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Evaluation of the effectiveness of different doses of ketamine / xylazine according to age and gender in rats

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ABSTRACT

Aim: To investigate the effect of different intraperitoneal (IP) doses of K/X on anesthesia according to age and gender.

Method: The rats were divided into three main groups according to the dose of K/X they received (GI:40/5, GII:60/7.5, and GIII: 90/10 mg/kg). These three groups were further subdivided into F and M. In each dose group, F and M groups were subdivided into five distinct age categories: 2-6 months, 7-12 months, 13-18 months, 19-24 months, and older than 25 months.

Results: There were differences in the muscle tone, pinch, palpebral and corneal reflexes at K/X administration doses of (GI), (GII), and (GIII) in ages and genders (Table 1). We detected that 20-25 minutes of superficial anesthesia was achieved with a dose of 40/5 mg/kg in 2-6 and 7-12 months rats both male and female. A dose of 60/7.5 mg/kg was sufficient for short-term (35-minute deep anesthesia) procedures in male rats aged 2-6 and 7-12 months. In addition, we found that 90/10 mg/kg ip dose was an effective anesthesia dose in all age groups and both genders.

Conclusion: Our findings indicate that the duration of anesthesia was prolonged with increasing age in all age groups. Furthermore, we observed that the duration of anesthesia was prolonged in males compared to females. We contend that a dose of 90/10 mg/kg represents the optimal balance between the induction of anesthesia and the total duration of anesthesia.

Keywords: Ketamine, xylazine, rat, anesthesia. age, gender.

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Introduction

Choosing appropriate anesthesia protocols is the most important starting phase of experimental studies with animals. According to the ethical rules, it is necessary to give the proper anesthetic substance and dose for the breed, species, sex, and age of the animal. The effects of many anesthetics are known to vary with gender and age [1]. The anesthetic drugs that are nontoxic, have rapid onset of action, a wide confidence interval, providing rapid induction and recovery are the ideal anesthetic drugs. Therefore, anesthesia protocols that provide a fast onset of action, sufficient surgical time, and rapid recovery should be preferred. Choosing the appropriate anesthetic drug,

administration route, and dose also affects the success of the study. An experienced researcher should choose the appropriate drug, route, and dose suitable for the breed and age of the animal, purpose of the experiment, duration, and content of the experiment [2,3].

Induction and maintenance of anesthesia in experimental studies can be performed by inhalation, intravenous (IV) route, intramuscular (IM) route, and intraperitoneal (IP) route. Inhalation agents are not preferred much due to lack of equipment, exposure to gas, and late-onset action although they are suitable for short-term procedures [4,5]. Intravenous drugs such as propofol, barbiturates, medetomidine, and ketamine-xylazine are preferred because of their ease of use in different ways, rapid onset of action, and long effects [6]. Ketamine is a phencyclidine analogue drug that is fast-acting, powerful analgesic, and mild anesthetic. It was first put into use in 1960. Its chemical structure is 2-o-chlorophenyl-2-methylaminocyclohexanone hydrochloride. It has a quite complex mechanism of action. It affects opiate, muscarinic, cholinergic, and nicotinic receptors. However, it shows its main effect by blocking the NMDA receptors in the brain [7,8]. It is more effective alone in rats than other rodents. However its muscle relaxant effect is weak, and its analgesia is inconsistent. It can be used intravenously, intramuscularly, and intraperitoneally. It reaches the maximum effect in 10 minutes (min), the effect can last 30-40 minutes, and it may take 1.5 hours to fully recover. It has the advantage of increasing cardiopulmonary function and a wide range of safety. However, it can cause increased muscle tone, tremors, tonic-clonic seizures, and salivation; one of its disadvantages is that it leads to wide variations in response between species and individuals. Muscle rigidity may occur when used alone. It is always used in combination with xylazine or sedative medication to avoid this. Therefore, Ketamine/xylazine (K/X) is the most used proper drug combination in laboratory studies [9,10].

