly longer in the FD group, likely due to droperidol having a longer half-life of 134 ± 13 min, as observed in humans (Cressman et al., 1973). During induction, animals in KM presented excitation (n = 1) and muscle spasms (n = 2) while FD was uneventful.

HR was similar between groups at all moments, except at T5, when KM was significantly higher ($p = 0.013$). No significant difference was found when comparing the different moments within the groups (Table 1). No abnormalities in heart rhythm were observed during the procedure. HR was stable during the procedure in the KM group, as previously observed in marmosets (Furtado et al., 2010). FD presented lower HR 5 min after administration compared to KM, likely due to increased vagal tone caused by fentanyl (Hendrix et al., 1995). This reduction in HR has also been observed in dogs that received fentanyl (15.7 μg/kg) and droperidol (0.5 mg/kg) intravenously (Santos et al., 2001). Studies in primates indicate that fentanyl (40-120 μg/kg) and droperidol (2-6 mg/kg) may cause respiratory depression and bradycardia (Field et al., 1966; Green et al., 1981; Martin et al., 1972). In this study, the HR decrease was not clinically important either in FD or KM. HR values found in this study are higher than those reported in conscious common marmosets (*Callithrix jacchus* Linnaeus, 1758) at rest (134-173 beats/min) (Horii et al., 2002).

RR between groups was not significantly different. RR in the KM group was higher at T0 compared with T10 ($p = 0.019$) (Table 1). Ketamine is known to cause slight and transient respiratory depression in different species. A decrease in RR was observed in marmosets treated with midazolam and ketamine or S(+)-ketamine, with significance only in the racemic group (Furtado et al., 2010). In rats, however, it has been demonstrated that, at supraspinal sites, the S(+) variant interacts with the MOR system contributing to S(+) ketamine-induced respiratory depression (Sarton et al.,

Table 1 – Physiological parameters of black-tufted marmosets (n = 26) immobilized with intramuscular S-(+)-ketamine–midazolam (KM) or fentanyl-droperidol (FD)

Time point	Heart rate (beats/min)		Respiratory rate (breaths/min)	
	KM	FD	KM	FD
T0	348±33	335±49	84±20*	88±21
T5	341±45*	295±44*	62±13	64±21
T10	315±44	292±42	62±24*	65±18
T20	320±53	291±43	69±22	66±18
T30	328±53	292±38	79±21	66±17
T45	299±56	297±36	59±16	67±14
T60	320±46	305±42	72±14	70±13

Data are shown as mean ± SD. *Indicates significant difference at $p < 0.05$.

Table 2 – Scores of black-tufted marmosets (n = 26) sedated with intramuscular S-(+)-ketamine-midazolam (KM) or fentanyl-droperidol (FD)

Group	Sedation	Antinociception	Muscle relaxation	Posture	Response to manipulation	Auditory response	Visual response
KM	6	2	3	3	3	3	3
KM	5	2	2	3	3	1	2
KM	6	2	3	3	3	3	3
KM	6	3	3	3	3	3	3
KM	6	1	3	3	3	3	3
KM	5	1	3	3	3	3	3
KM	4	1	1	3	3	1	2
KM	5	1	3	3	3	2	2
KM	4	1	1	3	3	3	2
KM	4	2	3	3	3	3	2
KM	5	1	2	3	3	3	3
KM	6	2	3	3	3	3	3
KM	6	2	3	3	3	3	3
FD	1	3	0	0	0	3	3
FD	1	3	0	0	0	3	3
FD	1	3	0	1	0	3	3
FD	1	2	0	0	0	2	2
FD	1	3	0	0	3	3	3
FD	1	3	0	0	0	3	3
FD	1	2	0	0	0	2	2
FD	1	2	0	0	1	2	2
FD	1	3	0	0	0	3	3
FD	1	3	0	0	0	3	3
FD	1	3	0	0	0	3	3
FD	1	2	0	0	0	3	2
FD	1	2	0	0	3	3	3
p-value	0.003*	0.001*	0.002*	0.001*	0.02*	0.709	0.862

*Significant difference at $p < 0.05$.

2001). Fentanyl (MOR agonist) has been shown to decrease RR in rhesus monkeys after intravenous administration of 2 µg/kg (Nussmeier et al., 1991) and 8 µg/kg (Valverde et al., 2000). In our study, however, we did not observe changes in the RR in FD, which remained higher than the reported RR in conscious common marmosets at rest (36-44 bpm) (Horii et al., 2002).

Animals in KM presented a higher degree of sedation (p = 0.003) and muscle relaxation (p = 0.002). Benzodiazepines promote sedative, anxiolytic, and muscle relaxant action due to their modulation of $GABA_A$ receptors (Rudolph & Knoflach, 2011). In addition, ketamine produces a cataleptic state in which animals are unresponsive to manipulation (Shiigi & Casey, 1999; Winters et al., 1972). Droperidol may also present extrapyramidal syndromes and induce a cataleptic state (Dupre et al., 1981). In pigs, droperidol has been shown to promote quieting and calming effects, with no apparent muscle relaxation (Nishimura et al., 1993). In this study, we observed satisfactory sedation to allow manipulation in both groups with more pronounced muscle relaxation in KM (Table 2).

