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Ketamine-Medetomidine-Midazolam and Ketamine-Dexmedetomidine-Midazolam anesthesia in gerbils: a clinical comparison of subcutaneous and intraperitoneal administration

Evaluación clínica y cardiovascular comparativa de la anestesia con Ketamina-Medetomidina-Midazolam y Ketamina-Dexmedetomidina-Midazolam en gerbillos

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ABSTRACT

This study investigated the anesthetic performance and cardiopulmonary effects of ketamine-medetomidine-midazolam and ketamine-dexmedetomidine-midazolam combinations administered by subcutaneous or intraperitoneal routes in gerbils. Thirty-two clinically healthy male gerbils were allocated to four experimental groups according to the anesthetic protocol and route of administration. All protocols produced satisfactory sedation and surgical anesthesia without mortality. Induction was achieved in all animals within a relatively short period, and dexmedetomidine-containing combinations tended to induce anesthesia more rapidly than medetomidine-based protocols. The duration of surgical anesthesia was significantly longer in animals receiving dexmedetomidine, indicating a more prolonged anesthetic effect. Throughout the observation period, significant alterations were recorded in heart rate, respiratory rate, body temperature, and peripheral oxygen saturation. Cardiopulmonary depression was observed in all groups but was more evident in animals receiving dexmedetomidine. Intraperitoneal administration generally resulted in a faster onset of anesthesia and more pronounced physiological effects compared with subcutaneous administration. Although reductions in cardiovascular and respiratory parameters were detected, no life-threatening complications occurred and although oxygen saturation values remained compatible with spontaneous recovery, transient decreases observed in dexmedetomidine-treated animals suggest that oxygen supplementation should be considered during prolonged anesthetic procedures. Recovery tended to occur earlier in medetomidine-treated gerbils, whereas dexmedetomidine produced a longer-lasting anesthetic state. The findings indicate that both anesthetic combinations can be safely used in gerbils under appropriate monitoring conditions. Dexmedetomidine provided a longer and deeper anesthetic effect, while subcutaneous administration offered a more gradual anesthetic profile with less pronounced physiological depression.

Key words: Gerbil; Ketamine; Medetomidine; Dexmedetomidine; Injectable anesthesia

RESUMEN

Este estudio investigó el desempeño anestésico y los efectos cardiopulmonares de las combinaciones ketamina-medetomidina-midazolam y ketamina-dexmedetomidina-midazolam administradas por vía subcutánea o intraperitoneal en gerbillos. Treinta y dos gerbillos machos clínicamente sanos fueron distribuidos en cuatro grupos experimentales según el protocolo anestésico y la vía de administración. Todos los protocolos produjeron una sedación adecuada y anestesia quirúrgica sin mortalidad. La inducción anestésica se logró en todos los animales en un período relativamente corto, y las combinaciones que contenían dexmedetomidina tendieron a inducir la anestesia más rápidamente que los protocolos basados en medetomidina. La duración de la anestesia quirúrgica fue significativamente mayor en los animales que recibieron dexmedetomidina, lo que indica un efecto anestésico más prolongado. Durante el período de observación se registraron cambios significativos en la frecuencia cardíaca, la frecuencia respiratoria, la temperatura corporal y la saturación periférica de oxígeno. Se observó depresión cardiopulmonar en todos los grupos, aunque fue más evidente en los animales tratados con dexmedetomidina. La administración intraperitoneal generalmente produjo un inicio anestésico más rápido y efectos fisiológicos más marcados en comparación con la administración subcutánea. Aunque se observaron reducciones en los parámetros cardiovasculares y respiratorios, no se registraron complicaciones potencialmente mortales. Asimismo, aunque los valores de saturación de oxígeno fueron compatibles con una recuperación espontánea, las disminuciones transitorias observadas en los animales tratados con dexmedetomidina sugieren que debería considerarse la administración de oxígeno suplementario durante procedimientos anestésicos prolongados. La recuperación tendió a producirse más tempranamente en los gerbillos tratados con medetomidina, mientras que la dexmedetomidina generó un estado anestésico de mayor duración. Los resultados indican que ambas combinaciones anestésicas pueden utilizarse de forma segura en gerbillos bajo condiciones adecuadas de monitorización. La dexmedetomidina proporcionó un efecto anestésico más profundo y prolongado, mientras que la administración subcutánea ofreció un perfil anestésico más gradual con una depresión fisiológica menos marcada.

Palabras clave: Gerbillo; Medetomidina; Dexmedetomidina; Anestesia inyectable; Ketamina

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INTRODUCTION

Gerbils (*Meriones unguiculatus*) are widely used in experimental studies and are also increasingly encountered in companion animal practice. Their value as research animals stems from their suitability for neurological, behavioral, auditory, and gastrointestinal investigations. Nevertheless, anesthetic management in gerbils can be challenging due to their small size, high metabolic activity, and sensitivity to stressful stimuli. As a result, these animals may be predisposed to complications such as hypothermia, respiratory depression, and cardiovascular alterations during anesthesia [1, 2]. For this reason, the development of safe and effective anesthetic protocols remains important for both animal welfare and the successful completion of clinical and experimental procedures [3, 4, 5].

Anesthesia plays a central role in surgical and invasive procedures by reducing pain perception, limiting stress responses, and facilitating immobilization. Appropriate anesthetic management is necessary to maintain animal welfare standards and to minimize physiological disturbances associated with handling and surgical interventions [1,2,4].

