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RESEARCH ARTICLE

Changes in Antioxidant Sytem, Lipid Peroxidation, Heart and Respiratory Rate and Rectal Temperature with Ketamine and Ketamine-Xylazine Anaestesia in Tuj Rams

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Summary

The objective of this study was to determine if a ketamine or ketamine-xylazine had any effect on the antioxidant defence system and on lipid peroxidation as thiobarbituric acid substance (TBARS) in rams. The experiment was carried out on 16 clinically healthy Tuj rams each weighing an average 56-60 kg and aged 3-4 years old. Rams were divided into two groups of eight ram in each. To produce anesthesia the Group I was given 10 mg/kg ketamine HCl, the Group II received 50 µg/kg xylazine HCl and 10 mg/kg ketamine HCl, intramuscularly. Blood samples were taken from the jugular vein before and at 5, 10, 15, 30 and 60th minutes after drugs injection to measure the levels of vitamin E and TBARS in plasma and the activity of glutathione peroxidase (GSHPX) and the levels of glutathione (GSH) in erythrocytes. Heart and respiratory rates and rectal temperature were also recorded for 60 min. in two groups. Although plasma levels of TBARS and vitamin E were significantly ($P<0.001$) lowered at the 15th min. after ketamine injection, the activity of GSHPx and levels of GSH in RBC were significantly increased at the 10th min. after ketamine injection ($P<0.001$) in Group I. While significant changes were observed in heart rate and respiratory rate at the 5th minute after anestesia. Also, the activity of GSHPx and levels of GSH in RBC in Group II were significantly increased at the 15th min. after ketamine-xylazine injection ($P<0.001$). Levels of TBARS in plasma and vitamin E in plasma were not changed with ketamine-xylazine injection. Heart and respiratory rates began to increase at the 10th min. after ketamine-xylazine injection and decreased to a value approximately that of before analgesia by the 15th min. and remained stable thoughout the anaesthesia. The plasma levels of TBARS in the ketamine group were lover than those in the ketamine-xylazine group at the 15 and 30th min. after injections ($P<0.001$). GSH levels and GSHPx activity in RBCs of rams have a similar increase in two groups. There were no significant differences observed in rectal temperature during anaesthesia in Group I and II. In conclusion, the results of this study shows that ketamine and kombination of ketamine-xylazine injection at a therapeutic concentration can suppress free radical generation and support antioxidant system of rams.

Keyword: *Ketamine, Xylazine, Ram, Lipid peroxidation, Antioxidants*

Koyunlarda Ketamin ve Ketamin-Xylazin Anestezisi ile Eritrosit Antioksidan Sistemi, Lipid Peroksidasyon, Kalp Hızı, Solunum Sayısı ve Rektal Isıda Meydana Gelen Değişiklikler

