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Effect of Butorphanol and Buprenorphine use on cardiac Troponin I and C-reactive protein concentrations in Ketamine-Dexmedetomidine-Midazolam anesthesia in rabbits

Efecto del uso de Butorfanol y Buprenorfina en la anestesia con Ketamina-Dexmedetomidina-Midazolam sobre las concentraciones de Troponina I cardíaca y proteína C reactiva en conejos

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ABSTRACT

The purpose of this study was to evaluate the effects of two anesthetic combinations, ketamine–dexmedetomidine–midazolam–butorphanol and ketamine–dexmedetomidine–midazolam–buprenorphine, on cardiac troponin I and C-reactive protein concentrations during short-term anesthesia in rabbits. Sixteen adult New Zealand White rabbits (8 males and 8 females) were included in the study and randomly divided into two groups, each consisting of four males and four females. Group 1 received ketamine (20 mg/kg IM), dexmedetomidine (35 µg/kg IM), midazolam (0.5 mg/kg IM) and buprenorphine (0.02 mg/kg IM), while Group 2 received ketamine (20 mg/kg IM), dexmedetomidine (35 µg/kg IM), midazolam (0.5 mg/kg IM) and butorphanol (0.2 mg/kg IM). Reflex responses (standing, ear and rear pedal reflex) and cardiopulmonary parameters (heart rate, respiratory rate and peripheral oxygen saturation) were recorded before anesthesia and at 5, 10, 15, 30, 45 and 60 min after drug administration. Blood samples for C-reactive protein and cardiac troponin I analysis were collected before anesthesia induction, at 30 min of anesthesia, and at 6, 12 and 24 h after anesthesia. Both anesthetic combinations provided adequate anesthesia for short surgical procedures in rabbits within the tested dose ranges. However, the butorphanol combination produced a longer duration of surgical anesthesia and deeper sedation compared with buprenorphine. Neither protocol caused significant increases in serum C-reactive protein or cardiac troponin I concentrations, suggesting that both anesthetic combinations can be safely used for short-term anesthesia in rabbits.

Key words: Ketamine, dexmedetomidine, cardiac Troponin-I, C-reactive protein, rabbit.

RESUMEN

El objetivo de este estudio fue evaluar los efectos de dos combinaciones anestésicas, ketamina-dexmedetomidina-midazolam-butorfanol y ketamina-dexmedetomidina-midazolam-buprenorfina, sobre las concentraciones de troponina I cardíaca y proteína C reactiva durante la anestesia a corto plazo en conejos. Se incluyeron dieciséis conejos adultos de Nueva Zelanda (8 machos y 8 hembras) en el estudio, que se dividieron aleatoriamente en dos grupos, cada uno compuesto por cuatro machos y cuatro hembras. El grupo 1 recibió ketamina (20 mg/kg IM), dexmedetomidina (35 µg/kg IM), midazolam (0,5 mg/kg IM) y buprenorfina (0,02 mg/kg IM), mientras que el grupo 2 recibió ketamina (20 mg/kg IM), dexmedetomidina (35 µg/kg IM), midazolam (0,5 mg/kg IM) y butorfanol (0,2 mg/kg IM). Las respuestas reflejas (de pie, de oreja y de pedal trasero) y los parámetros cardiopulmonares (frecuencia cardíaca, frecuencia respiratoria y saturación periférica de oxígeno) se registraron antes de la anestesia y a los 5, 10, 15, 30, 45 y 60 min después de la administración del fármaco. Se tomaron muestras de sangre para el análisis de proteína C reactiva y concentraciones de troponina I cardíaca antes de la inducción de la anestesia, a los 30 min de la anestesia y a las 6, 12 y 24 h después de la anestesia. Ambas combinaciones anestésicas proporcionaron una anestesia adecuada para procedimientos quirúrgicos cortos en conejos dentro de los rangos de dosis evaluados. Sin embargo, la combinación con butorfanol produjo una mayor duración de la anestesia quirúrgica y una sedación más profunda en comparación con la buprenorfina. Ninguno de los protocolos provocó aumentos significativos en las concentraciones séricas de proteína C reactiva o concentraciones de troponina I cardíaca, lo que sugiere que ambas combinaciones anestésicas pueden utilizarse de forma segura para la anestesia a corto plazo en conejos.