In gerbils, effective anesthetic management is particularly important during soft tissue surgeries, dental procedures, biopsies, and various experimental interventions. The selection of appropriate anesthetic agents and routes of administration can significantly improve anesthetic quality while reducing perioperative complications [6].

Ketamine is a dissociative anesthetic agent widely used in small laboratory animals. However, ketamine administered alone may result in inadequate muscle relaxation, spontaneous movements, and insufficient anesthetic depth in rodents [7, 8].

For this reason, Ketamine is commonly combined with alpha–2 adrenergic agonists and benzodiazepines to achieve more balanced anesthesia. Medetomidine and dexmedetomidine provide sedation, analgesia, and muscle relaxation, thereby enhancing the anesthetic effects of ketamine, while midazolam contributes additional sedation and muscle relaxation, improving overall anesthetic quality [9, 10].

In addition, alpha–2 adrenergic agonists are known to produce dose-dependent cardiovascular effects, including bradycardia, decreased cardiac output, and respiratory depression [11, 12]. Therefore, careful evaluation of cardiovascular parameters during anesthesia is essential in small rodents such as gerbils. Alpha–2 adrenergic agonists are also known to produce dose-dependent sedation, analgesia, bradycardia, and respiratory depression in laboratory animals [13, 14].

The route of administration may also influence anesthetic onset, depth, duration, and recovery characteristics in laboratory rodents. Intraperitoneal administration is commonly preferred because of its rapid absorption and ease of application, whereas subcutaneous administration may provide a slower and more controlled absorption profile with potentially reduced adverse effects [6]. However, comparative information regarding the effects of these administration routes in gerbils remains limited. Previous studies in laboratory rodents have demonstrated that anesthetic onset, anesthetic depth, and recovery quality may vary considerably according to the administration route [5].

The aim of the present study was to comparatively evaluate the anesthetic efficacy and the effects on clinical and cardiovascular parameters of ketamine-medetomidine-midazolam and ketamine-dexmedetomidine-midazolam combinations administered via subcutaneous and intraperitoneal routes in gerbils.

In this context, the effects of different anesthetic combinations and administration routes on induction time, anesthetic depth, duration of anesthesia, analgesic efficacy, and recovery time were assessed. Furthermore, changes in vital parameters including heart rate, respiratory rate, and body temperature during anesthesia were evaluated to determine the cardiovascular effects associated with both the selected alpha–2 adrenergic agonist and the route of administration [11, 12].

Providing safe and appropriate anesthesia in small laboratory animals is essential not only for animal welfare but also for the reliability and reproducibility of scientific data obtained from experimental studies. Although ketamine-based anesthetic protocols and their combinations with alpha–2 adrenergic agonists have been extensively investigated in rodents and shown to improve anesthetic quality [9, 10], studies specifically comparing the clinical and cardiovascular effects of ketamine-medetomidine-midazolam and ketamine-dexmedetomidine-midazolam combinations administered via different routes in gerbils remain limited.

Therefore, the findings of the present study are expected to contribute to the existing literature on gerbil anesthesia and assist in the development of safer and more effective anesthetic protocols for experimental and clinical applications.

To the authors' knowledge, comparative information regarding the clinical and cardiovascular effects of ketamine-medetomidine-midazolam and ketamine-dexmedetomidine-midazolam combinations administered by both subcutaneous and intraperitoneal routes in gerbils is currently limited. Therefore, the present study was designed to address this gap and provide data that may assist in anesthetic protocol selection for this species.

MATERIALS AND METHODS

Animal material

Thirty-two healthy male gerbils were used in the present study. Before the experimental procedures commenced, all animals were allowed a 7–d adaptation period to the laboratory environment. During this period, their health status was assessed through routine clinical examinations, and only animals showing no signs of disease or abnormality were enrolled in the study.

The animals were maintained under controlled environmental conditions throughout the experiment. Room temperature was kept between 20 and 24°C, relative humidity ranged from 40% to 60%, and a 12–h light/12–h dark cycle was applied. Housing and animal care procedures complied with accepted laboratory animal welfare standards [4, 5]. All gerbils were supplied by the Experimental Animal Unit of the Faculty of Veterinary Medicine, Aydın Adnan Menderes University. Sterile wood shavings served as bedding material and were replaced regularly as part of routine husbandry practices. Standard laboratory rodent feed and drinking water were available without restriction during the study period. Ethical

approval for the study was granted by the Aydın Adnan Menderes University Animal Experiments Local Ethics Committee (ADÜ–HADYEK; Approval No. 64583101/2026/075, dated 30.04.2026).

Experimental procedure

Before the administration of anesthesia, all gerbils were clinically evaluated and baseline physiological parameters were obtained. After completion of the acclimatization period, the animals were allocated randomly to four experimental groups, with eight male gerbils assigned to each group (n = 8).

Animals in Group 1 received a subcutaneous injection of Midazolam (5 mg·kg^{-1}), Medetomidine (0.1 mg·kg^{-1}), and Ketamine (50 mg·kg^{-1}). Group 2 was administered the same anesthetic protocol and dosages via the intraperitoneal route. For Group 3, anesthesia was induced by subcutaneous administration of Midazolam (5 mg·kg^{-1}), Dexmedetomidine (0.25 mg·kg^{-1}), and Ketamine (50 mg·kg^{-1}). Gerbils assigned to Group 4 received the identical drug combination at the same doses, but by intraperitoneal injection. In all groups, the anesthetic agents were administered as a single-dose combination through the designated route of administration [1]. "No pharmacological reversal with atipamezole was performed because the study objective was to evaluate the complete anesthetic profile of each protocol until spontaneous recovery. No supplemental analgesia or postoperative analgesic treatment was administered because no surgical or painful procedures were performed."

