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The Effect of Repeated Doses of Ketamine on Hematological Parameters in Rats

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Abstract: The aim of this study was to investigate the effect of repeated doses of ketamine on hematological parameters in rats. Ketamine, general and rapid-acting phencyclidine derivative, is a dissociative anesthetic. Ketamine, which is usually used in laboratory animals in combination with xylazine, can be used alone for short-term anesthesia in rats. General anesthesia applications repeated several times a day or on different days in animal experiments are used in pharmacokinetic studies where blood or other samples are required, in surgical procedures or drug applications on different days under anesthesia, in treatment applications such as painful wound care, regular dental care. In this study, 20 adult male Wistar albino rats, weighing between 180-230 g were used. Rats were divided into two groups as 10 rats in each group. The rats in the ketamine group received intraperitoneal injection of 10% Ketamine HCl at a dose of 50 mg/kg every other day for 12 days. The control group received 0.2 ml saline intraperitoneally at the same time. At the end of the study, 2 ml blood obtained by intracardiac route was placed in K_3EDTA tubes. Hematological parameters include total leukocyte (WBC), erythrocyte (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), erythrocyte distribution width (RDW), lymphocyte (LYM), monocyte (MON), neutrophil (NEU), lymphocyte%, monocyte%, neutrophil% levels were measured with autoanalyzer device. According to the results, ketamine has statistically decreased only lymphocyte count ($p < 0.05$). In conclusion, ketamine caused changes in the lymphocyte values according to the control group; however, since these changes were within the range of reference values reported for rats, it was concluded that ketamine can be used safely in laboratory animals. Investigation of the effect of the anesthetic agents on blood parameters in laboratory animals is important in terms of the reliability of experimental studies.

Keywords: Ketamine, Rat, Hematological parameters

Tekrarlanarak Uygulanan Ketaminin Sıçanlarda Hematolojik Parametreleri Üzerine Etkisi

Özet: Bu çalışmanın amacı tekrarlanan dozlarda uygulanan ketaminin sıçanlarda hematolojik parametreler üzerine etkisini incelemektir. Ketamin, genel ve çabuk etkili fensiklidin türevi dissosiyatif bir anesteziktir. Genellikle laboratuvar hayvanlarında ksilazin ile birlikte kullanılan ketamin, sıçanlarda kısa süreli anestezilerde tek başına kullanılabilmektedir. Hayvan deneylerinde günde birkaç kez veya farklı günlerde tekrarlanan genel anestezi uygulamaları; kanın veya başka numunelerin gerekli olduğu farmakokinetik çalışmalarda, anestezi altında farklı günlerde cerrahi prosedürlerin veya ilaç uygulamalarının yapıldığı çalışmalarda, ağrılı yara bakımı, düzenli diş bakımı gibi tedavi uygulamalarında başvurulan bir yöntemdir. Bu çalışmada Wistar-Albino ırkı 180-230 gram ağırlığında, aynı yaş grubu, 20 adet ergin erkek sıçanlar kullanılmıştır. Sıçanlar her grupta 10 adet sıçan olmak üzere 2 gruba ayrılmıştır. Ketamin grubundaki sıçanlara 12 gün boyunca gün aşırı 50 mg/kg dozunda %10 Ketamin HCl intraperitoneal yolla uygulanmıştır. Kontrol grubuna ise eş zamanlı olarak intraperitonal yolla 0,2 ml serum fizyolojik uygulanmıştır. Çalışmanın sonunda intrakardiyak yolla elde edilen kanlar EDTA'lı tüplere konulmuştur. Hematolojik parametrelerden Tam kanda total lökosit (WBC), eritrosit (RBC), hemoglobin (HGB), hematokrit (HCT), ortalama Alyuvar Hacmi (MCV), ortalama alyuvar hemoglobin konsantrasyonu (MCHC), ortalama alyuvar hemoglobini (MCH), eritrosit dağılım genişliği (RDW) ve lenfosit (LYM), monosit (MON), nötrofil (NEU), lenfosit %, monosit %, nötrofil % düzeyleri otoanalizör cihazında belirlenmiştir. Hematolojik olarak sadece LYM değerleri açısından gruplar arasında istatistiksel olarak anlamlı fark bulunmuştur ($p<0.05$). Elde edilen bulgulara göre ketamin istatiksel olarak sadece lenfosit sayısında düşüşe neden olmuştur. Ancak bütün ölçüm değerlerinin referans aralıklar içerisinde olduğu belirlenmiştir. Bu çalışmada, ketamin anestezi protokolünün lenfosit değerlerinde kontrol grubuna göre değişikliklere yol açtığı; ancak, bu değişikliklerin sıçanlar için bildirilen referans değerler aralığında olması nedeniyle, ketaminin laboratuvar hayvanlarında güvenle kullanılabileceği sonucuna ulaşılmıştır. Laboratuvar hayvanlarında anestezik maddenin kan parametrelerine etkisinin araştırılması, yapılacak deneysel çalışmaların güvenilirliği açısından önemlidir.