Palabras clave: Ketamina; dexmedetomidina; troponina I cardíaca; proteína C reactiva; conejo.

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INTRODUCTION

Cardiac troponin I (cTn-I) is considered one of the most sensitive and specific biomarkers for detecting myocardial injury. Increased circulating cTn-I concentrations may indicate myocardial cell damage resulting from ischemia, hypoxia, inflammation or physiological stress associated with anesthesia [1]. C-reactive protein (CRP) is an acute phase protein produced primarily by the liver and is widely used as a marker of systemic inflammatory responses. Changes in CRP concentrations may occur following tissue injury, inflammatory reactions or stress conditions [2]. Therefore, monitoring cTn-I and CRP levels during anesthesia may provide valuable information about possible cardiac and inflammatory effects of anesthetic protocols in rabbits

Myocardial lesions can occur during anesthesia or as a result of myocardial ischemia [3]. The effects of some anesthetic agents on myocardial status and their ischemia-reducing effects have been documented [4].

Inhalation anesthetic agents such as Isoflurane and Sevoflurane provide myocardial protection by accelerating myocardial recovery and reperfusion [5, 6, 7]. Injectable anesthetic agents such as Fentanyl and Propofol show less myocardial protective effect than inhalation anesthetics [8, 9].

In fact, although myocardial protection varies depending on the type of myocardial ischemia, the mechanism of ischemia, and the anesthetics used, different effects have been observed in many species such as rabbits (*Oryctolagus cuniculus*), rats (*Rattus norvegicus*), dogs (*Canis lupus familiaris*), and humans (*Homo sapiens*) [4, 8, 10, 11, 12].

In this study, the effect of anesthesia performed by adding Butorphanol and Buprenorphine to the Ketamine-Dexmedetomidine-Midazolam combination on some clinical findings and cTn-I and CRP levels was investigated.

MATERIAL AND METHODS

The animal material consisted of a total of 16 adult New Zealand breed rabbits, 1-2 years of age, weighing 2-3 kg, 8 males and 8 females (Neky Ticaret AŞ, Turgutlu, Türkiye). The rabbits were obtained from a certified private farm in Manisa and each was housed in separate cages throughout the study. After the animals were brought from the to the experimental housing facility, they were allowed to acclimate to their new surroundings for 14 days (d), after which they were used as study materials.

Throughout the study, their feed and bedding were checked daily, and their care and feeding were carried out under hygienic conditions. The ambient temperature was conditioned between 15-20 °C, and the humidity between 50-60 %. Lighting was provided for the animals in 12-hour (h) daylight and 12-h nightlight cycles.

Standard rabbit feed was used as the feed material, and the animals were given feed and clean drinking water *ad libitum*.

This study was conducted with the approval of the Aydın Adnan Menderes University Local Ethics Committee for Animal Experiments (ADÜ-HADYEK), dated 10.03.2022 and numbered 64583101/2022/005.

To ensure homogeneity of the groups, the animals were divided into two groups of eight, with four females and four males in each group. The day before the study, the animals underwent clinical examination, and their weight, respiratory rate, heart rate, and rectal temperature were measured and recorded (Edan Veterinary Monitor, IM 8 Vet, Hamburg, Germany) in a pre-prepared anesthesia protocol. For this purpose, a separate anesthesia protocol was prepared for each animal, and the results of the measurements, taken by the same person, were recorded in this protocol.

Animals that were ill or suspected of being ill were excluded from the study. Since this study used two different anesthetic combinations for the first time in rabbits, two male and two female rabbits were randomly selected from each group to observe whether the predicted dose produced the desired level of sedation or anesthesia.

Subsequently, based on literature data, the doses were adjusted in Group 1 rabbits to induce a total of 1 h of anesthesia by administering Ketamine (20 mg/kg IM), Dexmedetomidine (35 µg/kg IM), Midazolam (0.5 mg/kg IM), and finally Buprenorphine (0.02 mg/kg IM). In Group 2, the animals were administered Ketamine (20 mg/kg IM), Dexmedetomidine (35 µg/kg IM), Midazolam (0.5 mg/kg IM), and finally Butorphanol (0.2 mg/kg IM), respectively, to induce a total of 1 h of anesthesia. In this way, the study was initiated by administering the preclinically determined doses to all animals.