Evaluation of clinical and cardiovascular parameters

Physiological and anesthetic parameters were recorded before anesthesia (baseline) and at 0, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 min after anesthetic administration. Respiratory rate (breaths·min^{-1}) was determined by observing thoracic movements over one min. Heart rate (beats·min^{-1}) and peripheral oxygen saturation (SpO_2, %) were monitored using a Veterinary monitor and pulse oximetry. Body temperature was measured rectally using a digital thermometer. Heart rate, respiratory rate, and SpO_2 measurements were obtained using a Veterinary monitoring device (Edan Veterinary Monitor, IM8 Vet, Hamburg, Germany).

Anesthetic depth was assessed by evaluation of pedal withdrawal and palpebral reflexes. The pedal withdrawal reflex was evaluated every 5 min by applying a gentle clamp stimulus to the interdigital skin. Limb withdrawal, vocalization, or muscle contraction was considered a positive response. The palpebral reflex was assessed by gentle stimulation of the eyelid margin, and blinking or eyelid movement was considered a positive response.

Measurement schedule

All anesthetic procedures were performed between 13:00 and 14:00 h in order to minimize circadian variation. Baseline measurements were recorded immediately before anesthetic administration. Following anesthesia induction, measurements were recorded every 5 min during the first 30 min, every 15 min between 30 and 60 min, and every 30 min thereafter until the end of the observation period. At the end of the study, all gerbils survived and were retained for future experimental studies.

Statistical analysis

All statistical analyses were performed using SPSS version 22.0 (IBM Corp., Armonk, NY, USA). Data distribution was assessed using the Kolmogorov-Smirnov test. Parametric data were analyzed using two-way repeated measures ANOVA to evaluate the effects of time and treatment groups. Tukey's post hoc test was used for multiple comparisons. For non-parametric data, the Friedman test was used for repeated measurements within groups, while intergroup comparisons were performed using the Mann-Whitney U test. Results were expressed as mean ± standard deviation (SD), and a value of $P<0.05$ was considered statistically significant. Statistical methodology and sample size considerations were performed according to standard recommendations for laboratory animal studies and biostatistical analysis [15, 16].

RESULTS AND DISCUSSIONS

All anesthetic protocols successfully produced sedation and surgical anesthesia in the gerbils. No mortality or severe anesthetic complications were observed during the study period. Cardiopulmonary parameters including heart rate, respiratory rate, body temperature, and SpO_2 changed progressively throughout anesthesia in all experimental groups.

Induction time, sedation quality, and surgical anesthesia duration

All anesthetic protocols successfully produced sedation and surgical anesthesia in the gerbils without mortality or severe anesthetic complications.

All anesthetic combinations produced successful sedation, immobilization, and surgical anesthesia in gerbils without mortality or severe anesthetic complications. Induction was generally smooth and characterized by progressive loss of spontaneous activity, decreased response to environmental stimuli, muscle relaxation, and gradual disappearance of pedal withdrawal and palpebral reflexes. Comparative findings regarding induction time, total anesthesia duration, and surgical anesthesia duration are summarized in TABLE I.

The mean induction times were 80.25 ± 35.20 sec in Group 1 (SC ketamine-medetomidine-midazolam), 80.75 ± 12.96 sec in Group 2 (IP ketamine-medetomidine-midazolam), 69.38 ± 20.05 sec in Group 3 (SC ketamine-dexmedetomidine-midazolam), and 65.75 ± 8.35 sec in Group 4 (IP ketamine-dexmedetomidine-midazolam). Although induction times were numerically shorter in dexmedetomidine groups, no statistically significant intergroup difference was detected ($P = 0.412$). Nevertheless, intraperitoneal administration appeared to accelerate induction compared with subcutaneous administration.

The shorter induction times observed in dexmedetomidine-treated gerbils may be associated with the higher α2–adrenoceptor selectivity of dexmedetomidine, which produces stronger sedation and analgesia than medetomidine [11]. Similar observations have previously been reported in rodents anesthetized with α2 agonist-containing protocols [6, 7].

Total anesthesia duration was longest in Group 4 (257.63 ± 57.66 min) and shortest in Group 2 (187.88 ± 47.68 min), although the

TABLE I
Comparison of anesthetic induction, surgical anesthesia, and total anesthesia durations in gerbils receiving Ketamine-Medetomidine-Midazolam or Ketamine-Dexmedetomidine-Midazolam via subcutaneous and intraperitoneal administration

Parameter	Group 1 (SC KET-MED-MID)	Group 2 (IP KET-MED-MID)	Group 3 (SC KET-DEX-MID)	Group 4 (IP KET-DEX-MID)	*P* value
Induction Time (sec)	80.25 ± 35.20[a]	80.75 ± 12.96[a]	69.38 ± 20.05[a]	65.75 ± 8.35[a]	0.412
Total Sleep Duration (min)	242.13 ± 60.87[ab]	187.88 ± 47.68[a]	245.63 ± 45.00[b]	257.63 ± 57.66[b]	0.064
Surgical Anesthesia Duration (min)	64.38 ± 25.70[a]	20.63 ± 4.17[b]	99.38 ± 20.78[c]	103.13 ± 23.29[c]	<0.001

Data are presented as mean ± SD. Different superscript letters (a, b, c) within the same row indicate statistically significant differences between groups (P<0.05). KET: ketamine; MED: medetomidine; DEX: dexmedetomidine; MID: midazolam

difference among groups did not reach statistical significance (P = 0.064). The absence of statistical significance may be related to individual biological variability and the relatively limited sample size. However, dexmedetomidine-treated animals consistently demonstrated a tendency toward prolonged anesthetic duration.