Groups were significantly different regarding posture after drug administration (p = 0.001). All animals in KM assumed lateral recumbency after drug administration, while all animals in FD remained in a quadrupedal position. Response to manually changing the animal's position to dorsal recumbency was also significantly different between groups (p = 0.02). Animals in KM remained in dorsal recumbency and did not attempt to return to their previous position. Eleven animals in FD returned to their previous standing position or assumed a seated position while two remained in dorsal recumbency. In pigs, droperidol (2 mg/kg) promoted sedation, but the animals assumed lateral recumbency for a short time or did not assume lateral recumbency at all and remained in the standing position for the entire evaluation period (120 min) (Nishimura et al., 1993). Overall, marmosets in KM presented a loss of muscle tone and did not resist manipulation while the aforementioned procedures were being conducted. Similarly, animals in FD allowed safe and less stressful manipulation, but without losing muscle tone, thus being able to return to a convenient position when left untouched.

Animals in FD showed a higher score of antinociception (p = 0.001), which was similar to results obtained in rats where fentanyl-droperidol scored higher in an antinociceptive response to clamping than ketamine-diazepam (Wixson et al., 1987). In rhesus monkeys, fentanyl has been shown to produce analgesic effects at 4 µg/kg (Nussmeier et al., 1991). Thus, the dose used in this study (12.5 µg/kg) may have contributed to the higher score of antinociception observed.

Visual (p = 0.709) and auditory responses (p = 0.862) were decreased in both groups and not significantly different. Fentanyl-droperidol in dogs decreased the auditory response in only one-third of the animals (Pettifer & Dyson, 1993). In this study, however, all marmosets became unresponsive to visual and auditory stimuli as reported in several other primate species (Field et al., 1966).

During recovery, animals in FD presented excitation (n = 1), licking reflex (n = 1), vocalization (n = 3), and vomiting (n = 1). In KM, we observed excitation (n = 5), licking reflex (n = 1), ataxia (n = 1), and tremors (n = 1). Data on children suggest that premedication with oral fentanyl reduced anxiety, but increased postoperative nausea and vomiting when compared to a placebo (Binstock et al., 2004; Zanette et al., 2010) or midazolam (Tamura et al., 2003). Fentanyl likely caused the episode of vomiting observed in FD, but due to the limited number of subjects and the antiemetic action of droperidol, the cause is unclear. Similar to our findings, Furtado et al. (2010) reported licking reflex, involuntary movements, salivation, sternutation, and muscle spasms during the recovery period in marmosets anesthetized with ketamine-midazolam.

Limitations of this study include the single evaluation of sedation, muscle relaxation, and antinociception using a scoring system and the limited number of physiological variables recorded. Since evaluation was performed only at T20, a comparison is only possible between groups at one time point, and parameters cannot be compared over time in the same group. Several assessments would provide information about the duration of the effects. The limited equipment available in the wildlife rescue center allowed the monitoring of HR and rhythm. But we could not measure arterial blood pressure, cardiac output, or PCO_2, so the impact of these drugs on the cardiopulmonary system was not evaluated. Additionally, time for recovery was not recorded for all subjects, so the comparison was not possible. Further research is required to evaluate the fentanyl-droperidol protocol in marmosets regarding cardiorespiratory parameters and recovery time.

Conclusion

This study demonstrated the efficacy of S-(+)-ketamine-midazolam and fentanyl-droperidol in the immobilization of marmosets. Fentanyl and droperidol produced a lesser degree of sedation and muscle relaxation but a higher degree of antinociception compared to ketamine and midazolam. KM presented the advantage of a shorter onset time and shorter effective time. Both protocols resulted in adequate sedation, but fentanyl-droperidol may be more suitable for procedures that require more intense analgesia in black-tufted marmosets.

Conflict of Interest

The authors declare no conflicts of interest.

Ethics Statement

This research was approved by the Ethics Committee on the Use of Animals of the School of Veterinary Medicine and Animal Science (University of São Paulo) under protocol number 1224020916 and is following ethical treatment for primates.

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Appendix 1 – Scoring criteria for the anesthetic effects of fentanyl-droperidol and ketamine-midazolam in black-tufted marmosets. Modified from Selmi et al. (2003)

Score	Sedation
0	Awake, walking, normal consciousness
1	Awake, but does not pay attention to the environment, able to walk slowly
2	Able to walk with loss of balance
3	Unable to stand
4	Holds head up
5	Eyes open, but unable to hold the head up
6	No response

Score	Antinociception (response to interdigital pad pinch)
0	Normal (flight response)
1	Mild (exaggerated limb movements and trying to get up)
2	Moderate (slight limb movements)
3	Profound (lack of response)

Score	Muscle relaxation
0	Normal (tense or hypertonic)
1	Mild relaxation
2	Moderate relaxation
3	Profound relaxation

Score	Posture
0	Standing
1	Sitting/ataxic
2	Sternal recumbency
3	Lateral recumbency

Score	Visual response
0	Normal (closes eyelids to the approach of objects; gazing/interest in the object)
1	Mild (closes the eyelids and brief interest in the object without gazing)
2	Moderate (closed eyelids without interest or object tracking)
3	No response (no reaction to stimuli)

Score	Auditory response (a handclap close to the animal's ears)
0	Normal
1	Mild (eye and body movement)
2	Moderate (eye movement without body movement)
3	Absence of response (no reaction to stimuli)

Score	Response to manipulation (manually changing the animal's position)
0	Normal (returns to the previous position)
1	Mild (postural recovery after several attempts)
2	Moderate (unsuccessful attempts of returning to original position)
3	Absence of response (no reaction to stimuli)