The doses obtained from the preclinical study for each rabbit were administered. The final doses used in the present study were determined based on preliminary observations and previously published literature. During the preclinical phase, a small number of animals were used to evaluate whether the predicted drug combinations produced an adequate level of sedation and surgical anesthesia without causing severe cardiopulmonary depression. The selected doses were found to provide stable anesthesia lasting approximately one h.

In addition, the dose ranges used in this study were consistent with those previously reported for ketamine-based anesthetic combinations in rabbit [13, 14] and the following parameters were monitored within the same time frame. These parameters were reflexes (standing, ear and rear pedal, respectively) measured before anesthesia and at the 5th, 10th, 15th, 30th, 45th, and 60th minutes (min) after anesthesia, and cardiopulmonary parameters (heart rate, respiratory rate, peripheral oxygen saturation).

For this purpose, all animals were monitored before, during, and after anesthesia via a probe from a monitor (Edan Veterinary Monitor, IM 8 Vet, Hamburg, Germany), and the evaluations were recorded in the anesthesia protocol. Reflex measurements were taken by compressing the reflexes with toothless forceps, and any reaction in the rabbits was recorded as positive or negative in the anesthesia protocol. Leg retraction, vocalization, muscle twitching, or deep sighing were considered positive [13, 14].

During anesthesia, all rabbits received supplemental oxygen to prevent hypoxemia. Oxygen was administered via mask throughout the anesthetic procedure and oxygen saturation was continuously monitored using a pulse oximeter.

For biochemical measurements, blood samples were taken from all animals before anesthesia, at the 30th min of anesthesia, and 6, 12, and 24 h after anesthesia. For this purpose, the outer surface of the ear skin was disinfected and then shaved. Using a light source held from below to facilitate exposure of the vein, a size 22 intravenous catheter (Mediflon, Global Medicit Limited, İndia) or cannula was inserted into the Vena auricularis marginalis, and 2 mL of venous blood was withdrawn from the patients via this catheter. These serum samples were then placed in Eppendorf tubes (Biogen, Türkiye), labeled, and stored at -20 °C.

Serum C-reactive Protein and cTn-I levels were determined using rabbit-specific antibodies and Enzyme-Linked Immunosorbent Assay (ELISA)-based analyses. A specific ELISA kit (Bioassay Technology Laboratory) was used for rabbit CRP analysis. In this context, after adding 120 µL of diluent to 120 µL of standard solution, standards (150 ng/L, 300 ng/L, 600 ng/L, 1200 ng/L, and 2400 ng/L) were prepared by serial dilution processes. Samples were placed in 40 µL wells, and 10 µL of anti-CRP antibody was added.

Subsequently, 50 µL of streptavidin-HRP was added, and the samples were incubated at 37 °C for 60 min (Memmert, Germany).. After incubation, the plate was washed five times with buffer washing solution, and 50 µL of substrate A solution and 50 µL of substrate B solution were added to each well sequentially. The wells were then incubated for 10 min at 37 °C in the dark. After the second incubation, the reaction was stopped by adding 50 µL of stop solution, and the optical densities (OD) were determined using a microplate reader (Thermo Scientific, Multiskan Go) at a wavelength of 450 nm.

For cTn-I, analyses were performed similarly as indicated in the ELISA kit (Bioassay Tech. Lab.). For this purpose, standards (200 ng/L, 400 ng/L, 800 ng/L, 1600 ng/L, and 3200 ng/L) were prepared by serial dilution after adding 120 µL of diluent to 120 µL of standard solution. Then, samples were placed in 40 µL wells, and 10 µL of anti-cTn-I antibodies were added. Subsequently, 50 µL of streptavidin-Horseradish Peroxidase was added, the plate was covered, and incubated at 37 °C for 60 min (Memmert, USA).

