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Evaluation of Xylazine Ketamine Anesthesia in Rabbits Undergoing Tendon Surgery: A Prospective Randomized Controlled Study

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ABSTRACT: *This study was designed to evaluate the efficacy of xylazine-ketamine anesthesia on reflexes and vital signs during and after tendon surgery in rabbits. Twenty healthy adult male rabbits (2-2.5 kg) were randomly assigned to two groups Xylazine-ketamine (XK) group and ketamine (K) group. Intramuscular injections of xylazine (5 mg/kg) and ketamine (35 mg/kg), were given to rabbits. The clinical parameters including rectal temperature, heart rate, and respiratory rate were estimated prior to injection 0, 5, 15, 30, 45, 60, 75, 90, and 120 minutes post-anesthetic injection, As well as reflexes ear pinch reflex, and pedal reflex were measured before and after anesthetic injection. In the XK group, we observed that rectal temperature increased considerably ($P < 0.05$) at 5 and 15 minutes after induction and then gradually fell to preanesthetic control values. During the anesthetic phase, both groups' heart rates and respiration rates were reduced significantly. In XK-injected rabbits, the return of reflexes was delayed compared to K injected groub. Surgical anesthesia in XK group lasted longer than K group. The XK combination provided sufficient anesthesia for rabbits, as evidenced by a prolonged anesthetic period, and good cardiovascular and other clinical indices.*

KEYWORDS: Anesthesia, ketamine, rabbit, Tendon, xylazine

1. Introduction

Rabbits have been extensively employed in biomedical research due to their diminutive size, facile handling, and the availability of a diverse array of tissues and organs for investigative purposes [1]. Tendon surgery is a commonly performed procedure in rabbit models, contributing to our understanding of tendon healing, biomechanics, and the tendon-bone interface [2]. However, while these surgical procedures are instrumental in advancing scientific knowledge, the potential pain and discomfort experienced by the animals during the procedures necessitate the inclusion of anesthesia to alleviate their distress [3]. Various anesthetic agents are utilized in rabbit surgery, including ketamine, xylazine, and a combination of both [4]. Ketamine, a dissociative anesthetic, provides sedation, analgesia, and amnesia[5]. Xylazine, on the other hand, acts as a sedative, inducing muscle relaxation, analgesia, and anxiolysis [6]. The combination of xylazine and ketamine offers a more profound level of anesthesia compared to either agent alone, which is favored for its safety and efficacy [7] and is frequently employed in rabbit surgery [8] Nonetheless, there is a paucity of research on the use of xylazine-ketamine anesthesia specifically in rabbits undergoing tendon surgery. As a result, this study aims to assess the effectiveness of xylazine-ketamine anesthesia in rabbits undergoing tendon surgery. To the best of our knowledge, this is the first study to explore the application of xylazine-ketamine anesthesia in rabbits undergoing tendon surgery. The outcomes of this study are expected to offer valuable insights to the scientific community regarding the efficacy of xylazine-ketamine anesthesia in this context. Furthermore, this research will contribute to the ongoing endeavors to mitigate pain and discomfort in animals utilized for scientific research.

2. Materials and Methods

2.1. Animal Selection

Twenty clinically healthy, adult male rabbits, aged between 6-8 months, with a weight range of 2-2.5 kg, were included in the study. These animals were individually

housed in cages for a minimum of 7 days prior to the experiments for acclimatization. During this period, they were provided with commercial pellet food and ad libitum access to fresh water. However, food intake was suspended for at least 12 hours before the experiments. The study was conducted following the approval of the study protocol by the ethical committee of the Faculty of Veterinary Medicine

2.2. Anesthetic Protocols

The rabbits were randomly allocated to two distinct groups. The XK group (n = 10) received intramuscular injections of a mixture of xylazine-ketamine hydrochloride (Xylazine: 5 mg/kg B.W (Xylaject® 2%, ADWIA Co., Egypt) and Ketamine: 35 mg/kg B.W (Ketamine® 5%, Sigma-Tec Co., Egypt). In contrast, the K group (n = 10) were anesthetized solely by intramuscular injection of Ketamine: 35mg/kg B.W.