Özet

Bu çalışma, ketamin ve ketamin-xylazinin koyunlarda kullanılan anestezik dozunun plazma ve eritrosit antioksidan sistemi ve thiobarbituric acid substance (TBARS) düzeyleri üzerine etkilerini belirlemek amacı ile 3-4 yaşında, ortalama 56-60 kg ağırlığında 16 klinik olarak sağlıklı koç üzerinde yürütüldü. Her grupta 8 koç olacak şekilde iki grup oluşturuldu. Anestezi oluşturmak maksadıyla I. Gruba 10 mg/kg ketamin HCl, II. Gruba 10 mg/kg ketamin HCl ve 50 µg/kg Xylazin HCl IM yolla enjekte edildi. Plazma E vitamini ve TBARS olarak lipit peroksidasyon düzeylerini, glutatyon peroksidaz (GSHPX) aktivitesini ve eritrosit glutatyon (GSH) düzeylerini belirlemek için, kan numuneleri ketamin ve ketamin-xylazin enjeksiyonundan önce ve 5, 10, 15, 30 ve 60. dakikalarda v. jugularis'ten alındı. Kalp hızı, solunum sayısı ve rektal ısı da aynı zaman aralıklarında 60 dak süresince belirlendi. I. Grupta plazma TBARS ve E vitamini düzeylerinin ketamin enjeksiyonundan 15 dak sonra azaldığı ($P<0.001$) gözlenirken, eritrosit GSHPx aktiviteleri ve GSH düzeylerinin ketamin enjeksiyonundan 10 dak sonra önemli düzeyde arttığı ($P<0.001$) belirlendi. I. Grupta, kalp hızı ve solunum sayısının anestezinin 5 dak sonra azaldığı gözlenirken, rektal ısıda bir farklılık tespit edilmedi. II. Grupta eritrosit GSHPx aktiviteleri ve GSH düzeylerinin ketamin-xylazin enjeksiyonundan 15 dak sonra arttığı belirlenirken, plazma E vitamini ve TBARS düzeylerinin ketamin-xylazin enjeksiyonu ile değişmediği belirlendi. Plazma TBARS düzeyleri I. Grupta anestezinin 15 ve 30. dak'larında II. Gruba göre daha düşük bulundu ($P<0.001$). GSHPx aktiviteleri ve GSH düzeylerinin her iki grupta da benzer şekilde arttığı gözlendi. II. Grupta kalp hızı ve solunum sayısının anestezinin 10. dak'sına kadar arttığı, ancak 15. dak'dan sonra başlangıç değerlerine döndüğü ve anestezi süresince bu değerlerde bir değişiklik belirlenmediği gözlenirken, rektal ısıda bir farklılık saptanmadı. Çalışma sonuçları, ketamin ve ketamin-xylazin enjeksiyonunun serbest radikal oluşumunu baskıladığını ve antioksidan sistemi desteklediğini göstermektedir.

Anahtar sözcükler: *Ketamin, Xylazin, Koyun, Lipit Peroksidasyon, Antioksidanlar*

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INTRODUCTION

In clinical veterinary practice there is demand for a safe and reliable injectable method of anaesthesia for use in routine surgery of short to intermediate duration. Ketamine, a dissociative anaesthetic, have been used for this purpose by intra venously and intra muscularly. Ketamine produce poor muscle relaxion, good somatic analgesia and an increased sympathetic tone. The major problems occur during recovery and include tonic-clonic cramps and convulsion-like signs and also increases blood pressure. However, it does not affect heart rate or respiratory rate when used for general anaesthesia or premedication [1,2].

On the other hand, evidences suggest some interactions between anaesthetic agents and reactive oxygen species (ROS) [3]. Also, some anaesthetic agents which used in routine surgery such as ketamine, thiopental, midazolam and propofol were investigated in terms of antioxidative effects and effects on lipid peroxidation production. Clinical plasma concentrations of ketamine have minimal effects on ROS production [4]. Lupp et al.[4] suggested that ketamine act as a radical scavenger and inhibitor of the oxidative function of microsomal cytochrome P_{450} that has a role in the metabolism of endogenous pools of arachidonic acid and also play important physiological roles in the control of tissue and body homeostasis. Studies from several laboratories have suggested important roles for the P_{450} arachidonic acid monooxygenase in the pathophysiology of experimental hypertension. Also, it is reported that ketamine inhibit nitric oxide synthase activity in rat brain [5].

An alpha 2 agonist-xylazine combination with ketamine is often used in veterinary anaesthesiology for anaesthetizing or immobilizing laboratory or domestic animals [6,7]. Depending on the dose of ketamine has sedative, analgetic, psychomimetic, cataleptic and anaestetic effects. It stimulates blood circulation [8]. It does not significantly alter the pattern of breathing [8]. Xylazine markedly reduces ketamine dosage and the subsequent reduction of the undesirable psychomimetic efects [9]. It markedly potentiates hypnotic and analgetic effect of ketamine [10]. Breathing is slightly influenced when clinical doses are used [11]. Xylazine reduce the dose of ketamine by 40–60%, induce muscle relaxation and reduce the occurrence of psychomotor symptoms after ketamine. It is known that, breathing is not significantly influenced with this combination and ketamine-xylazine anaesthesia so reliable with respect to the incidence of cardiac arrhythmias [6,12].

Therefore this study was undertaken in order to evaluate the effect of ketamine and combination of ketamine-xylazine anaesthesia on the antioxidant system and lipid peroxidation in rams.