Surgical anesthesia duration differed significantly among groups (P<0.001). Group 3 (99.38 ± 20.78 min) and Group 4 (103.13 ± 23.29 min) exhibited markedly prolonged surgical anesthesia compared with medetomidine groups. In contrast, Group 2 demonstrated the shortest surgical anesthetic duration (20.63 ± 4.17 min). The results obtained in the present study suggest that anesthetic protocols containing dexmedetomidine were associated with a more consistent and prolonged surgical anesthetic depth.

This observation may be related to the pharmacological characteristics of dexmedetomidine, which has been reported to produce more pronounced sedative and analgesic effects than several other α2–adrenergic agonists, including Medetomidine and Xylazine [12]. From a practical perspective, the longer duration of surgical anesthesia observed in these groups may be beneficial for experimental procedures requiring sustained immobilization and effective analgesia.

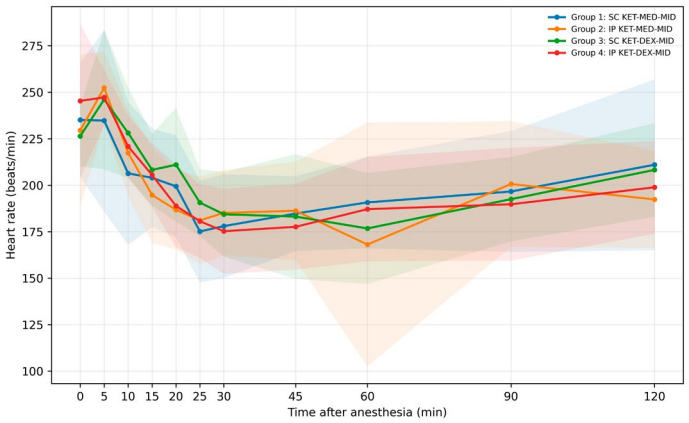

FIGURE 1. Temporal changes in heart rate in gerbils during anesthesia. Group 1: SC ketamine-medetomidine-midazolam. Group 2: IP ketamine-medetomidine-midazolam. Group 3: SC ketamine-dexmedetomidine-midazolam. Group 4: IP ketamine-dexmedetomidine-midazolam

Cardiovascular effects

A reduction in heart rate was observed after anesthetic administration in all experimental groups. Baseline values were relatively high, which is expected in gerbils and other small rodent species because of their naturally elevated metabolic and cardiovascular activity [1, 2]. Following induction of anesthesia, heart rate declined in all groups, with the most marked and sustained decreases recorded in animals receiving dexmedetomidine-containing protocols.

The greatest reductions were observed in Groups 3 and 4 during the intermediate stages of anesthesia (FIG. 1). Statistical analysis demonstrated significant changes in heart rate over time within each group (P<0.05). Comparisons among groups showed that heart rate values were generally lower in dexmedetomidine-treated animals at several monitoring intervals.

The more pronounced cardiovascular depression observed in the dexmedetomidine groups may be explained by the known physiological effects of α2–adrenergic agonists. These agents reduce sympathetic nervous system activity while enhancing vagal influence, leading to decreases in heart rate [11, 12]. Dexmedetomidine has been reported to produce marked bradycardia through both central and peripheral mechanisms, particularly when administered at clinically effective sedative doses [12, 13]. Recent studies have continued to demonstrate the marked influence of dexmedetomidine-containing anesthetic protocols on cardiovascular function in laboratory animals. Variations in heart rate and other physiological parameters have been reported in both rodent and rabbit models, supporting the view that α2–adrenergic agonists remain major determinants of anesthetic depth and cardiopulmonary responses during injectable anesthesia [16, 17, 18].

One of the most remarkable findings of the present study was the significantly longer duration of surgical anesthesia and recovery observed in gerbils receiving dexmedetomidine-containing protocols compared with those receiving medetomidine. This difference is most likely explained by the pharmacological characteristics of dexmedetomidine rather than by differences in ketamine or midazolam, since these drugs were administered at identical doses in both protocols.

Dexmedetomidine is the pharmacologically active dextrorotatory enantiomer of medetomidine and possesses a substantially greater selectivity for α_2–adrenoceptors. The α_2:α_1 selectivity ratio of dexmedetomidine has been reported to be approximately 1,620:1, whereas medetomidine exhibits a lower selectivity ratio because it is a racemic mixture containing both dexmedetomidine and the less active levomedetomidine isomer. Consequently, dexmedetomidine produces a more selective activation of central

α_2–adrenoceptors, resulting in more pronounced sedation, muscle relaxation, analgesia and sympatholysis while reducing undesirable α_1–mediated effects [19, 20].

The higher α_2–adrenoceptor selectivity of dexmedetomidine also contributes to the prolongation of anesthetic depth through several complementary mechanisms. Activation of presynaptic α_2–receptors within the locus coeruleus suppresses norepinephrine release, producing profound sedation and decreasing central sympathetic outflow. Simultaneously, stimulation of α_2–receptors in the dorsal horn of the spinal cord inhibits nociceptive neurotransmission and enhances analgesia [19, 20]. These central actions may reduce arousal responses during anesthesia and contribute to the significantly longer maintenance of surgical anesthesia observed in the dexmedetomidine-treated gerbils.