Anahtar Kelimeler: Ketamin, Sıçan, Hematolojik parametreler

1. Introduction

Anesthesia is a reversible and controllable condition where the perception of pain and other stimulants in the central nervous system is prevented. Anesthesia is used for anatomy-physiology demonstrations, experimental injection, rectal drug/substance applications and surgical interventions. The aim of anesthesia is to reduce the fear of the experimental animal, to prevent pain related to the interference, to provide safe and comfortable interventions to the experiment, to protect the researcher (Flecknell, 1996; Tremoleda et al., 2012). Interventions in laboratory animals used in experimental studies should not affect the result of the experiment due to pain or anxiety, and for the scientific and ethical reasons, the use of appropriate anesthetics, analgesics or tranquilizing agents should be used (Carbone and Austin, 2016).

Rats are the most preferred animals for experimental use among all laboratory animals. All physiological events in humans and other vertebrate animals can be easily studied in rats that provide excellent experimental working conditions in laboratory conditions (Flecknell, 1996). Rodents can be anesthetized with either inhalant gas or injectable drugs. Criteria of choice are the ethical use of animals, safety for the animals and personnel, cost and equipment available in the laboratory setting as well as the impact of these compounds or techniques on blood parameters (Tremoleda et al., 2012)

Ketamine, general and rapid-acting phencyclidine derivative, is a dissociative anesthetic. It was synthesized in 1962 by Calvin Stevens. It was first used in the clinic in 1965. It was released in 1970 for clinical use (Liu et al., 2016). Ketamine is the only anesthetic with analgesic, hypnotic and amnesic effects and, unlike other intravenous anesthetics, has no depressant effect. The analgesic effect of ketamine is strong and the narcotic effect is mild. It has the lowest hallucinogen potential in phencyclidine derivatives (Gao et al., 2016). Ketamine crosses the blood-brain barrier quickly because it has low molecular weight and high lipid solubility. The maximum effect occurs in about 1 min. Ketamine does not cause respiratory and cardiovascular depression complications that occur frequently during the use of other anesthetic agents and provides good anesthesia. The duration of action is dose-dependent, but its effect is short-time due to its rapid dissipation from brain tissue to other tissues. Ketamine causes a cataleptic condition called central dissociative anesthesia in the central nervous system, in which communication with the patient cannot be established but the patient can be seen as awake, there are skeletal muscle movements and tonus independent of the painful stimulus, and the patient is under amnesic and deep analgesic effects. Patients have deep analgesia, eyes are open, protective reflexes (cornea, coughing, swallowing, retching) are active in ketamine anesthesia. Also, blood pressure and heart rate increase in this anesthesia (Lodge and Mercier, 2015; Gao et al., 2016; Jonkman et al., 2017).

Although its mechanism of action is not well understood, ketamine appears exerts complex pharmacological actions including inhibition of biogenic amine uptake, binding to opioid receptors, and inhibition of N-methyl D-aspartate (NMDA) receptors. Because of the involvement of spinal NMDA receptors in the process of central sensitization, this agent may reduce pain perception and induce sedation (Kohrs and Durieux, 1998).

Ketamine is used as a general anesthetic in all kinds of large and small operations in animals. It can be safely used alone in inspections for diagnosis, in the capture of wild animals and in situations requiring anesthesia. It is also used to create short-term anesthesia to facilitate intubation before anesthesia by inhalation. Ketamine, which is usually used in laboratory animals in combination with xylazine, can be used alone for short-term anesthesia in rats (Hall and Clarke, 1991; Stoelting, 1999).