MATERIAL and METHODS

The experiment was carried out on 16 clinically healthy male Tuj sheep, each weighing an average 56-60 kg and aged 3-4 years old. The sheeps were divided into two groups of eight sheep in each. To produce sedation the first group (Group I) was given 10 mg/kg ketamine HCl (Ketamine, Parke Davis, Eczacıbası), the other group (Group II) received 50 µg/kg xylazine HCl (%2 Alfazyne, Alfasan) injectable solution and 10 mg/kg ketamine hydrochloride (Ketamine, Parke Davis, Eczacıbası) five min after xylazine administration. Drugs were injected intra muscularly into the rams.

Blood samples were taken from the jugular vein before ketamine injection and at 5, 10, 15, 30, 45 and 60 minutes to measure the levels of vitamin E and lipid peroxidation (LPO) in plasma and the activity of glutathione peroxidase (GSHPX; EC: 1.11.1.9) and the levels of glutathione (GSH) in erythrocytes.

Hearth rate (beats/per min), respiratory rate (breaths/ per min) and rectal temparature were recorded for each animal at the above mentioned time intervals. Hearth rate was calculated from ECG records. The ECGs were recorded by a direct writing electrocardiograph. All ECGs were standardized at 10 mm/mV, with chart speed of 25 mm/min. The rectal temparature was obtained by a termometer placed into the rectum. Respiratory rate was determined by direct observation. Motor functions were tested as full response to needle prick to various areas of the body. Sensory functions were tested as response to palpebral and corneal reflexes.

Blood was collected using heparinized vacutainer tubes. The plasma and the red blood cells (RBC) were separated by centrifugation (2500 g, for 15 min at 4°C). The plasma was frozen (-20°C) until

further analysis. The RBC samples were washed three times with 0.9% sodium chloride and then haemolysed by exposure to a nine-fold volume of redistilled water followed by freezing (-20°C for 18 h) and thawing before analysis. The plasma specimens were used for the determination of vitamin E and LPO, and the RBC samples were used for the determination of GSHPX and GSH.

The end product of polyunsaturated fatty acid peroxidation, malondialdehyde (MDA), reacting with thiobarbituric acid (TBA) in serum samples was determined by the method of Matkovichs et al.[13]. The values of MDA reactive material (TBARS) were expressed in terms of the malondialdehyde (MDA) content (nmol/ml plasma), which served as a standard of 1,1,3,3-tetraethoxypropane (Sigma, Chemical Company St. Louis, MO, USA).

The vitamin E (α-tocopherol) levels of plasma were determined in the frozen serum samples by the method described by McMurray et al.[14]. The relevant wavelengths for vitamin E detection were 292 and 330 nm. Calibration was performed using a standardt solution of α-tocopherol in methanol.

The GSH levels of haemolysed RBC were measured spectrophotometrically using Ellman's reagent [15]. GSH-Px (EC 1.11.1.9) activity was determined using cumene hydroperoxide and reduced GSH as co-substrates, and the loss of GSH following enzymic reaction was measured spectrophotometrically with Ellman's reagent at 37°C and 412 nm according to Lawrence and Burk [16]. The haemoglobin concentration in lysed erythrocytes was determined by the cyan-methemoglobin method [17].

Measurements were compared with baseline values (time: 0) by performing One-way parametric ANOVA test and post-hoc differences in variables between groups performed by Tukey test using MINITAB statistical program (Minitab,1998). All results were expressed as the mean±standard deviation (SD). P value <0.05 was considered to be statistically significant

RESULTS

Plasma TBARS and vitamin E concentrations and erythrocytes GSH levels and GSHPx activity according to time and to the experimental groups are shown in the *Figures 1*. A, B, C and D respectively.