In contrast, medetomidine contains both dexmedetomidine and levomedetomidine in equal proportions. Experimental studies have suggested that levomedetomidine contributes minimally to α_2–mediated sedation and may partially antagonize some of the pharmacological effects of dexmedetomidine. Consequently, administration of an equivalent dose of racemic medetomidine results in a lower effective amount of the active dexmedetomidine isomer, which may explain the comparatively shorter anesthetic duration observed in the present study.

Another possible explanation for the prolonged anesthetic effect is the pharmacodynamic interaction between dexmedetomidine and ketamine. Alpha$_2$–adrenoceptor agonists markedly reduce the minimum anesthetic dose required for dissociative anesthetics and potentiate ketamine-induced hypnosis by decreasing central catecholamine release. Midazolam further enhances this effect through positive modulation of GABA_A receptors, providing additional muscle relaxation and anxiolysis. The combination of these three agents therefore produces balanced anesthesia with synergistic rather than merely additive pharmacological effects.

Although dexmedetomidine produced significantly longer anesthesia, the differences in heart rate between the treatment groups were numerically smaller than might be expected based on the known sympatholytic properties of α_2–adrenoceptor agonists. This apparent discrepancy highlights the importance of distinguishing statistical significance from biological significance. Small differences may achieve statistical significance because of low within-group variability while exerting limited clinical impact. Furthermore, gerbils possess species-specific cardiovascular adaptations and relatively high basal sympathetic tone, which may attenuate the magnitude of observable bradycardia compared with other laboratory rodents. Therefore, interpretation of cardiovascular effects should consider both statistical outcomes and their physiological relevance.

Differences associated with the route of administration were also evident. Animals receiving intraperitoneal injections tended to exhibit cardiovascular depression earlier than those treated subcutaneously. A more rapid absorption profile following intraperitoneal administration may account for this observation. Comparable route-related variations have been reported in studies involving other laboratory rodent species [5].

Although heart rate decreased significantly during anesthesia, no clinically important arrhythmias, cardiovascular failure, or anesthesia-related deaths were encountered. Overall, the cardiovascular responses remained within acceptable limits for all anesthetic protocols when appropriate monitoring was provided. Detailed cardiopulmonary measurements obtained during the anesthetic period are summarized in TABLE II.

"Although arterial blood pressure and electrocardiography would have provided additional cardiovascular information, these parameters were not available in the present experimental setting. Therefore, cardiovascular assessment was limited to heart rate and SpO_2. Future studies including invasive or non-invasive blood pressure monitoring and electrocardiography would allow a more comprehensive evaluation".

Respiratory effects

Respiratory activity declined following anesthetic administration in all experimental groups. While the reduction was relatively moderate in animals receiving medetomidine-containing protocols, gerbils anesthetized with dexmedetomidine exhibited a more pronounced decrease in respiratory rate, particularly during periods corresponding to deeper anesthesia (FIG. 2).

The lowest respiratory rate measurements were recorded in Group 4 during the middle phase of anesthesia. This observation suggests that the combination of dexmedetomidine and intraperitoneal administration produced the greatest respiratory depressive effect among the protocols evaluated. Significant fluctuations in respiratory rate over time were detected within all groups (P<0.01).

Ketamine is generally recognized for maintaining spontaneous respiration more effectively than many other injectable anesthetic agents. Nevertheless, the addition of α2–adrenergic agonists and benzodiazepines may enhance respiratory depression during anesthesia [1]. The greater reduction in respiratory rate observed in the dexmedetomidine groups may therefore be associated with the stronger sedative and muscle-relaxant properties of this drug.

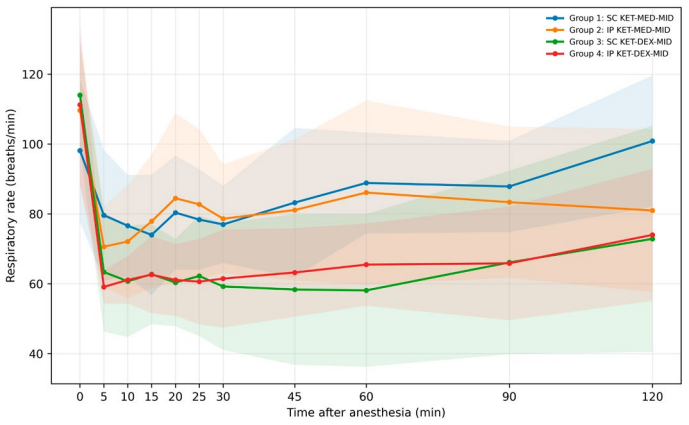

FIGURE 2. Temporal changes in respiratory rate in gerbils during anesthesia. Group 1: SC ketamine-medetomidine-midazolam. Group 2: IP ketamine-medetomidine-midazolam. Group 3: SC ketamine-dexmedetomidine-midazolam. Group 4: IP ketamine-dexmedetomidine-midazolam

TABLE II
Temporal changes in heart rate, respiratory rate, body temperature, and peripheral oxygen saturation during anesthesia in gerbils receiving different anesthetic protocols