General anesthesia applications repeated several times a day or on different days in animal experiments are used in pharmacokinetic studies where blood or other samples are required, in surgical procedures or drug applications on different days under anesthesia, in treatment applications such as painful wound care, regular dental care (Albrecht, 2014). Specifically, during experimental wound studies, experimental animals are exposed to anesthetics such as ketamine 3-5 times before biopsy from the wound to examine the wound healing stages. It is not clear how and in what time hematological functions are affected by anesthesia which is thought to temporarily suppress cognitive functions. Therefore, the aim of this study was to investigate the effect of repeated doses of ketamine on hematological parameters in rats.

2. Materials and Method

2.1. *Drugs*

Ketamine was obtained from Richter pharma ag, Austria (Ketasol® %10 flacon).

2.2. *Animals*

In this study, 20 adult male Wistar albino rats, weighing between 180-230 g were used. After the necessary permission was received (Protocol No: 2013/294) from Y.Y.Ü Animal Experiments Local Ethics Committee, animals obtained from Y.Y.Ü Experimental Medicine Research and Application Center were housed in rooms with 12 hours light - 12 hours dark lighting up, automatically adjusted temperature (22 ± 2 ^{0}C) and humidity (%45-50) again in the same place during experiment, and they were fed with city water supply and standard pellet diet (Van Feed Factory), food and water intake were made free.

2.3. *Experimental design*

20 male rats were divided into two groups, as 10 rats in each group. These groups were made up as follows, including one of them with the application of ketamine, and one of them as the control group. The rats in the ketamine group received an intraperitoneal injection of 10% Ketamine HCl at a dose of 50 mg/kg every other day for 12 days. The control group received 0.2 ml saline intraperitoneally at the same time. At the end of the study, rats were sacrificed under ketamine anesthesia. Blood samples obtained by intracardiac route were placed into EDTA capped bottles.

2.4. *Hematological analysis*

Hematological parameters include Total leukocyte (WBC), Erythrocyte (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH), Erythrocyte Distribution Width (RDW), lymphocyte (LYM), monocyte (MON), neutrophil (NEU), lymphocyte%, monocyte%, neutrophil%, levels were measured with autoanalyzer device (Abacus Junior Vet 5, Diatron, Hungary).

2.5. *Statistical analysis*

The statistical analysis of the data was carried out with the SPSS software 10.0 (SPSS Inc., Chicago, IL, U SA). Paired t test were used to compare the hematologic parameters. Values obtained were expressed as mean ± S.D. The differences were considered to be significant when $P < 0.05$.

3. Results

The results of hematological parameters obtained from the blood taken at the end of the study in the experimental groups are given in Table 1.

Table 1. Mean (±SEM) values of hematological parameters

Parameters	Control	Ketamine	P	Reference
WBC	9.58±1.05	8.18±4.11	0.187	2.1-19.5
LYM	7.40±0.73[b]	5.59±2.93[a]	0.021	2-14.1
MON	0.37±0.16	0.48±0.43	0.402	0-0.98
NEU	1.81±0.43	2.11±2.07	0.543	0.1-5.4
LY%	77.30±1.45	72.22±15.40	0.206	55-97
MO%	3.95±1.73	5.48±3.65	0.221	0-5
NE%	18.72±3.04	24.29±19.11	0.105	2-31
RBC	8.84±0.47	8.59±1.04	0.461	5.3-10
HGB	14.60±0.62	12.54±1.47	0.218	14-18
HCT	48.80±1.85	48.79±5.07	0.993	35-52
MCV	55.00±0.81	56.95±2.05	0.077	50-62
MCH	16.52±0.65	16.72±1.95	0.714	16-23
MCHC	29.92±0.76	23.92±17.76	0.129	31-40
RDWC	15.27±0.68	15.14±0.82	0.741	-

Different letters in the same coloumn indicate statistical significance ($p<0.05$).

In this study, there were no significant differences between groups for hematological parameters. Only, ketamine statistically decreased lymphocyte count ($p <0.05$).