2.3. Surgical procedure

All rabbits in both groups underwent surgical exposure of the Achilles tendon. An animal model was created by splitting the Achilles tendon, which was subsequently utilized in experimental studies on tendon healing. After aseptic preparation, a curved incision of approximately 1 cm in length was made on the lateral side of the Achilles tendon to form a skin flap. Care was taken to ensure that the tendon remained unharmed beneath the skin wound. Two parallel incisions, spaced 0.5 mm apart and measuring 5 mm in length, were then created within the tendon, extending from the point where the tendon attaches to the calcaneus to the middle of the tendon. Utilizing micro scissors, the central portion of the tendon was excised, resulting in a full-thickness defect at the mid-substance. Subsequently, the respective biomaterials were applied in each experimental group, and the subcutaneous tissue and skin were closed through standard procedures (Fig. 4). Following the closure of the skin, the treated limb was immobilized using a splint cast, and the rabbits were kept in a warm and monitored environment until they regained consciousness.

2.4. Anesthesia Evaluation

Post-injection, the depth of anesthesia was assessed based on pedal withdrawal, ear pinch, and righting reflexes, with evaluations conducted every 5 minutes. The induction time was determined as the time elapsed from the injection to the loss of the righting reflex. Ear pinch reflex was gauged as the response to pinching the pinna with the thumb and index finger. The pedal withdrawal reflex was measured as the reaction to pinching one digit with the thumb and index finger. The recovery from anesthesia was evaluated by observing the animal's ability to regain the righting reflex.

2.5. Measured Physiological Parameters

Heart rate, respiratory rate, and body temperature were monitored prior to injection (0 min) and at 5, 15, 30, 45, 60, 75, 90, and 120 minutes' post-anesthetic injection. The heart rate was ascertained using a stethoscope placed on the lower left lateral thoracic wall, respiratory rate was visually counted, and body temperature was recorded using a digital rectal thermometer. Following the administration of XK, the animals underwent surgery, which included splitting tenotomy. The depth of anesthesia was considered by observing the animal's response to a surgical incision.

2.6. Statistical Analyses

Comparison of each parameter among different groups was performed using an independent-sample t-test. Within the same group, the changes in the value of each parameter after anesthetic injection were compared using a paired-sample t-test. Significance was determined when $p < 0.05$, and all statistical analyses were conducted using SPSS software. Results are presented as means ± standard deviations (SD).

2.7. Ethical approval

This study was approved for research ethics from the committee of research ethics, faculty of Veterinary Medicine, New Valley University with Ref. Numb. 04-2023-100068.

Table 1: Induction time, duration, and recovery times

Criteria	XK group	K group
Induction time (min)	1.8	2.0
Duration of loss of ear pinch reflex (min)	55	30
Duration of loss of pedal withdrawal reflex (min)	50	28
Recovery time (min)	45	30