Group	Parameter	0 min	15 min	30 min	60 min	120 min	Intragroup *P*
Group 1 (SC KET-MED-MID)	Heart Rate	238 ± 39^{aA}	$192 \pm 26^{aB*}$	$175 \pm 28^{ac*}$	$186 \pm 31^{aBc*}$	$201 \pm 42^{aB*}$	< 0.05
	Respiratory Rate	106 ± 19^{aA}	$73 \pm 18^{aB*}$	$78 \pm 14^{aB*}$	$91 \pm 16^{ac*}$	$96 \pm 20^{ac*}$	< 0.01
	Body Temperature	37.4 ± 0.4^{aA}	$35.2 \pm 0.5^{aB*}$	$34.1 \pm 0.6^{ac*}$	$33.4 \pm 0.7^{aD*}$	$34.8 \pm 0.6^{ac*}$	< 0.001
	SpO_2	95 ± 4^{aA}	$91 \pm 5^{aB*}$	$89 \pm 5^{aB*}$	$92 \pm 4^{aB*}$	96 ± 3^{aA}	< 0.05
Group 2 (IP KET-MED-MID)	Heart Rate	229 ± 35^{aA}	$198 \pm 20^{aB*}$	$181 \pm 24^{ac*}$	$191 \pm 26^{aB*}$	$194 \pm 37^{aB*}$	< 0.05
	Respiratory Rate	111 ± 22^{aA}	$70 \pm 16^{aB*}$	$74 \pm 13^{aB*}$	$82 \pm 15^{ac*}$	$89 \pm 18^{ac*}$	< 0.01
	Body Temperature	37.5 ± 0.3^{aA}	$35.0 \pm 0.4^{aB*}$	$34.0 \pm 0.5^{ac*}$	$33.2 \pm 0.6^{aD*}$	$34.6 \pm 0.5^{ac*}$	< 0.001
	SpO_2	96 ± 3^{aA}	$92 \pm 4^{aB*}$	$90 \pm 5^{aB*}$	$91 \pm 5^{aB*}$	95 ± 4^{aA}	< 0.05
Group 3 (SC KET-DEX-MID)	Heart Rate	239 ± 33^{aA}	$209 \pm 18^{bB*}$	$196 \pm 17^{bc*}$	$205 \pm 19^{bB*}$	$208 \pm 29^{bB*}$	< 0.01
	Respiratory Rate	115 ± 18^{aA}	$58 \pm 13^{bB*}$	$56 \pm 11^{bB*}$	$61 \pm 10^{bB*}$	$69 \pm 12^{bc*}$	< 0.001
	Body Temperature	37.3 ± 0.5^{aA}	$34.3 \pm 0.5^{bB*}$	$33.1 \pm 0.5^{bc*}$	$32.4 \pm 0.5^{bD*}$	$33.5 \pm 0.6^{bc*}$	< 0.001
	SpO_2	96 ± 3^{aA}	$88 \pm 6^{bB*}$	$86 \pm 7^{bB*}$	$88 \pm 6^{bB*}$	$93 \pm 5^{bA*}$	< 0.01
Group 4 (IP KET-DEX-MID)	Heart Rate	242 ± 36^{aA}	$220 \pm 17^{bB*}$	$201 \pm 16^{bc*}$	$210 \pm 18^{bB*}$	$215 \pm 24^{bB*}$	< 0.01
	Respiratory Rate	121 ± 16^{aA}	$54 \pm 12^{bB*}$	$52 \pm 10^{bB*}$	$56 \pm 9^{bB*}$	$63 \pm 11^{bc*}$	< 0.001
	Body Temperature	37.4 ± 0.4^{aA}	$34.1 \pm 0.6^{bB*}$	$32.9 \pm 0.6^{bc*}$	$32.2 \pm 0.6^{bD*}$	$33.2 \pm 0.7^{bc*}$	< 0.001
	SpO_2	95 ± 4^{aA}	$86 \pm 7^{bB*}$	$85 \pm 7^{bB*}$	$87 \pm 7^{bB*}$	$92 \pm 6^{bA*}$	< 0.01

Different lowercase superscript letters (a, b) within the same column indicate statistically significant intergroup differences ($P<0.05$). Different uppercase superscript letters (A, B, C, D) within the same row indicate statistically significant intragroup temporal differences ($P<0.05$). * indicates significant difference compared with baseline (0 min) within the same group. KET: ketamine; MED: medetomidine; DEX: dexmedetomidine; MID: midazolam

Comparable decreases in respiratory function have been described in studies evaluating ketamine-based anesthetic combinations in rodents and rabbits (*Oryctolagus cuniculus*) [9, 10].

Despite the reductions observed in respiratory rate, all animals maintained spontaneous ventilation throughout the anesthetic period. No severe respiratory adverse events were encountered, indicating that the protocols evaluated in the present study provided an acceptable level of respiratory safety when adequate monitoring was maintained.

Body temperature changes

A gradual decline in body temperature was observed in all experimental groups during anesthesia. Following anesthetic induction, temperature values decreased steadily and remained lower than pre-anesthetic measurements throughout the monitoring period (FIG. 3). Significant time-dependent reductions in body temperature were observed in all groups ($P<0.001$).

The most severe hypothermia occurred in dexmedetomidine groups, especially Group 4. The prolonged anesthetic depth and reduced metabolic activity associated with dexmedetomidine may explain these findings (FIG. 3).

Gerbils and other small rodents are highly susceptible to anesthesia-associated hypothermia because of their high metabolic rate and large surface area-to-body mass ratio [1]. Furthermore, muscle relaxation and decreased thermoregulatory capacity during anesthesia contribute substantially to heat loss. Hypothermia is considered one of the most common anesthetic complications in small rodents because of rapid heat loss and reduced metabolic thermoregulation during anesthesia [2, 4].