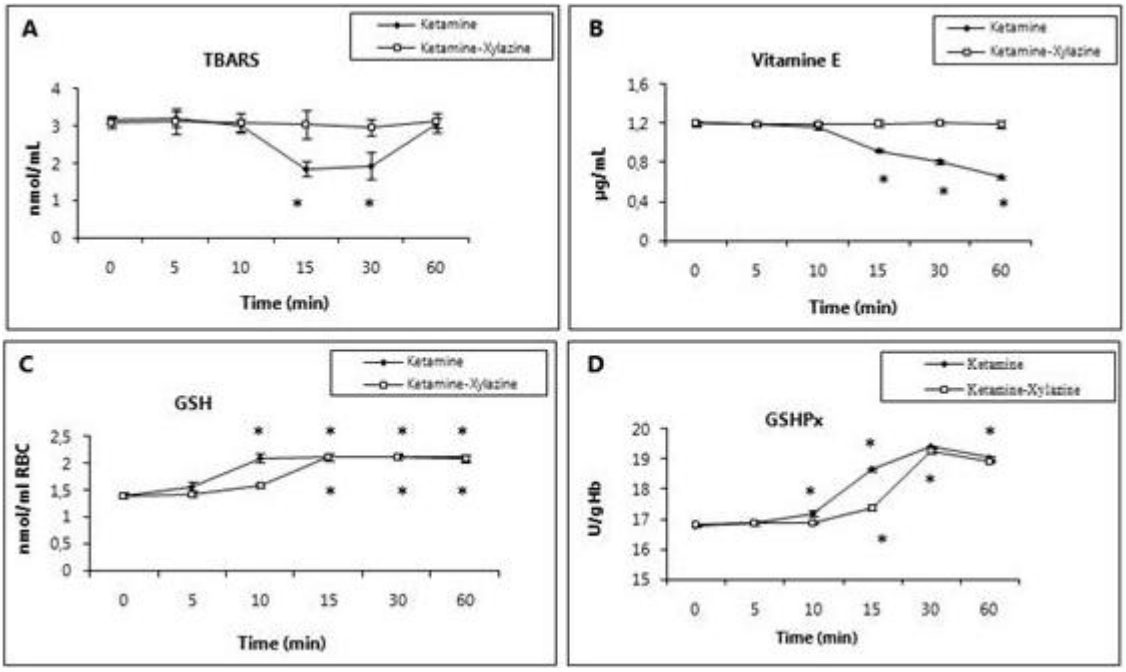

Fig 1. Line graphs showing the plasma TBARS and vitamin E concentrations and erythrocytes GSH levels and GSHPx activity at different time points from the experimental groups. * P<0.001 in comparision to the 0th point of the time. Values are expressed as mean ±SD

Şekil 1. Grafikler ketamin ve ketamin-ksilazin enjeksiyonu ile plazma TBARS ve E vitamini düzeyleri ile eritrosit GSH ve GSHPx aktivitelerinde zamana göre belirlenen değişiklikleri göstermektedir. *: P<0.001 (0. dakikaya göre istatistiksel önemliliği göstermektedir. Değerler Ortalama±Standart Sapma olarak verilmiştir