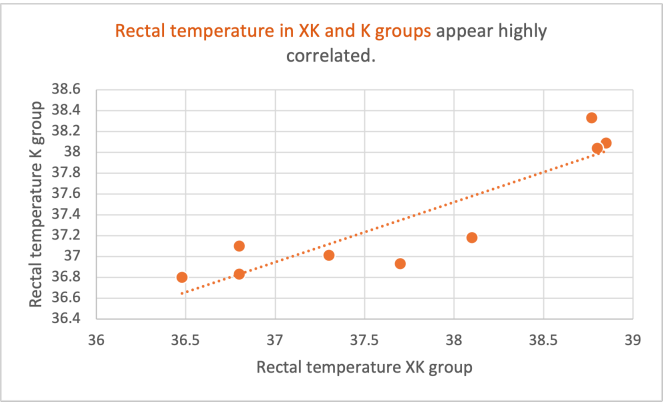

Figure 1: Graph 1

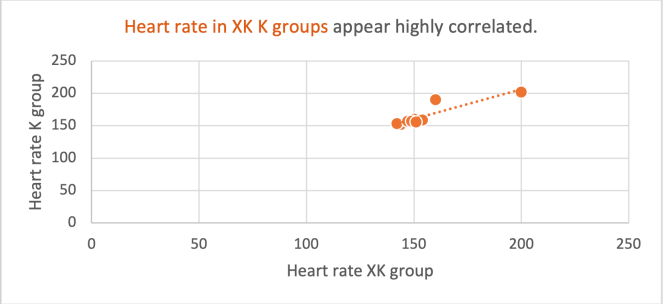

Figure 2: Graph 2

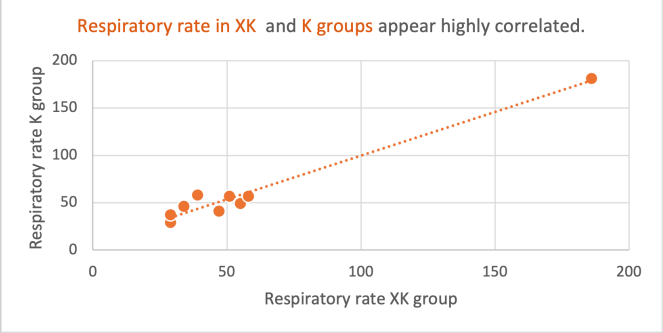

Figure 3: Graph 3

3. Results

timeline (Table 2). In the XK group, the induction time, representing the time from injection to the loss of the righting reflex, was observed to be 1.8 minutes. Additionally, the duration of the loss of the ear pinch reflex, indicative of anesthetic depth, was measured at 55 minutes, while the duration of the loss of the pedal withdrawal reflex was 50 minutes. The recovery time in the XK group was 45 minutes. In the K group, which received a different anesthetic regimen, the induction time was slightly longer, recorded at 2.0 minutes. The duration of the loss of the ear pinch reflex was notably shorter, at 30 minutes, compared to the XK group. Similarly, the duration of the loss of the pedal withdrawal reflex was 28 minutes. The recovery time in the K group mirrored that of the XK group, being 30 minutes. These findings highlight the differences in anesthetic parameters between the two groups, providing valuable insights into the anesthesia's effects on the rabbits undergoing tendon surgery (Table 1.) In both the XK group and the K group, various physiological parameters were monitored at different time intervals during the experimental procedure. The heart rate, measured in beats per minute (bpm), exhibited specific trends over time. At the outset (0 minutes), the XK group had a heart rate of 200 bpm, slightly higher than the K group, which recorded a rate of 202 bpm. As time progressed, both groups showed a decrease in heart rate. At 120 minutes, the XK group had a heart rate of 151 bpm, while the K group had a heart rate of 156 bpm. Similarly, respiratory rate, measured in breaths per minute (bpm), varied throughout the experiment. At the beginning (0 minutes), the XK group displayed a respiratory rate of 186 bpm, whereas the K group exhibited a rate of 181 bpm. Over time, the respiratory rates in both groups fluctuated, and by the end of the experiment (120 minutes), the XK group had a respiratory rate of 58 bpm, whereas the K group had a rate of 57 bpm. Rectal temperature, measured in degrees Celsius (°C), was also recorded at different time points. At the start of the experiment, the XK group had a rectal temperature of 38.77°C, while the K group had a temperature

of 38.33°C. The temperature values showed variations over time, with both groups experiencing a slight decline. At 120 minutes, the XK group had a rectal temperature of 36.80°C, and the K group recorded a temperature of 37.10°C. This comprehensive data provides a detailed insight into the dynamic physiological responses of rabbits in both groups undergoing tendon surgery and anesthesia, highlighting the changes in heart rate, respiratory rate, and rectal temperature throughout the experimental