The present findings emphasize the importance of active thermal support during prolonged anesthesia in gerbils. Failure to maintain body temperature may prolong recovery time and increase anesthetic risk in small rodents. The reduction in body temperature observed in the present study is consistent with recent reports

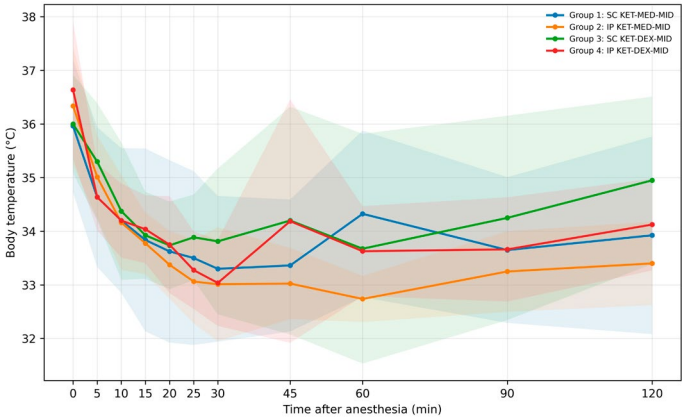

FIGURE 3. Temporal changes in body temperature in gerbils during anesthesia. Group 1: SC ketamine-medetomidine-midazolam. Group 2: IP ketamine-medetomidine-midazolam. Group 3: SC ketamine-dexmedetomidine-midazolam. Group 4: IP ketamine-dexmedetomidine-midazolam

describing hypothermia as one of the most common adverse effects of α2 agonist-based injectable anesthesia in laboratory rodents. Tashiro and Tohei [20] emphasized the importance of dose selection and perioperative thermal support to minimize anesthetic-induced hypothermia in mice.

Peripheral oxygen saturation

Peripheral oxygen saturation showed only moderate fluctuations during the anesthetic period, although temporary decreases were observed at time points corresponding to deeper levels of anesthesia (FIG. 4). Statistical analysis revealed significant changes in SpO_2 values over time within the experimental groups ($P<0.05$).

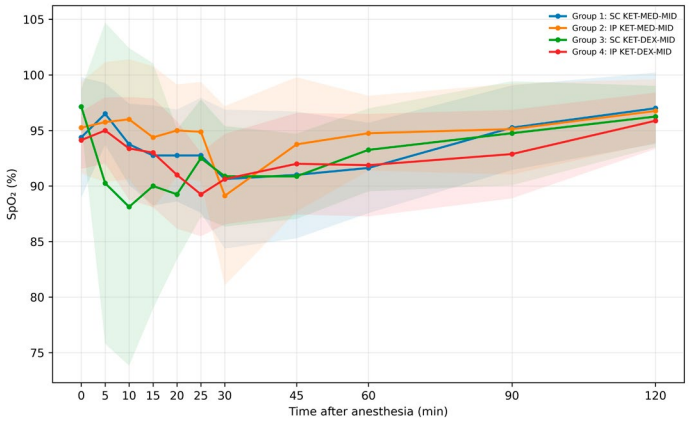

FIGURE 4. Temporal changes in peripheral oxygen saturation in gerbils during anesthesia. Group 1: SC ketamine-medetomidine-midazolam. Group 2: IP ketamine-medetomidine-midazolam. Group 3: SC ketamine-dexmedetomidine-midazolam. Group 4: IP ketamine-dexmedetomidine-midazolam

Lower oxygen saturation values were recorded in dexmedetomidine-treated gerbils during several monitoring intervals when compared with animals receiving medetomidine-containing protocols. This observation may be associated with the greater degree of respiratory depression documented in the dexmedetomidine groups.

Despite these reductions, SpO_2 measurements generally remained within clinically acceptable ranges, and no animal developed severe hypoxemia during anesthesia. Comparable transient decreases in oxygen saturation have been described previously in rodents anesthetized with ketamine combined with α2–adrenergic agonists [8].

Although oxygen saturation values remained sufficient to allow spontaneous recovery in all animals, the lowest SpO_2 values recorded in the dexmedetomidine groups (approximately 85–86%) should not be interpreted as entirely benign. In both veterinary and human anesthesia, oxygen saturation values below 90% are generally considered indicative of clinically relevant hypoxemia and may reflect impaired pulmonary gas exchange or reduced ventilatory function. The transient decrease observed in the present study is likely attributable to the central respiratory depressant effects of α_2–adrenoceptor agonists, which may reduce respiratory rate and tidal volume, despite the partial respiratory-sparing properties of ketamine. Importantly, none of the gerbils exhibited clinical signs of severe respiratory compromise or failed to recover uneventfully, suggesting that the observed hypoxemia was temporary and clinically manageable under the experimental conditions.

Nevertheless, these findings indicate that supplemental oxygen and close respiratory monitoring should be considered when dexmedetomidine-containing anesthetic protocols are used, particularly during prolonged procedures or in animals with pre-existing cardiopulmonary disease.

Overall, the results indicate that although dexmedetomidine produced more pronounced cardiopulmonary effects, oxygen delivery appeared to remain sufficient throughout the anesthetic period under the monitoring conditions used in this study.

Reflex suppression and recovery characteristics

Loss of pedal withdrawal and palpebral reflexes was observed in all groups after anesthetic administration. The duration of reflex suppression was notably longer in gerbils receiving dexmedetomidine-containing protocols, particularly in Groups 3 and 4. This finding is consistent with the longer surgical anesthetic periods recorded in these animals and supports the greater anesthetic depth achieved with dexmedetomidine.