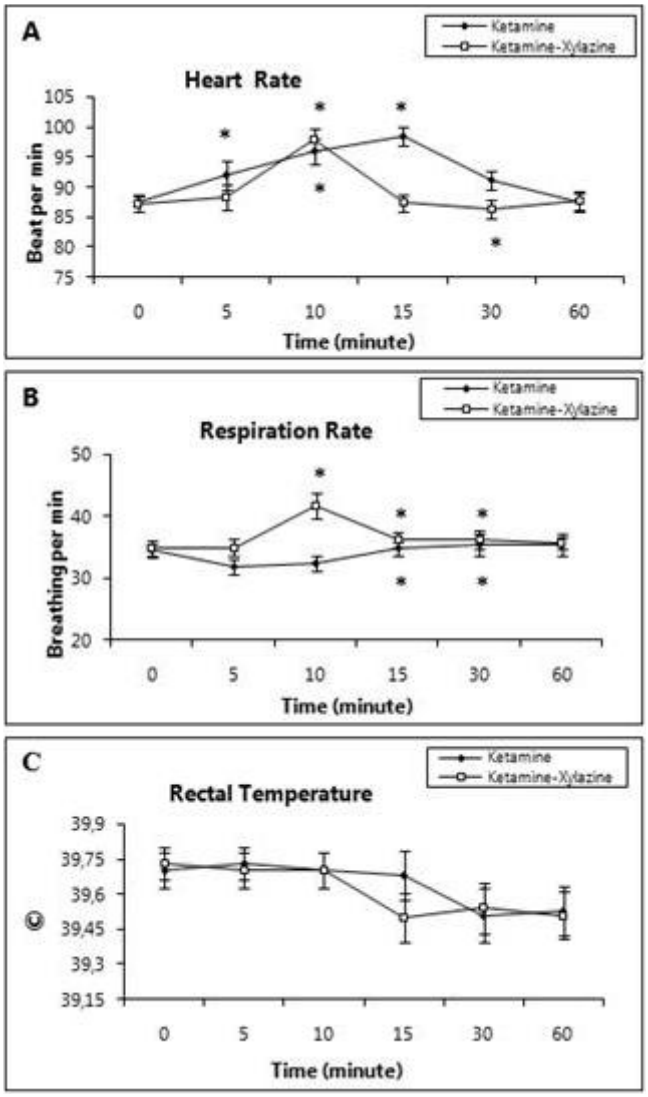

Fig 2. Line graphs showing the alteration in heart rate, respiratory rate and rectal temperature at differrent time points from the experimental groups. * P<0.001 in comparision to the 0th point of the time. Values are expressed as mean±SD

Şekil 2. Grafikler ketamin ve ketamin-ksilazin enjeksiyonu ile kalp hızı, solunum sayısı ve rektal ısıda zamana göre belirlenen değişiklikleri göstermektedir. *: P<0.001 (0. dakikaya göre istatistiksel önemliliği göstermektedir. Değerler Ortalama±Standart Sapma olarak verilmiştir

Altough plasma levels of TBARS and vitamin E content was significantly (P<0.001) lowered at the 15th minute after ketamine injection, the activity of GSHPx and levels of GSH in RBC were significantly increased at the 10th minute after ketamine injection (P<0.001) in Group I when compared baseline values. Also, the activity of GSHPx and levels of GSH in RBC in Group II were significantly increased at the 15th minute after ketamine-xylazine injection (P<0.001). Levels of TBARS in plasma and vitamin E in plasma were not changed with ketamine-xylazine injection incomparision with baseline values. On the other hand when compared groups, plasma levels of vitamin E were higher than in the ketamine-xylazine group than in the ketamine group at the 15, 30, 60th min after anestesia (P<0.001). TBARS concentration remained relatively constant in the two groups until 15th min. The plasma levels of TBARS in the ketamine group were lover than those in the ketamin-xylazine group at the 15 and 30th min after injections (P<0.001) GSH levels and GSHPx activity in RBCs of rams have a similar increase in two groups. There is no significant difference between groups in GSH levels. GSHPx activity in RBCs of rams in the ketamine group higher than that in the ketamine-xylazine group only at the 15th min after anestesia.

Figure 2 (A, B and C) shows the alteration in heart rate, respiratory rate and rectal temperature before and after injections in the experimental groups. In Group I, significant increase were observed in heart rate and respiratory rate at the 5th minute after anestesia, then decreased to a value approximately that of the control (0. min) by the 60th min. On the other hand, in Group II, heart and respiratory rates began to increase at the 10th minutes after ketamine-xylazine injection and and decreased to a value approximately that of before analgesia by the 15th min and remained stable thoughout the anaesthesia. There were no significant differences observed in rectal temperature during anaesthesia in Group I and II.

DISCUSSION

In this study, we analyzed the effect of widely used dose of ketamine and combination of ketamine/xylazine anesthesia on oxidant-antioxidant parameters as well as the effects of these agents on cardio-pulmoner activity in sheep. It is known that a cyclohexylamine analogue, ketamine, produces a cataleptoid state involving unconsciousness and somatic analgesia but no muscular relaxation. Also, ketamine induces anaesthesia rapidly and causes minimal depression of the respiratory and cardiovascular systems, and has a wide margin of safety [18,19]. Because of these advantages of ketamine, it is widely used in veterinary practise by alone or in combination with xylazine [12]. On the other hand, xylazine is a potent hypnotic with powerful central muscular relaxant properties. Its main disadvantages are that it produces significant cardiac arrhythmias in all

species, interfering with normal electrical activity in the heart [7]. This study showed that respiration and hearth changed significantly (P<0.001) after ketamine injection. However, from the clinical point of view, cardiovascular stability is good and breathing is not significantly influenced with combination of ketamine/xylazine. Similar to results of previous studies [6,9,20], it is observed that combination of ketamine/xylazine effectively reduced some of the undesirable effects of ketamine, such as tachycardia, apnea on cardio-pulmonary activity. However, niether ketamin nor kombination of ketamin-xylazine effected rectal temparature.

