Effects of premedication with Melatonin and L-Theanine on Ketamine induced anesthesia in New Zealand White Rabbits

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ABSTRACT

Melatonin, a pineal gland secreted hormone has different effects on mammalian physiology. Recently, specific receptors of melatonine have been found in the central nervous system and spinal cord of rabbits. L - Theanine is a unique amino acid in the tea plant (*Camellia sinensis*). The effects of this amino acid is based on increasing GABA and dopamine levels in the brain and has a weak effect on the NMDA receptors, receptors that inhibitory effects of ketamine on them have been proven. The aim of this study was to evaluate the effect of oral combination of melatonin and L-Theanine on analgesia and anesthesia characteristics, induced by intramuscular injection of ketamine hydrochloride in New Zealand white rabbits. Five rabbits were anesthetized by intramascular injection of 35 mg/kg of 10% ketamine hydrochloride *(Control group)*. The same rabbits, after 2 weeks, and complete clearance of the drugs from their bodies, were administered orally with the combination of 25 mg L-Theanine and 3 mg melatonin, 60 min prior to ketamine injection *(Experimental group)*. In both groups, time spending to induction of anesthesia, duration of anesthesia, anesthetic depth and vital signs including rectal temperature, heart rate, respiratory rate, SPO2 and palpebral, pedal and righting reflexes were observed. The results showed no significant differences in onset of anesthesia, duration of anesthesia and anesthetic depth, heart rate, respiratory rate, body temperature and oxygen saturation of hemoglobin. As a conclusion, administration of 3 mg melatonin and 25 mg L-theanine could not effect on ketamine hydrochloride anesthesia in New Zealand white rabbits.

Keywords: Ketamine; Melatonin; L-theanine; Anesthesia; Rabbit.

INTRODUCTION

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone secreted by the pineal gland which affects different receptors in different tissues and has different effects on mammalian physiology. One of its most important effects is interference in the 24-hour biological rhythms (Circadian Rhythm) [1,2]. Thus, different indications have been proposed for this hormone in humans where the most important one is treatment of sleep disorders. Recently, specific receptors for this hormone have been found in the central nervous system and spinal cord of rabbits [3,4]. In the pineal gland, serotonin is converted to melatonin by N-acetylation and O-methylation [5].

Melatonin applies its effects through two G-protein-coupled receptors (GPCR) which are known as MT1 and MT2 [6]. Adenylyl cyclase inhibition which is the result of activation of MT1 receptor leads to reduction of cAMP and then continues with decreased activity of protein kinase A (PKA). Furthermore, activation of phospholipase C (PLC) by MT1 affects the ion channels and regulation of the diffusion of ions into the cell.

However, binding of melatonin with MT2 receptor inhibits adenylyl cyclase and reduces cAMP. Likewise, affects the guanylyl cyclase and reduces cGMP. MT2 activation probably affects PLC and increases the activity of protein kinase A. Activation of this receptor can lead to the entry of ions into the cell [7].

L - theanine (gamma-glutamylethylamide) is a unique amino acid which is often individually

present in the tea plant (*Camellia sinensis*). Theanine constitute 1-2% of the dry weight of green tea leaves, constitutes approximately 50% of the amino acids in tea and is the only free amino acid which is not present in the protein. L-Theanine was discovered by Sakato (1949) as one of the main components of green tea [8] and in 1964, it was approved as a food supplement in Japan. L - theanine is a water soluble compound and in the case of oral administration it is absorbed in the small intestine. This amino acid has the ability to cross the blood-brain barrier. In rats, its maximum plasma concentration can be achieved after 30 min of oral administration [9]. In rats, theanine increases the production of serotonin and dopamine when it reaches the brain [10]. Theanine is hydrolyzed to glutamic acid and ethyl amines by glutaminase in the kidney [9]. Regardless of the mechanism, theanine raises alpha brain wave activity which is a sign of relaxation [11]. Theanine competitively inhibits the transport of glutamate in tumor cells which results in a decrease in intracellular levels of glutathione (GSH). Theanine prevents the destruction of normal cells by chemotherapy through the antioxidant activity, particularly through the preservation of cell GSH levels [12-15]. The effects of this amino acid is based on increasing GABA levels and increased levels of dopamine in the brain and has a weak effect on the NMDA receptors (receptors that inhibitory effects of ketamine on them have been proven). Oral administration of this amino acid in human reduces the stress levels [11]. Thus, it seems that the combination of melatonin and L-theanine can alter the characteristics of ketamine-induced anesthesia in rabbits.

Several studies have been conducted within and outside the country on various drugs, different injection techniques and protocols of different combinations of anesthetics, analgesics and tranquilizers on a variety of laboratory animals such as rat, rabbit, guinea pig and hamster. However, no similar articles were found in review of literature from inside and outside the country on the effect of oral administration of L - theanine and melatonine on anesthesia induced by intramuscular injection of ketamine hydrochloride in New Zealand white rabbits. For example, in 1996, Wan and colleagues worked on the accumulation and characterization of melatonin in rabbit spinal cord [3]. In 1998, Yokogushi and colleagues studied theanine dependent serotonin concentration decrease in the rat brain [16]. In 2007, Kimura and colleagues conducted a study on reduction of physiological and psychological stress reactions due to administration of L-theanine [17]. Furthermore, in the same year, Gomez-Ramirez and colleagues conducted a study of high-density electrical mapping on the effects of theanine [18].