After the incubation process, all wells were washed 5 times. After the washing process, 50 µL of substrate A and 50 µL of substrate B were added to wells respectively, and a second incubation was initiated at 37 °C for 10 min. At the end of the second incubation period, 50 µL of stop solution was added to each well, and readings were taken using an ELISA reader at a wavelength of 450 nm to determine the optical densities (Thermo Scientific, Multiskan Go). For both ELISA analyses, optical density curves were derived from the optical densities obtained from the standards, and the equation of the curves was obtained. Calculations performed from the equation allowed for the determination of CRP and cTn-I levels in the animals.

Statistical analyses

The homogeneity tests of the values obtained from the study were performed, and whether the data showed a normal distribution was determined according to the Shapiro-Wilk analysis. Descriptive statistics were performed on all data, and the data were presented in tabular form as mean and standard error. Since the data of the reflex measurements did not show a normal distribution, the Cochrans' Q test, a non-parametric test technique, was used for comparisons.

In addition, for the data of the laboratory findings that did not show a normal distribution, a logarithmic transformation was performed, and homogeneity tests were repeated, and it was determined that they showed a normal distribution. In the comparison of data showing a normal distribution, two-way Analysis of Variance was used, and Tukey test was performed for post hoc evaluations. SPSS 26.0 (IBM, USA) program was used to determine the changes in reflex parameters. In the statistical evaluation of the data, cases where the p-value is less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Reflex disappearance time

The sedative effect after injection occurred on average in 131.3 ± 17.67 seconds (s) in the Buprenorphine group and 133.8 ± 23.52 s in the Butorphanol group. The duration of surgical anesthesia was determined to be 1650 ± 247.1 s (approximately 27 min) on average in the Buprenorphine group and 1950 ± 98.5 s (approximately 32 min) in the Butorphanol group.

The pupillary reflex disappearance duration was statistically significantly longer in the Butorphanol group 2025 ± 147.3 s, compared to the Buprenorphine group of 1388 ± 149.3 s. The duration of anesthesia was statistically significantly longer in the Butorphanol group compared to the Buprenorphine group (TABLE I). The resulting anesthesia was sufficient for minor surgical procedures.

TABLE I

Comparison of some reflex disappearance time and total sleep and surgical anesthesia duration for Butorphanol and Buprenorphine groups (mean ± SE)

Parameters	Group	
	Buprenorphine	Butorphanol
Duration for the standing reflex to disappear[b]	131.3 ± 17.67	133.8 ± 23.52
Duration for the ear reflex to disappear	1463 ± 256.3	1215 ± 360.1
Duration for the tail reflex to disappear	1350 ± 289.1	1575 ± 202.4
Duration for the pupillary reflex to disappear	1388 ± 149.3	2025 ± 147.3*
Total sleep duration	4095 ± 417.8	3705 ± 105
Duration of surgical anesthesia	1650 ± 247.1	1950 ± 98.5*

*: Values are marked in the same row are statistically significant between groups ($P < 0.05$). [b] was measured in seconds.

Throughout the study, no deaths or anesthetic complications were observed during the study were observed in rabbits in either group. In a similar study in cats (Felis catus), using Dexmedetomidine (25 µg/kg) and Ketamine (3 mg/kg) in combination with different narcotic analgesics such as Butorphanol (0.2 mg/kg), Hydromorphone (0.05 mg/kg) or Buprenorphine (30 µg/kg) for castration purposes did not result in any complications such as vomiting or salivation in any of the cats [15].

In another study conducted on dogs, the cardiovascular effects of the Dexmedetomidine (15 µg/kg)- Ketamine (3 mg/

kg) combination with the addition of Butorphanol (0.2 mg/kg), Hydromorphone (0.05 mg/kg), and Buprenorphine (40 µg/kg) for castration purposes were compared [16]. Similar results were observed in dogs, meaning no complications were observed during any of the anesthesia stages or recovery.

In a study on rabbits conducted by Henke *et al*. [13], it was reported that the loss of the standing reflex occurred in 1.7 ± 0.4 min after intravenous injection of Medetomidine (0.25 mg/kg) and Ketamine (35 mg/kg), and the total sleep duration was 149.7 ± 38.7 min. In another report, it was stated that the standing reflex disappeared 1.4 ± 1.1 min after intranasal administration of the Dexmedetomidine-midazolam-butorphanol combination in rabbits [17].