Differences were also evident during recovery. Animals treated with medetomidine-based protocols generally regained spontaneous movement and reflex responses earlier than those receiving dexmedetomidine. In contrast, dexmedetomidine-treated gerbils remained sedated for a longer period and showed delayed return of normal activity.

The significantly longer anesthetic duration observed in the dexmedetomidine groups may primarily be explained by the greater pharmacological selectivity of dexmedetomidine for α_2–adrenoceptors. Dexmedetomidine is the pharmacologically active dextrorotatory isomer of medetomidine, whereas medetomidine is a racemic mixture containing equal proportions of dexmedetomidine and levomedetomidine. Because levomedetomidine exhibits considerably lower affinity for α_2–adrenoceptors, administration of racemic medetomidine effectively delivers only half of the active isomer. Consequently, dexmedetomidine produces more potent central sympatholysis, sedation, muscle relaxation and analgesia than an equivalent dose of medetomidine [19, 21]. The pharmacological actions of dexmedetomidine are mediated predominantly through α_2A-adrenoceptors located within the locus coeruleus, producing profound sedation and hypnosis through inhibition of noradrenergic neuronal activity. Activation of α_2–receptors within the dorsal horn of the spinal cord suppresses the release of substance P and glutamate, thereby reducing nociceptive transmission and enhancing analgesia. Peripheral α_2B-receptor stimulation initially produces transient vasoconstriction, whereas the subsequent central sympatholytic effect predominates during maintenance of anesthesia. These receptor subtype-specific actions explain the balanced sedative and analgesic profile of dexmedetomidine.

Although both medetomidine and dexmedetomidine belong to the α_2–adrenoceptor agonist class, their pharmacodynamic properties differ considerably. Dexmedetomidine produces a more selective receptor activation and less α_1–mediated activity, resulting

in deeper sedation and greater analgesic efficacy with lower doses. Previous studies have demonstrated that dexmedetomidine provides more predictable anesthetic depth and prolonged recovery compared with racemic medetomidine in several animal species. The findings of the present study are consistent with these observations and indicate that these pharmacodynamic differences are also evident in Mongolian gerbils.

Several complementary mechanisms may explain the prolonged anesthetic duration observed with dexmedetomidine. Activation of central α_2–adrenoceptors within the locus coeruleus suppresses norepinephrine release, thereby reducing neuronal excitability and prolonging sedation. In addition, α_2–adrenoceptor stimulation within the spinal cord inhibits nociceptive neurotransmission, enhancing analgesia. Dexmedetomidine also reduces the anesthetic requirement of ketamine through synergistic pharmacodynamic interactions, while midazolam further potentiates inhibitory neurotransmission via GABA_A receptors [19, 21]. The combined activation of these pathways probably produced a synergistic rather than merely additive anesthetic effect, explaining the significantly longer duration of anesthesia observed in the present study [22, 23, 24].

The extended recovery period observed in the dexmedetomidine groups may be beneficial in situations where prolonged analgesia and immobilization are required. On the other hand, longer recovery times may increase the need for postoperative observation until normal physiological function is fully restored.

Effects of administration route

The route of administration affected several anesthetic variables, including induction time, cardiopulmonary responses, and recovery characteristics. Intraperitoneal injection generally resulted in a more rapid onset of anesthesia and a greater degree of physiological depression, whereas subcutaneous administration was associated with a slower progression of anesthetic effects and less pronounced alterations in cardiopulmonary parameters.

These observations may be explained by differences in the absorption characteristics of the two administration routes. Because the peritoneal cavity has an extensive vascular supply, drugs administered intraperitoneally can enter the systemic circulation relatively quickly. In contrast, subcutaneous administration is usually associated with a slower and more gradual absorption profile [5].

Comparable route-related differences have been documented in previous studies evaluating ketamine-medetomidine anesthesia in hamsters. Kilic *et al.* [25] reported faster anesthetic induction and more marked physiological depression following intraperitoneal administration when compared with subcutaneous injection in hamster (*Cricetinae*). Similar observations have also been described in other laboratory rodent species receiving injectable anesthetic combinations [25].

CONCLUSIONS

Selection of the administration route should therefore be based on the objectives of the procedure. Intraperitoneal administration may be preferable when rapid induction and a deeper anesthetic plane are required, whereas subcutaneous administration may offer greater physiological stability and a more gradual anesthetic course. Recent investigations have also highlighted the importance of administration route in determining anesthetic performance. Iwanaga *et al.* reported that dual-route anesthetic protocols may influence anesthetic depth, physiological responses, and recovery quality, emphasizing the role of administration strategy in refinement of laboratory animal anesthesia.

Both ketamine-medetomidine-midazolam and ketamine-dexmedetomidine-midazolam combinations provided effective anesthesia in gerbils following subcutaneous or intraperitoneal administration. Protocols containing dexmedetomidine produced a longer surgical anesthetic period and greater anesthetic depth, although these effects were accompanied by more pronounced reductions in heart rate, respiratory rate, and body temperature. Intraperitoneal administration was associated with faster induction and stronger physiological effects, whereas subcutaneous administration produced a more gradual anesthetic profile. Although measurable physiological changes occurred during anesthesia, all protocols were completed without mortality or severe complications, indicating that each regimen can be used safely under appropriate monitoring conditions.

Conflict of interest

The authors confirm that there are no conflicts of interest related to this study.

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