In this study, we aimed to determine the most effective intraperitoneal K/X dose by researching the duration and depth of anesthesia according to age and gender by using low doses different from the doses used in literature. The study also aimed to determine the most effective doses of anesthetic.

Materials and Methods

The effect of age and sex on the efficacy of K/X at different doses was investigated in this study. The current study was conducted on male and female albino Wistar rats obtained from Bolu Abant İzzet Baysal University (BAIBU) Experimental Animals Application and Research Center. The experiments were conducted in accordance with the approval granted by the BAIBU Animal Research Local Ethical Committee on 13 November 2015, with the approval number 2015/48. All procedures were conducted in accordance with the US National Research Council's Guide for the Care and Use of Laboratory Animals (Eighth Edition, 2011), and adhered to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices.

Procurement of experimental animals and study groups: The experimental animals to be used in the study were obtained from BAIBU Experimental Animals Application Research Center. The animals were kept for 12 hours light/dark, relative humidity 55-60 % in the same center from the start to the end of the study and fed ad libitum.

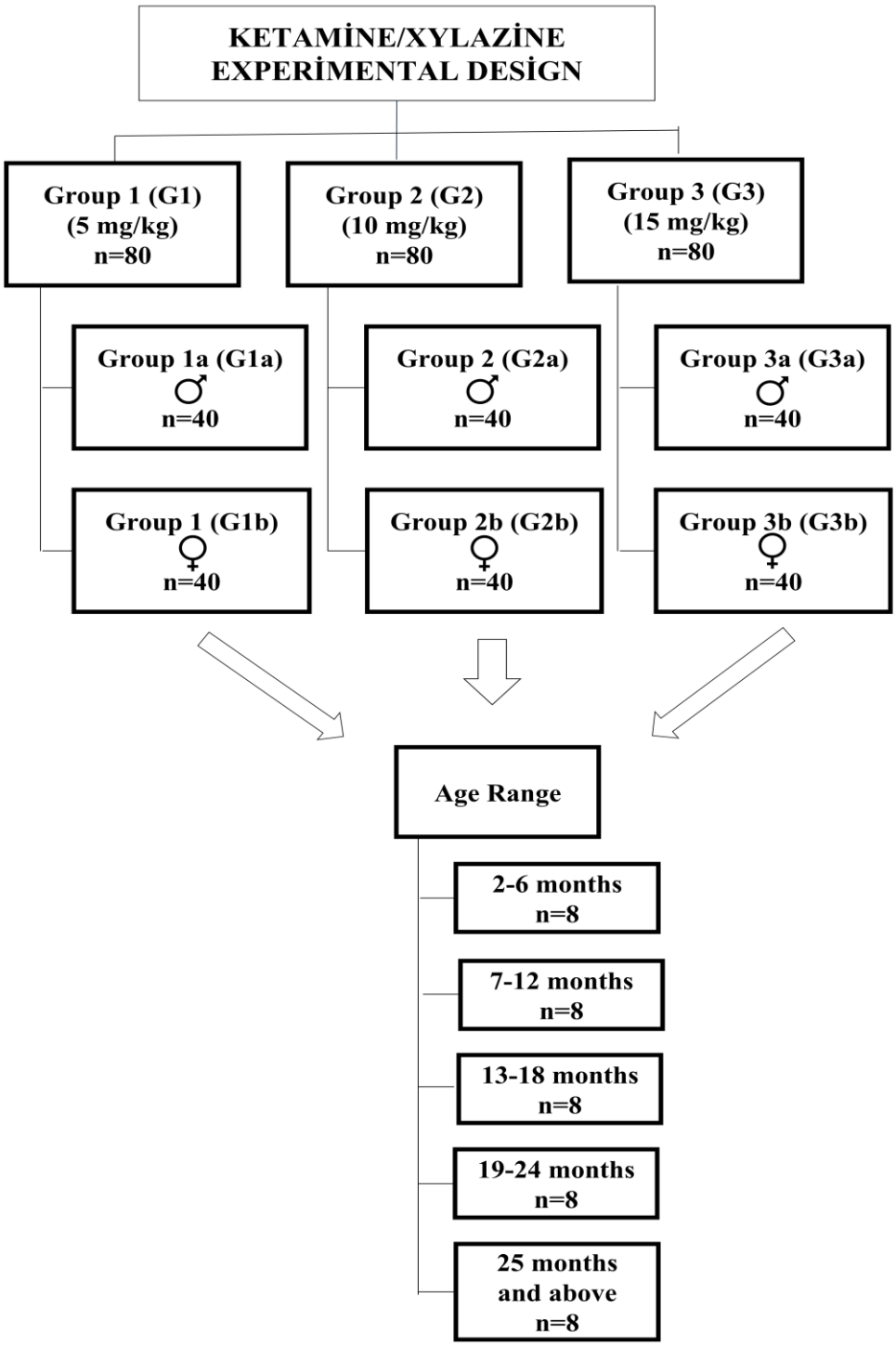

Figure 1. Ketamine/xylazine experimental design.

In the present study, the age ranges of rats were determined by comparing the average age of rats with that of humans, based on previously published data by Robert Quinn [11] and Sengupta [12]. The average age of rats was assumed to be three years. Similarly, the average human age was assumed to be 80 years. The calculation is based on 26.7 human days divided into 1 rat day and 13.7 rat days divided into 1 human year. To determine the age ranges

and groups for this study, the above equation and assumptions were used.

Male and female rats were divided into 5 subgroups based on their gender: 2-6 months (0-12 years of age = Childhood), 7-12 months (12-18 years of age = Adolescent), 13-18 months (30-45 years of age = Young Adults), 19-24 months (45-60 years of age = Adulthood), and 25 months over (65 years of age = Elderly).