In yet another study, it was observed that the standing reflex disappeared 185 sec after intranasal administration of Midazolam (1 mg/kg), Medetomidine (0.05 mg/kg), and Ketamine (20 mg/kg) in rabbits [18]. In this study, the reflexes disappeared later compared to the study by Henke *et al*. [13]. This is because while the intravenous route was used in that study, the intramuscular route was preferred in this study.

Furthermore, it was observed that in rabbits, the response to the pedal reflex (PR) disappeared at 4.5th min after intramuscular administration of Dexmedetomine (25 µg/kg) and Ketamine (30 mg/kg), and was regained at 105th min; and with the administration of Ketamine (30 mg/kg)-Xylazine (4 mg/kg), the response to the PR disappeared at 6th min and was regained at 102.5th min [19].

In this study, the average total sleep duration was observed to be 68 min and 61 min with the administration of Ketamine (20 mg/kg IM), Dexmedetomidine (35 µg/kg IM), Midazolam (0.5 mg/kg IM), and finally, Butorphanol (0.2 mg/kg IM) or Buprenorphine (0.02 mg/kg IM). It is thought that the most important reason for the shorter awakening time in this study is the lower dose of Ketamine used.

In this study, it was observed that the duration of surgical anesthesia was statistically significantly longer in the Butorphanol group compared to the Buprenorphine group. In a similar study in cats, Ko *et al*. [15] during the operation, isoflurane supplementation was deemed necessary in 7 out of 10 cats in the Buprenorphine group. On the other hand, only 1 out of 10 cats required additional isoflurane supplementation in the Butorphanol group. When comparing both groups, additional isoflurane supplementation was statistically significantly higher in the Buprenorphine group. This difference is thought to be due to Buprenorphine having a less sedative effect than Butorphanol in cats [15].

Another reason reported is that the effect of Buprenorphine may have started later than that of Butorphanol. In another study, Buprenorphine was used alone and in combination with dexmedetomidine in cats, and while no sedation was observed at Buprenorphine doses of 10 and 20 µg/kg, IM, Dexmedetomidine produced profound sedation at doses of 20 and 40 µg/kg, IM [20].

Another study in dogs compared the suitability of the dexmedetomidine-butorphanol combination for hip joint radiography. This study showed that the dexmedetomidine-buprenorphine combination provided inadequate sedation and was therefore deemed unsuitable for such procedures. In parallel with these studies, our study also observed a longer surgical anesthesia duration in the Butorphanol group compared to the Buprenorphine group [21].

The differences observed between Butorphanol and Buprenorphine in terms of sedative and anesthetic effects may be explained by their distinct pharmacological mechanisms. Butorphanol acts primarily as a kappa (κ) opioid receptor agonist and a partial antagonist at mu (µ) opioid receptors, producing moderate analgesia and pronounced sedative effects. In contrast, Buprenorphine is a partial agonist at µ-opioid receptors with very high receptor affinity and prolonged receptor binding. Although buprenorphine provides potent and long-lasting analgesia, its sedative effects are generally less pronounced compared to Butorphanol. These pharmacodynamic differences may explain the longer duration of surgical anesthesia and deeper sedation observed in the Butorphanol group in the present study. Similar differences between these opioid agents have also been reported in veterinary anesthetic protocols in rabbits and other small animal species [15, 21].

Respiratory system findings

Respiratory rate was higher in the Butorphanol group, but at the end of anesthesia, it was observed to be within physiological values in both groups. No statistically significant difference was observed between the two groups (within and between groups).

Peripheral oxygen saturation decreased in both groups at the 5th min of anesthesia, but was measured at 95 %, close to the baseline value, at the 60th min of anesthesia in both groups. In all groups, patients were externally oxygenated throughout anesthesia to protect them from hypoxia.

In this study, apnea lasting 1 min was observed in animal number 4 in the Buprenorphine group and in animal number 6 in the Butorphanol group. Respiratory rate decreased from 60/min to 39/min in the Butorphanol group at the 5th min of anesthesia, while it increased from 34.7/min to 46.5/min in the Buprenorphine group.

Respiratory depression develops secondarily to central nervous system depression resulting from the stimulation of α2-adenoreceptor agonists. However, the degree of depression caused by α2-adenoreceptor agonists alone is lower than that caused when used in combination with other sedatives at sublethal doses [22].