Regarding the effects of melatonin and L - theanine in the body and the background of related research, it seems that the combination of melatonin and L-theanine may be able to alter the characteristics of ketamine-induced anesthesia in the rabbit.

MATERIALS AND METHODS

Five healthy male New Zealand white rabbits weighing 2 to 2.5 kilograms were selected. Rabbits were held in proper condition according to the Helsinki Convention and in accordance with the regulations of the University Ethics Committee.

Each rabbit kept in a separate cage and had free access to food and water during the study and were kept in a stress free environment including 12 hours light and 12 hours darkness. Standard chow pellet *(Pars Animal Feed Co.,Iran)* were used to feed all the rabbits

Five Rabbits were anesthetized by intramuscular injection of 35 mg per kg of body weight of ketamine hydrochloride 10% *(Alfasan, Holland)* and were considered as the control group. The same rabbits, after 2 weeks of being held in the same condition and complete clearance of the drugs from the body, were fed with the combination of 25 mg L-theanine and 3 mg of melatonin (Schiff Melatonin Plus, America) and after 60 min, 35 mg/kg of ketamine hydrochloride was injected intramuscular into each rabbit and they were considered as the experimental group.

In both groups, time spending to induction of anesthesia, duration of anesthesia, anesthetic depth and vital signs including rectal temperature, heart rate, respiratory rate, SPO2 and palpebral,

pedal and righting reflexes were observed, measured and recorded.

The information obtained were compared and evaluated using T-test and ANOVA in the case of parametric data and Kruskal–Wallis test in the case of non-parametric data with a confidence level of 95% ($P < 0.0.5$).

RESULTS

The mean comparison of the time period between the injection and the onset of anesthesia in the experimental and control groups showed little differences between the two groups which is not statistically significant at confidence level 95%. The mean comparison of the duration of anesthesia in the experimental and control groups showed little differences between the two groups which is not statistically significant at confidence level 95%. Surprisingly, no analgesia and deep anesthesia were seen in both control and experimental group, therefore, no differences were found between the two groups in these evaluation parameters (Fig 1).

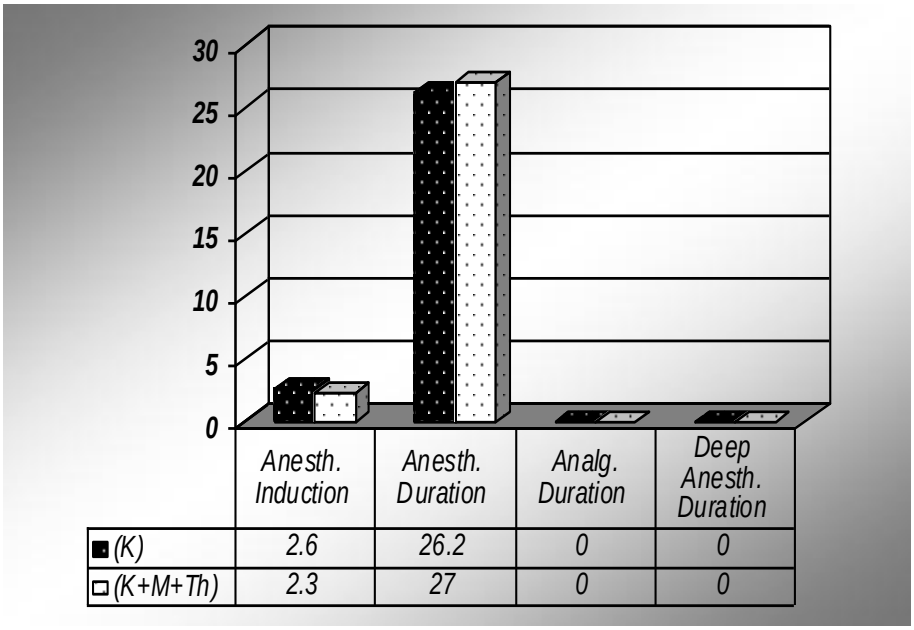

Figure 1. Evaluation of the mean time period between injection and onset of anesthesia, duration of anesthesia, duration of analgesia and duration of deep anesthesia in the control group (Ketamine) and the experimental group (Ketamine + Melatonin + L. Theanine) ($P < 0.05$, $n = 5$)

The mean comparison of the time period between the injection and the onset of anesthesia in the experimental and control groups showed little differences between the two groups which is not statistically significant at confidence level 95% (Fig 2).

The mean comparison of heart rate, respiratory rate and rectal temperature in the control and experimental groups did not show any significant difference and was not statistically significant at confidence level 95% (Fig 3 and 4).