Ketamine / xylazine was administered in three different doses as; G1, 40/5 mg / kg; G2, 60 / 7.5 mg / kg; G3, 90/10 mg / kg. Ketamine/xylazine were administered in male and female rats based on their genders (G1a-b; G2a-b; G3a-b) and age groups (G1a-b♀♂; G2a-b♀♂; G3a-b♀♂) (Figure 1).

Determination of anesthetic agent doses: Ketamine / xylase was used in three different doses. Drug doses were determined as the most intraperitoneally [13] used classical dose (60 / 7.5 mg/kg), half of the classical dose (40/5 mg/kg) and 1.5 times the classical dose (90/10 mg/kg).

Determining the depth of anesthesia: Efficacy and depth of anesthesia were assessed at 1, 5, 10, 20, 30, 40, 50, 60, and 80 min after ketamine/xylazine administration by measuring the palpebral, pinch, and corneal reflexes and muscle tone (by pulling the lower jaw or extremities).

During the deepening of the anesthesia, the scoring was as follows:

1. Palpebral reflex: If the eyelids blink when touched, there is superficial anesthesia.
2. Pedal reflex: Superficial anesthesia when the animal pulls on the toe with a pinch
3. DA; Corneal reflex: Deep anesthesia if the animal does not blink to cotton/thread touch.
4. Jaw Ton: Deep anesthesia, if there is no pull in the lower jaw or the extremities.
5. Observing the absence of these reflexes, the anesthesia exit time (AE) was determined.

Statistical Analysis: Descriptive data derived from the results of the study were presented as mean ± standard deviation. The distribution of the variables was evaluated by the Kolmogorov-Simirnov test. Normally distributed data obtained from the experimental groups were analyzed with the one-way ANOVA test. The intergroup differences were evaluated with the Bonferroni post-hoc test. Non-normally distributed data were analyzed with Kruskal Wallis test and intergroup differences were evaluated with Mann Whitney test. A p-value of <0.05 was accepted as statistically significant. All statistical analyses were performed with SPSS (IBM® SPSS® Statistics V22.0).