In rabbits, intranasal administration of dexmedetomidine (0.1 mg/kg)-midazolam (2 mg/kg)-butorphanol (0.4 mg/kg) resulted in a statistically significant decrease in respiratory rate from 100/min to 33/min after 40 min [17].

Another study in rabbits also showed that intramuscular administration of a Ketamine-Dexmedetomidine combination immediately reduced respiratory rate from 77.5/min to 49/min after 5 min, and remained below the baseline value throughout anesthesia with an average of 49-59/min [19]. In this study, similar decreases in respiratory rate were observed in the Butorphanol group.

However a higher respiratory rate was measured in the Buprenorphine group compared to the Butorphanol group at all times following injection. The baseline respiratory rate decreased from 72/min in both groups to a range of 25-41/min in the

Butorphanol group, while in the Buprenorphine group, this value was measured as 49.8 ± 12.4. Similarly, a higher respiratory rate was observed in the Buprenorphine group compared to Butorphanol in this study as well.

Because Buprenorphine has a less pronounced analgesic effect than Butorphanol, it was observed that more pain was generated in response to external stimuli, and the respiratory rate was also higher. Surgical anesthesia duration and total sleep duration were also shorter in the Buprenorphine group compared to Butorphanol. This supports these findings.

Deeper analgesia and a longer sleep duration were observed in the Butorphanol group compared to Buprenorphine. In this study, it was observed that the respiratory rate decreased more in the Butorphanol group compared to buprenorphine following injection. This decrease was measured within physiological limits at all times.

Peripheral oxygen saturation decreased in both groups at the 5th min of anesthesia, while it was measured at 95 %, close to the baseline value, at the 60th min of anesthesia in both groups. In all groups, patients were externally oxygenated throughout anesthesia to protect them from hypoxia.

In all rabbits in both groups, peripheral oxygen saturation (SpO2) dramatically approached the 85 % mark within 1 min following anesthesia and was immediately inhaled with 100 % oxygen. However, in this way, an attempt was made to create a safe anesthesia by maintaining SpO2 above 90 % in all animals.

Hypoxemia (SpO2 dropping to 85-89 %) was observed in 2 cats in the Butorphanol group and in 1 cat in the hydromorphone and buprenorphine group. In parallel with this study, hypoxemia was observed in our study within the first 5 min following anesthesia, and all animals responded positively to external oxygen supplementation, with oxygen levels rising back above 95 % in all animals.

Body temperature changes

Body temperature remained close to the baseline values in both groups, but showed a sharp decrease after the 15th min of anesthesia and remained below the baseline value at the 60th min of anesthesia. Throughout the anesthesia, the animals were heated with a hot water bottle from below to protect them from hypothermia.

No significant differences were observed within or between groups in terms of body temperatures. The changes in body temperature, respiratory rate, heart rate, and SpO2 levels over time for patients in the Butorphanol and Buprenorphine groups are shown in TABLE II.

In this study, body temperature remained close to the baseline values in both groups, but showed a sharp decrease after the 15th min of anesthesia and remained below the baseline value at the 60th min of anesthesia. Throughout the anesthesia, animals were kept warm with a hot water bottle from below to prevent from hypothermia. No significant differences were observed within or between groups in terms of body temperature.

In other study, Santangelo *et al*. [17] observed statistically significant decreases in body temperature from 39 °C to 38.4 °C and 38.2 °C, respectively, 45 and 60 min after intranasal administration of Dexmedetomidine-Midazolam-Butorphanol in rabbits. In another study conducted on rabbits, anesthesia induced by Dexmedetomidine-Ketamine and Xylazine-Ketamine was compared, and it was observed that body temperature decreased from 39.4 °C to 37.8 °C in the Dexmedetomidine-Ketamine group, while it decreased from 39.3 °C to 38.1 °C in the Xylazine-Ketamine group. This decrease in both groups was statistically significant [19]. α2-adrenoreceptor agonists have been reported to cause a decrease in body temperature in many animal species.