Discussion

Surgical procedures are associated with a complex stress response characterized by neurohormonal, immunological, and metabolic alterations which are intimately related to the severity of the lesion, duration of surgery, and the clinical status of the patient[9]. These responses induce a wide range of alterations in diverse organs and systems, which have resulted in various studies aimed at minimizing or inhibiting these alterations. Acute pain is the result of a traumatic, surgical, or infectious event. Acute pain has a biological function in that it serves as a warning that something is wrong. Nociceptive stimulation of surgical site causes hyperventilation, increased hypothalamic neural sympathetic tone, and increased release of catecholamines and other endocrine hormones which consequently increase blood pressure, cardiac work, and myocardial oxygen consumption. In addition, there is an increased secretion of cortisol [10, 11]. Pain is generally alleviated using analgesic or anesthetic drugs during and post-surgery. It is very important to use an anesthetic procedure to alleviate pain of the patient under surgical operation. Less expensive and easy to use, injectable anesthesia is preferable than inhalation anesthesia for small animal surgeries. Xylazine plus ketamine is an injectable anesthesia used in small animal surgeries. Xylazine is one of the alpha-2 adrenergic agonists and produces dose-dependent sedation, analgesia, and muscle relaxation. However, alpha-2 agonists profoundly alter cardiovascular function by producing bradycardia, hypertension followed by hypotension, decreased myocardial contractility, and dysrhythmias [10, 12, 13] Throughout the anesthetic period, there were slightly significant changes in rectal temperature in group K in this investigation. Similar findings have been reported by others [14]. In group XK, however, the rectal temperature was slightly higher at 5 and 15 minutes compared to preanesthetic control values. The increase in body temperature could be due to anesthetics (xylazine, ketamine) eliciting the thermoregulatory center and causing animals to become hyperthermic [15] (Fig. 1). Heart rate reduced significantly from 5 to 45 minutes (group XK) and 15 to 30 minutes (group K) compared to preanesthetic control values, then increased and returned to baseline values. This conclusion was in line with the findings of [15] When xylazine is given, it causes peripheral vasoconstriction, which causes an increase in arterial blood pressure and a drop-in heart rate [16] (Fig. 2). In this investigation, the RR was first reduced from its preanesthetic control values, then fluctuated up to recovery in all groups of animals (Fig. 3). According to [17, 18] , xylazine's respiratory effects are normally clinically negligible, but it can cause respiratory depression, with a decrease in tidal volume and respiratory rate, when used in combination with other medicines. The surgical anesthetic duration was measured using ear pinch reflex and pedal withdrawal reflex, as described in the literature. In adult rabbits, [19, 20] found that reflex loss and return periods varied according to the dose of anesthetic regimens, with stronger doses providing longer sedation durations and longer pedal withdrawal reflex return times. The results of this study provide valuable insights into the physiological responses of rabbits undergoing tendon surgery with different anesthesia protocols. Specifically, we observed notable differences in heart rate, respiratory rate, and rectal temperature over time in both the XK group and the K group and agreed with Casas-Díaz et al. [21]. Moreover, the study investigated anesthesia-specific parameters, including induction time and duration. Induction time, defined as the time from the administration of anesthesia to the loss of the righting reflex, is a critical measure of anesthetic efficiency [22] . In our study, the XK group demonstrated a shorter induction