Oxygen free radicals are formed continuously in the organism as part of enzymatic reactions or as a product of oxidation processes. They play an important role in various pathophysiological events, including respiratory distress and myocardial vasoconstruction [21]. In sheep, however, there is little information available on oxidant-antioxidant change with the anaesthesia induced by ketamine and a combination of xylazine and ketamine. A study by Berlinskii and Berlinskii [22] suggested that ketamine in combination with clofelin helped reduce the activity of free radical lipid peroxidation generation and showed minimal changes in the activities of red cell peroxide resistance, also lowest level of accumulation of MDA in children. Moreover, ketamine has been shown to be a cytochrome P_{450} inducer. Also, Lupp et al.[4] demonstrate that ketamine decreased LPO and H_2O_2 production and also act as radical scavengers and inhibitor of the oxidative function of P_{450}. On the other hand, there is strong evidence of formation of free radicals during ketamine use that are responsible for increased lipid peroxidation process and products in serum [23]. In addition, exposure to an anaesthetic agent, may reduce flowed body and pulmonary oxygen content, as seen in xylazine anaesthesia [24,25]. Cases of pulmonary oedema and death have been reported after administration of xylazine in sheep (26). Pulmonary oedema caused by increased permeability is characterized by the presence of cellular damage and toxicity mediated by oxygen free radicals [27]. However, there is limited study about antioxidant activity of combination of ketamine/xylazine. A study by Helmer et al.[28] suggest that xylazine/ketamine combination is capable of diminishing lipopolysaccaride-induced nitric oxide synthase in vivo. In our study, we found that while ketamine reduce TBARS production, combination of ketamine/xylazine did not affect on lipid peroxidation. This result may be the result of an antioxidant activity of ketamine and also the similar response to increased oxidative stress of this combination.

Oxidative stress occurs when production of active oxidants overwhelms the antioxidant defense mechanisms [29,30]. Cells are protected from the damaging effects of oxidant by superoxide dismutase (SOD), glutathione peroxidase (GSHPx) and catalase (CAT). GSHPx plays a major role in detoxification of lipid peroxides that are formed in vivo especially in the absence of vitamin E and other antioxidants or in animals undergoing oxidative stress. GSHPx system may be a compensatory physiological adaptive response to reduce the oxidative stress. Increased GSHPx activity may be due to the guantity of H_2O_2 and other organic peroxides. Also, increase of GSHPx activity protected the cells from the formation of lipid peroxides by reducing H_2O_2 levels which in turn attenuated OH• generation. Reduced GSH serves as substrate for GSHPx providing reducing equivalents for the metabolism of H_2O_2 and lipid peroxides. It is the major non-protein thiol in mammalian cells and tissues and also plays an important role in the cellular defenses against free radicals and peroxides [31,32]. Therefore, the role of GSH in the detoxification of highly reactive oxygen species (ROS) is important [33]. The present study found a significant increase (P<0.001) in GSHPx activity and levels of GSH 10-15 min after ketamine and combination of ketamine/xylazine injection in RBC of rams respectively. This increase may be explained by antioxidant activity of this anaesthesic agent prevent the utilization of antioxidant enzyme.

Our literature review found no studies related to the levels of vitamin E in plasma during anaesthesia or analgesia. Vitamin E has been known an essential structural companent of biological membranes contributing to their stability. This lipid-soluble compound is needed for the mitochondria electron-transport function and prevents oxidation of various compounds. Also, vitamin E diminishes the peroxidation of PUFA through scavenging and chain breaking free radicals [32]. We showed in our results that oxidative stress may have been potentiated by diminished

vitamin E concentration with ketamine anesthesia. Stable levels of vitamin E during xylazine/ketamine anesthesia may be explained by the antioxidant properties of this combination supress the utilization of these antioxidants.

In conclusion, the results of this study shows that ketamine and kombination of ketamine-xylazine injection at a therapeutic concentration can suppress free radical generation and support antioxidant system of rams.

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