Results

In this study, Ketamine/xylazine was administered to rats in five different age groups (2-6 months, 7-12 months, 13-18 months, 19-24 months, 24 months and above) in three different combinations (40/5, 60/7.5 and 90/10 mg/kg body weight) was administered IP (Figure 1). Muscle tone, pinch reflex, corneal reflex and palpebral measurements were used to assess the depth of anesthesia (Table 1). Depth of anesthesia and duration of anesthesia were observed in all three combinations. However, anesthesia depth and duration were different (Table 1-2).

Evaluation of the Effect of Sex on the Administered Doses: In rats aged 19–24 months and over 24 months, administration of 40/5 mg/kg intraperitoneal ketamine/xylazine resulted in statistically significant sex-related differences in pedal reflex and muscle tone ($p < 0.05$). While the mean reflex score was lower in

Table 1. Effect of Ketamine/ xylazine doses on anesthesia depth in different age range rats.

Age	Female									Male								
	40/5 mg/mg			60/7,5 mg/mg			90/10 mg/mg			40/5 mg/mg			60/7,5 mg/mg			90/10 mg/mg		
	SA	DA	RA	SA	DA	RA	SA	DA	RA	SA	DA	RA	SA	DA	RA	SA	DA	RA
2-6 months	5.	6.	30.	2.	5.	50	2.	3.	70.	5.	7.	25.	3.	6.	40	3.	4.	60.
7-12 months	5.	6.	30.	2.	5.	50	2.	3.	80.	5.	7.	25.	3.	6.	40	3.	4.	80.
13-18 months	5.	6.	30.	2.	3.	80.	2.	3.	80.	2.	5.	50	5.	6.	30.	3.	4.	80.
19-24 months	7.	8.	20.	2.	5.	50	2.	3.	80.	3.	6.	40	3.	4.	80.	3.	4.	80.
24 months over	2.	3.	80.	2.	3.	80.	2.	5.	50	7.	10.	20.	-	-	-	3.	4.	60.

SA: Entry minute of superficial anesthesia, DA: Entry minute of deed anesthesia, RA: Recovery from anesthesia

Table 2. Evaluating different Ketamine/xylazine doses on particular reflexes in different age groups of rats on both sex.

Age	Reflexes	Female			Male		
		40/5 mg/mg	60/7.5 mg/mg	90/10 mg/mg	40/5 mg/mg	60/7.5 mg/mg	90/10 mg/mg
0–6 months	PER	–	–	–	–	–	–
	JT	–	–	–	–	–	–
7–12 months	PER	–	–	–	–	–	–
	JT	–	–	–	–	–	–
13–18 months	PER	–	–	–	–	–	–
	JT	–	–	–	–	–	–
19–24 months	PR	+	+	–	+	–	–
	PER	+	–	–	–	–	–
	CR	+	+	–	+	–	–
	JT	–	–	–	–	–	–
≥24 months	PR	–	–	+	+	+	+
	PER	–	–	–	–	+	–
	CR	–	–	+	+	+	+
	JT	–	–	–	+	+	–

PR: Palpebral Reflex, CR: Corneal Reflex, PER: Pedal Reflex, JT: Jaw Tone

females in the 19–24-month age group, it was lower in males in rats aged over 24 months.

Following administration of 60/7.5 mg/kg intraperitoneal ketamine/xylazine, statistically significant differences between sexes were observed in the palpebral reflex in rats aged 2–6 months, in all reflex parameters in rats aged 13–18 and 19–24 months, and in pedal reflex and muscle tone in rats aged over 24 months. Mean reflex scores were higher in female rats not only in the 13–18-month age group but also in rats aged 24 months and older.

After administration of 90/10 mg/kg intraperitoneal ketamine/xylazine, statistically significant sex-related differences were detected only in the palpebral reflex in rats aged

2–6 months. No statistically significant differences were observed in any reflex parameters in the remaining age groups. Mean reflex scores were similar between male and female rats across all age groups.

Evaluation of the Effect of Age on the Administered Doses: Muscle tone, pinch reflex, corneal reflex, and palpebral reflex were used as scoring parameters. Depth of anesthesia was assessed by recording the time to entry into superficial anesthesia (LA), time to entry into deep anesthesia (DA), and time to recover from anesthesia (AC). The results are presented in Table 2.