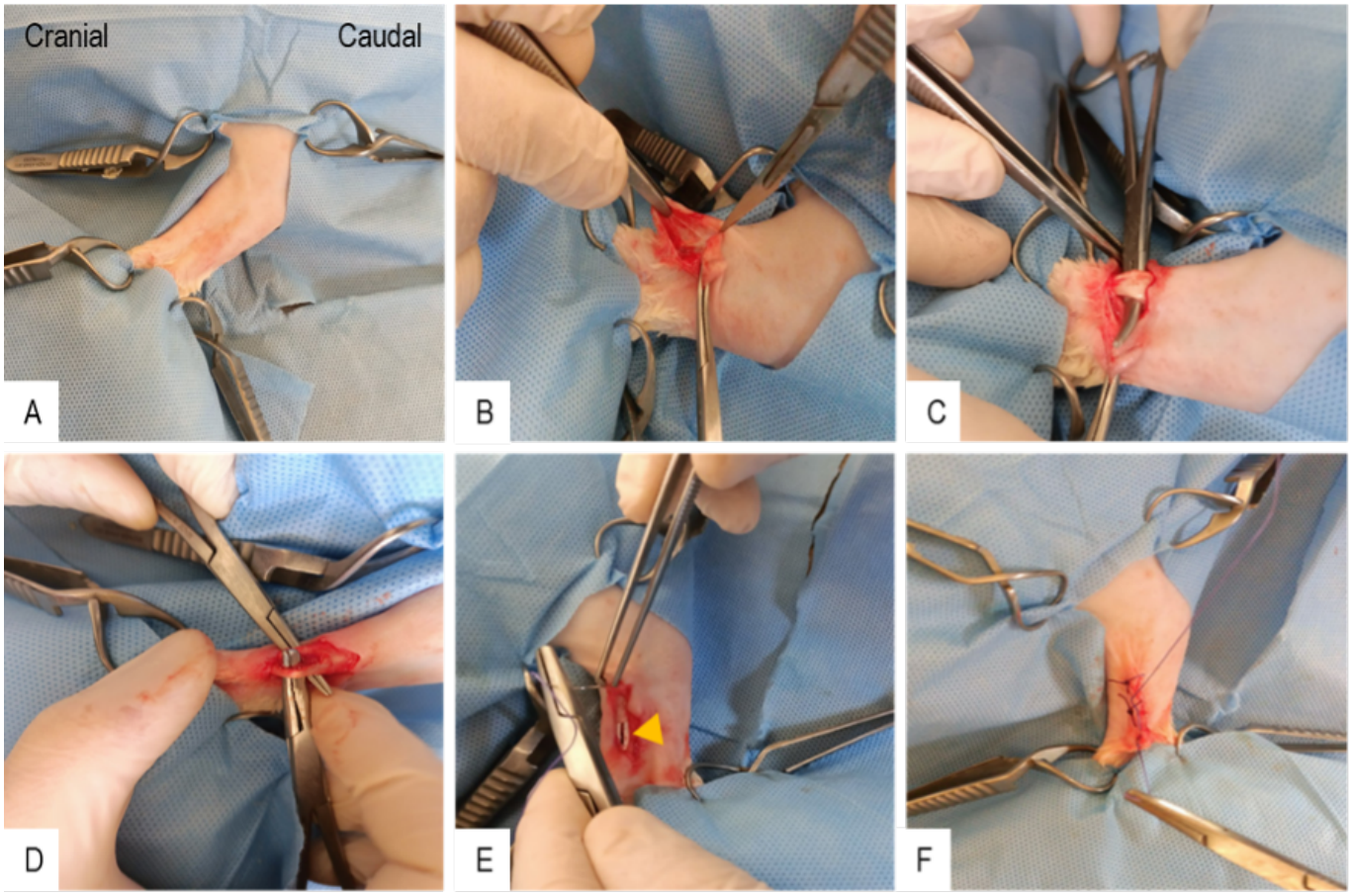

Figure 4: Achilles tendon splitting rabbit model surgery. A- preparation of the surgical site, B- skin and subcutaneous dissection, C-Achilles tendon exposure, D- splitting of the tendon, E- the yellow arrowhead indicates the split site, and F- closure of the skin wound.

Table 2: Means of physiological parameters among examined groups

Time (min.)	Heart rate		Respiratory rate		Rectal temperature	
	XK group	K group	XK group	K group	XK group	K group
0	200	202	186	181	38.77	38.33
5	160	190	55	49	38.85	38.09
15	150	160	47	41	38.80	38.04
30	144	152	29	29	38.10	37.18
45	142	153	29	37	37.70	36.93
60	147	157	34	46	37.30	37.01
75	149	157	39	58	36.80	36.83
90	154	159	51	57	36.48	36.80
120	151	156	58	57	36.80	37.10

time of 1.8 minutes, while the K group exhibited a slightly longer induction time of 2.0 minutes. This variance could be attributed to the presence of xylazine in the XK group, which, as a sedative, likely facilitated a quicker onset of sedation compared to the K group, which received ketamine alone [23] . A shorter induction time is favorable in clinical practice, as it minimizes the stress and discomfort experienced by the animals. The duration of the loss of the ear pinch and pedal withdrawal reflexes, which reflects the depth of anesthesia, is also of great importance [24] . In our study, the XK group displayed a longer duration of the loss of the ear pinch reflex (55 minutes) and the pedal withdrawal reflex (50 minutes) compared to the K group, which showed shorter durations (30 and 28 minutes, respectively) according to [25] . This observation indicates that the xylazine-ketamine combination in the XK group resulted in a more prolonged and deeper state of anesthesia than ketamine alone in the K group [26] . This finding aligns with the known characteristics of xylazine, which provides a more profound sedation and muscle relaxation. The physiological parameters, including heart rate, respiratory rate, and rectal temperature, exhibited dynamic changes throughout the experiment. Both groups experienced a decrease in heart rate and respiratory rate, which is expected with the administration of anesthesia. The marginal differences in these parameters between the two groups suggest that, despite the distinct anesthesia regimens, they reached a similar level of respiratory depression, likely due to the pain-relieving and muscle relaxant properties of the anesthetics. Rectal temperature also decreased, with the XK group exhibiting a greater reduction, potentially indicating a more profound level of anesthesia.

Conclusion

In conclusion, the study not only sheds light on the dynamic physiological responses of rabbits to different anesthesia regimens during tendon surgery but also highlights the importance of induction time and duration as critical factors to consider when selecting anesthetic agents. These findings can guide future research and clinical practice in optimizing anesthesia protocols to ensure the well-being and safety of animals undergoing surgical procedures. Further investigations may be warranted to delve deeper into the specific impacts of xylazine-ketamine anesthesia on these physiological and anesthetic parameters and their clinical implications for veterinary medical sciences.

Conflict of interest

The authors declare that there is no conflict of interest.

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