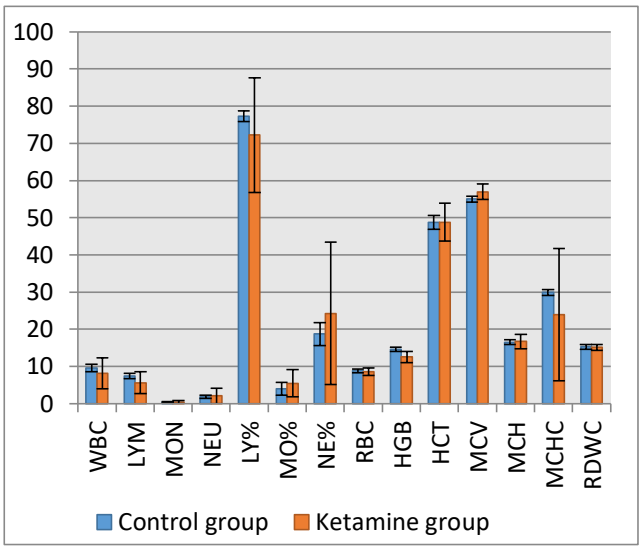

Figure 1. Hematological parameters

4. Discussion

Nowadays, it is required that anesthesia or analgesia be used during surgery in experimental animals because of the concerns regarding animal welfare. Therefore, it is important to understand how anesthesia affects the health conditions of experimental animals. Hematological parameters are important indices of the physiological and pathological status for both humans and animals (Adeneye et al., 2006).

We determined that there are no hematological changes in rats after ketamine administration in this study. But only, there is a decrease in lymphocyte counts according to the control group; however, this decrease was within the range of reference values for rats according to the autoanalyzer device. Çamkerten et al. (2013) reported that there were not significant differences between baseline and during anesthesia values of WBC, RBC, PLT, HCT and HGB of xylazine-ketamine anesthesia on Bozova greyhounds. Alsobayıl et al. (2018) evaluated the anesthetic effects on young hamadryas baboons (*Papio hamadryas*) of xylazine–ketamine compared to diazepam–ketamine. They observed no significant difference in complete blood count**.** Chauhan and Pandey (2006), determined that PCV, HGB, RBC and WBC count did not show any significant change in fentanyl-ketamine combinations in dogs. However, lymphocytosis with neutropenia was noticed. Some authors (Tobias and Schertel, 1992; Gülanber et al., 2001; Atalan et al., 2002) emphasised that there are no significant alterations in venous RBC, WBC, HCT and HGB values for the combination of ketamin and xylazine and this indicated good tissue perfusion during the anesthesia in dogs. Our finding is in agreement with the findings of these studies.

In another study with results contradictory to ours, Hashemnia et al. (2018) evaluated the effects of long-term administration of ketamine on some hematological in Sprague-Dawley rats. Ketamine caused an increase in the number of WBC as compared to the control group at the days of 0, 20, 40, and 60 post-injection. These differences between our study and this study may be due to the time of application and the breed of animal. Also, Lelovas et al. (2017) examined the effect of three different anaesthetic protocols (dexmedetomidine/ketamine intramuscularly (0.25 mg/kg and 50 mg/kg respectively), or isoflurane 0.2 ml on cotton inside a syringe case, or isoflurane administered by vaporiser (5% induction and 2-3% maintenance of anaesthesia, delivered in oxygen flow 1 L/min) on hematological and biochemical parameters of Wistar Albino rats. They stated that the different anesthetic protocols had no statistically significant effect on WBC, NEU, MON, EU, BA, and platelets, while their impact on RBC, HCT and HGB values was statistically significant ($p<0.05$). Demirkan et al. (2002) were reported that a decrease PCV count and an increase of WBC count in ketamine- xylazine anesthesia in the dog. Yohannes (2018) stated that after the administration of ketamine alone, and xylazine-ketamine combinations, HGB, WBC, RBC, LYM, MON were decreased non-significantly, but NEU was increased non-significantly in dogs.

5. Conclusions

In conclusion, ketamine caused changes in the lymphocyte values according to the control group; however, since these changes were within the range of reference values according to the autoanalyzer device, it was concluded that ketamine can be used safely in laboratory animals. Hematological indexes are very important parameters used in the evaluation of physiological conditions. Lymphocytes are involved in a variety of immunological functions, such as immuno-globulin production and modulation of immune defense (Campbell, 1996). There are many different reasons for the decrease in the number of lymphocytes in the blood. Nutritional deficiencies, stress, and prolonged fasting can lead to a decrease in lymphocyte count. Investigation of the effect of the anesthetic agent on blood parameters in laboratory animals is important in terms of the reliability of experimental studies.

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Conflict of interest disclosure:

We have no conflict of interest with any people or organization.

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