Evaluation of the Effect of Age on the Administered Doses in Male Rats: When 40/5 mg/kg ketamine/xylazine was administered intraperitoneally in male rats, superficial anesthesia durations were observed as follows: up to 20 minutes in the 2–6 and 7–12 month age groups, 45 minutes in the 13–18 month group, 35 minutes in the 19–24 month group, and only 10 minutes in rats aged over 24 months.

Following administration of 60/7.5 mg/kg intraperitoneal ketamine/xylazine, no anesthetic induction was observed in male rats aged 24 months and older. In the 2–6, 7–12, and 13–18-month age groups, superficial anesthesia durations were 35, 35, and 25 minutes, respectively. In male rats aged 19–24 months, 20 minutes of superficial anesthesia and 75 minutes of deep anesthesia were recorded.

Administration of 90/10 mg/kg ketamine/xylazine resulted in deep anesthesia across all age groups. Deep anesthesia duration was 50 minutes in rats aged 2–6 months and over 24 months, and 75 minutes in rats aged 7–12, 13–18, and 19–24 months.

Evaluation of the Effect of Age on the Administered Doses in Female Rats: When 40/5 mg/kg intraperitoneal ketamine/xylazine was administered in female rats, superficial anesthesia durations of up to 25 minutes were observed in the 2–6, 7–12, and 13–18 month age groups, whereas only 10 minutes of superficial anesthesia was observed in rats aged 19–24 months. In contrast, deep anesthesia lasting up to 70 minutes was observed in female rats aged over 24 months.

Following administration of 60/7.5 mg/kg intraperitoneal ketamine/xylazine, deep anesthesia was observed in female rats aged 2–6, 7–12, 13–18, and 19–24 months for up to 10 minutes, while deep anesthesia lasting up to 70 minutes was observed in rats aged 13–18 months and over 24 months.

Administration of 90/10 mg/kg ketamine/xylazine resulted in deep anesthesia across all age groups. Deep anesthesia durations were up to 60 minutes in rats aged 2–6 months, 75 minutes in rats aged 7–12, 13–18, and 19–24 months, and 45 minutes in rats aged over 24 months.

Discussion

The use of anesthetic drugs in laboratory animals covers a wide range of procedures, from minor interventions such as blood sampling, imaging applications, and injections to major surgical operations. Rats are among the most preferred animals in experimental studies, as they allow human physiological phenomena to be investigated under controlled experimental conditions [14].

One of the most frequently used routes of drug administration in rats is the intraperitoneal (IP) route due to its ability to accommodate large volumes, rapid absorption, ease of application, and advantages over other administration methods. However, IP injections are performed blindly, particularly by inexperienced practitioners, and technical errors during intra-abdominal injection may result in organ or tissue damage, leading to experimental failure [15]. The large omental

surface area and the presence of intra-abdominal organs facilitate the IP absorption of hydrophobic drugs. A substantial proportion of drug absorption occurs via the portal system, resulting in significant first-pass hepatic metabolism. In addition, drug loss to the gastrointestinal tract and extraperitoneal space may occur. Consequently, the minimum drug concentration required to achieve adequate induction of anesthesia may not always be attained. For these reasons, the minimum effective dose of anesthetic agents may vary according to age and sex [16,17].

Despite common practice, no single ketamine–xylazine formulation is likely to be satisfactory for all experimental conditions. Therefore, doses and dose ratios should be adjusted according to the expected pain level of the procedure and the required duration of anesthesia [18].