Cardiovascular system findings

The heart rate was found to be slightly higher in the Buprenorphine group at the 0th min of anesthesia compared to the Butorphanol group. Subsequently, a slight increase was observed in the Butorphanol group at 5th min of anesthesia, while a slight decrease was observed in both groups throughout the entire anesthesia period.

In our study, heart rate was slightly higher in the Buprenorphine group at 0th min of anesthesia compared to the Butorphanol group. Subsequently, a slight increase was observed in the butorphanol group at 5th min of anesthesia, while a slight decrease was observed throughout the anesthesia in both groups. In a comparative study in dogs using the dexmedetomidine (15 µg/kg)-ketamine (3 mg/kg) combination with the addition of Butorphanol (0.2 mg/kg) and buprenorphine (40 µg/kg) for castration purposes, significant decreases in heart rate of up to 50 % were observed in both groups. Decreases in heart rate after administration of β-2 agonists have been well studied and established in all animal species in the study [13, 23].

TABLE II

Changes in body temperature, respiratory rate, heart rate, and peripheral oxygen saturation levels over time in patients in the Butorphanol and Buprenorphine groups (mean ± SE).

Time	Body Temperature (°C)		Respiratory Rate (min^{-1})		Heart Rate (min^{-1})		SpO_2 (%)	
	Buprenorphine	Butorphanole	Buprenorphine	Butorphanol	Buprenorphine	Butorphanole	Buprenorphine	Butorphanol
0 min	38.8 ± 0.1	38.9 ± 0.1[a]	34.7 ± 6.2	59.6 ± 13.1	235 ± 7.9[a]	221.5 ± 13.7	95.7 ± 0.9	97 ± 0.7
5 min	38.9 ± 0.4	39.1 ± 0.2	46.5 ± 8.6	39.9 ± 7.5	233.4 ± 10.0	240.4 ± 5.6	92.9 ± 2.1	94 ± 2.1
15 min	39.0 ± 0.2	39.0 ± 0.2	69.1 ± 14.3	35.7 ± 8.0	187 ± 25.2	218.4 ± 5.2	94.1 ± 1.0	92.9 ± 1.7
30 min	38.7 ± 0.3	38.7 ± 0.2	49.4 ± 11.9	37.2 ± 3.6	195.1 ± 13.7	198.9 ± 9.7	92.4 ± 2.1	93.5 ± 2.3
45 min	38.7 ± 0.3	38.6 ± 0.2	42.2 ± 11.6	37.9 ± 5.3	199.2 ± 12.6	205.6 ± 6.9	94 ± 2.5	95.6 ± 1.2
60 min	38.7 ± 0.2	38.5 ± 0.3[b]	40.9 ± 7.2	44.9 ± 8.4	194.6 ± 15.6[b]	201 ± 9.3	95.5 ± 1.1	95.9 ± 1.4

[a,b]: Data indicated by different letters in the same column are statistically significant (Differences are presented relative to 0 min).

A slight decrease in heart rate was also recorded in cats following the dexmedetomidine-ketamine-Butorphanol combination, and this decrease was also observed in our study. This condition, while a very serious bradycardic state in dogs, can be interpreted as species sensitivity to dexmedetomidine in cats and rabbits. A slight decrease in heart rate has also been reported in rabbits after intramuscular injection of ketamine-dexmedetomidine [24]. Similarly, a slight decrease in heart rate has been observed in rabbits after intranasal dexmedetomidine-butorphanol-midazolam combination [17]. In this study, unlike ours, ketamine was not used. Ketamine's positive inotropic effect on the heart is known. In other words, when it is used alone, it causes increases in heart rate. The increase observed at 5th min in the Butorphanol group in our study can be attributed to this effect of ketamine.

Biochemical parameters

It was determined that there were no statistically significant differences in the changes in cTn-I and CRP levels in the animals in both groups subjected to anesthesia applications in terms of group, time and group-time relationships (TABLE III). It was also observed that neither of these applications caused any possible inflammatory process and changes that could negatively affect the heart muscle in the animals in the groups.

TABLE III
Changes in C-reactive protein and cardiac troponin I values over time in subjects in the Butorphanol and Buprenorphine groups (mean ± SE).