Waterman et al. (1978) divided rats aged 1, 2, 3, 4, 6, 8, 12, and 16 weeks into male and female groups and administered a single intraperitoneal dose of ketamine hydrochloride (75 mg/kg). The onset time (loss of the righting reflex) and total sleep duration were recorded. They reported that the onset of action was prolonged, while sleep duration shortened with increasing age in male rats. Two one-week-old rats died during the anesthesia period. Drug onset time was significantly shorter in one-week-old rats (10–20 min) compared to other age groups, increased progressively until the eighth week (approximately 70 min), and then declined thereafter. No significant differences were observed between rats aged 8, 12, and 16 weeks. Sleep duration was significantly longer during the first four weeks of life (approximately 90–100 min at one week and 20–30 min at four weeks), with no significant differences observed after the fourth week. No sex-related differences were found in the onset of anesthesia; however, females exhibited longer sleep durations than males at nearly all ages [19].

In the present study, ketamine was used in combination with xylazine. Sleep durations were similar between sexes across all age groups; however, overall sleep times were shorter than those reported by Waterman et al.
Jiron et al. compared the safety, physiological effects, and clinical efficacy of three anesthetic protocols (isoflurane, intraperitoneal ketamine–dexmedetomidine, and intraperitoneal ketamine–xylazine) in rice rats. Ketamine/xylazine was administered at a dose of 50/4 mg/kg via the IP route. Induction time, non-surgical anesthesia period, surgical anesthesia duration, and recovery time were recorded. The authors reported an induction time of 8.8 ± 4.0 min, non-surgical anesthesia time of 13.1 ± 5.2 min, surgical anesthesia duration of 41.1 ± 7.8 min, and recovery time of 11.1 ± 7.4 min [20].

Payton et al. evaluated the safety and efficacy of ketamine/xylazine anesthesia in hamsters. The duration of anesthesia was defined as the interval between loss and recovery of the righting reflex. Surgical anesthesia was defined as the period between disappearance and reappearance of the pedal withdrawal reflex. Two ketamine/xylazine doses (50/10 mg/kg and 150/10 mg/kg) were administered. At the 50 mg/kg ketamine dose, anesthesia duration was 20.8 ± 1.6 min; however, painful stimulus responses and the pedal reflex persisted, indicating the absence of surgical anesthesia. At the 150 mg/kg ketamine dose, anesthesia duration was 85.1 ± 9.2 min, with disappearance of pain responses and a surgical anesthesia duration of 37.1 ± 8.2 min [21].

Several studies have also investigated ketamine–xylazine dosing in mice [22,23].

Giroux et al. reported dose-dependent mortality in both young and old mice [22]. In another study involving young mice, mortality rates were higher in females than in males. In contrast, although mortality was reported in these mouse studies, no sex-related differences in mortality were observed among young male and female rats in the present study. These discrepancies may be attributed to differences in animal species (mice versus Wistar rats), body weight, body mass index, and metabolic rate. This interpretation is supported by the absence of mortality in another rat study similar to ours [24].

Rats are larger animals compared to mice and hamsters; therefore, anesthesia duration was shorter than that reported in these species. In the present study, anesthesia duration increased with advancing age across all age groups. Additionally, anesthesia duration was longer in males than in females. Based on induction time and total anesthesia duration, we consider the ketamine/xylazine dose of 90/10 mg/kg to be optimal.

In this study, the effects of three ketamine/xylazine doses (40/5, 60/7.5, and 90/10 mg/kg), administered under normal conditions, were evaluated across all age and sex groups. Study limitations include the absence of ketamine/xylazine plasma level measurements, assessment limited to sleep–wake duration, and the inability to record concurrent cardiac and respiratory parameters reflecting anesthesia depth.

In conclusion, intraperitoneal administration of anesthetic agents offers practical advantages in experimental studies. Selecting appropriate anesthetic doses based on age and sex may facilitate timely completion of experiments while minimizing harm to animals and ensuring compliance with ethical standards. Consequently, more reliable scientific data can be obtained. Further comprehensive studies are needed to evaluate surgical anesthesia duration and its variation according to age and gender.

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Conflict of Interest: *The authors declare that they have no conflict of interest.*

Ethical statement: *This study was approved by the Bolu Abant İzzet Baysal University Animal Research Local Ethics Committee (Date: 13.11.2015; Approval No: 2015/48).*

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