Time	CRP (ng/L)		cTn-I (ng/L)	
	Buprenorphine	Butorphanol	Buprenorphine	Butorphanol
Baseline	993.3 ± 122.6	773 ± 74.5	827 ± 95.2	681.3 ± 49.2
30 min	852.2 ± 86.9	656.4 ± 53.9	810 ± 110.7	655.3 ± 66
6 h	721.1 ± 72.9	960.1 ± 150.1	683.4 ± 66.3	725 ± 56.3
12 h	1061.3 ± 333.8	812.4 ± 99.6	604.3 ±1 26.4	683.2 ± 5 9.8
24 h	664.6 ± 80.9	616.4 ± 31.1	767 ± 78.5	565 ± 104.9

CRP: C-reactive protein, cTn-I: cardiac troponin I, min: minute,h:hours

In the presented study, CRP levels were measured to be higher in the Buprenorphine group compared to the Butorphanol group. In both groups, following a gradual increase 12 h after anesthesia, levels approached or decreased to baseline values one d after anesthesia. In this case, it was concluded that neither anesthetic combination caused any inflammatory conditions.

Serum CRP levels and cardiac biomarkers (atrial natriuretic peptide and angiotensin-converting enzyme were investigated in five different healthy rabbit breeds (New Zealand rabbit, Belgian giant rabbit, Dutch lop rabbit, Giant French butterfly rabbit, and American fuzzy lop rabbit). The study revealed significant differences both between species and individually. These differences were statistically significant. Therefore, the need to consider this finding is emphasized [25]. In this study, New Zealand rabbits were used as well, and CRP levels were measured to be higher in the Buprenorphine group compared to the Butorphanol group in healthy animals.

In another study, the effect of anesthesia and surgery on acute phase proteins and cortisol levels in rabbits was investigated. Plasma adrenaline levels 24 h after the operation were found to be statistically significantly lower in the laparoscopic group compared to the classic group. No statistically significant differences were observed between groups in adrenocorticotropic hormone, norepinephrine, IL-6, and CRP levels [26].

In this study, serum cTn-I levels were lower in the Butorphanol group compared to the Buprenorphine group. In almost all measured ranges, slight increases or decreases within physiological limits that were not statistically significant were recorded. Based on this, it was concluded that neither of the two anesthetic combinations caused any adverse effects on the heart muscle during the specified anesthesia period.

In another study conducted on rabbits, cTn-I and CRP levels, which are indicative of possible side effects on the heart from injectable anesthetic combinations such as Medetomidine-Ketamine-Midazolam and Xylazine-Ketamine, inhalation anesthetics such as isoflurane and sevoflurane, and injectable anesthetic combinations such as Propofol and Fentanyl, were comparatively investigated [18].

As a result, in parallel with this study, no significant difference was determined in CRP and cTn-I values between the groups and compared to baseline values. Again, in another study conducted on rabbits, the effects of anesthesia induced by Ketamine-Medetomidine and Fentanyl-Propofol were comparatively investigated in terms of some hemodynamic parameters and cTn-I and CRP levels [27].

Serum CRP levels decreased over time compared to baseline values in both groups. In addition, cTn-I levels reached their highest point at the 6th h in the Medetomidine-Ketamine group, while they fell below baseline in the Propofol-Fentanyl group during the same time period. Similar studies have been conducted in different animal species and similar results have been obtained. In another study by Saunders *et al*. [28], cTn-I and CRP levels were compared in dogs scheduled for ovariohysterectomy and intraabdominal cryptorchid castration using two different anesthesia protocols [29]. In this study, no surgical procedure was performed, and no increase in cTn-I values was observed during or after anesthesia, as well.

CONCLUSIONS

In conclusion, both the Ketamine-Dexmedetomidine-Midazolam-Butorphanol and the Ketamine-Dexmedetomidine-Midazolam-Buprenorphine combinations were found to be suitable for short-term surgical procedures in rabbits within the recommended dose range.

However, the Butorphanol combination appears superior to Buprenorphine because it provides a longer duration of surgical anesthesia and a deeper level of sedation. Neither combination caused an increase in serum CRP and cTn-I concentrations, and therefore it was concluded that they can be used safely within this dose range and for short-duration anesthesia.

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Conflict of interest

The authors declare no conflict of interest.

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