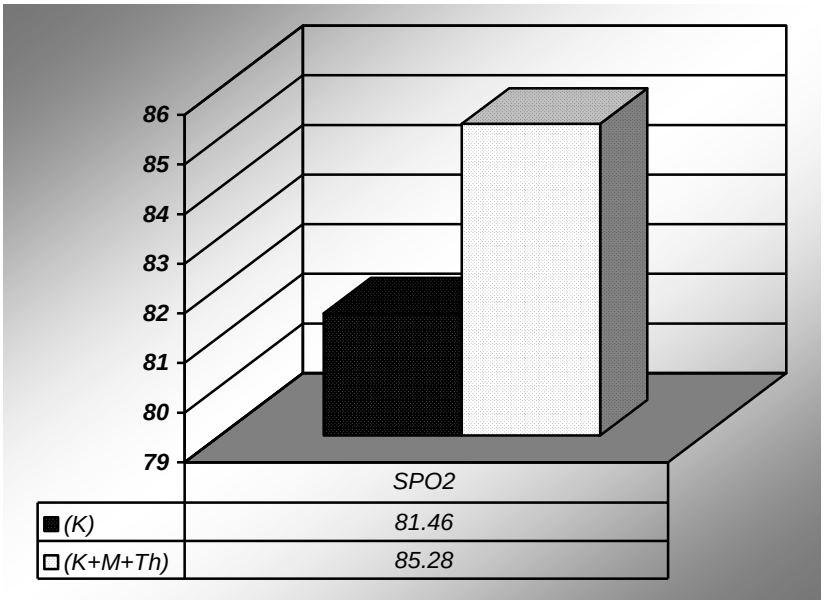

Figure 2. The diagram of mean comparison of oxygen saturation percentage of hemoglobin in the control group (Ketamine) and the experimental group (Ketamine + Melatonin + L - Theanine) (P <0.05, n = 5)

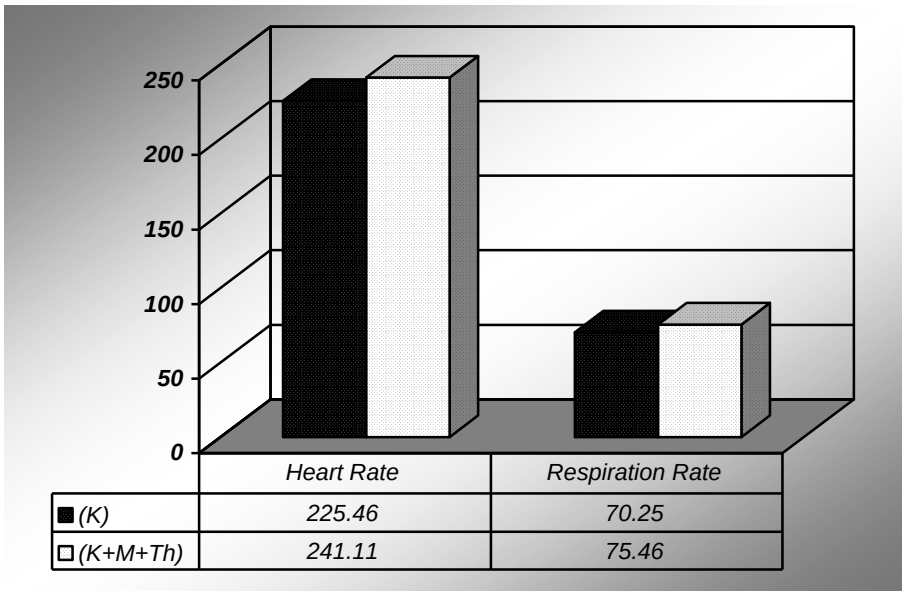

Figure 3. The diagram of mean comparison of heart rate and respiratory rate per minute in the control group (Ketamine) and the experimental group (Ketamine + Melatonin + L- Theanine) (P <0.05, n = 5)

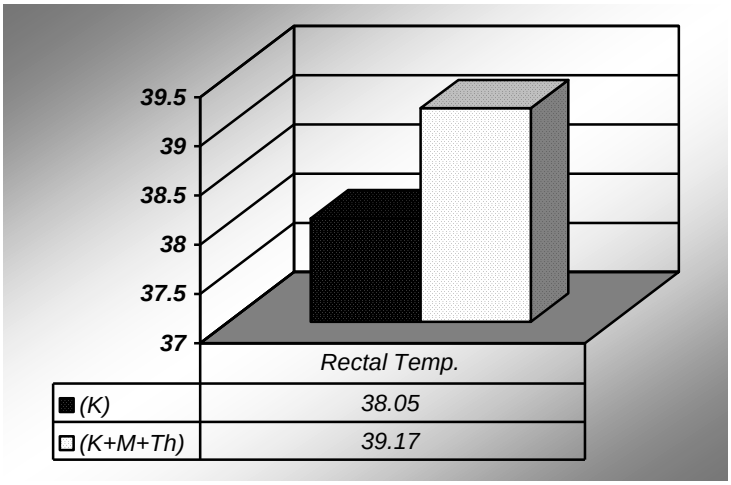

Figure 4. The diagram of mean comparison of rectal temperature in the control group (Ketamine) and the experimental group (Ketamine + Melatonin + L - Theanine) ($P < 0.05$, $n = 5$)

Table 1. Mean comparison and standard error of the mean (SEM) in the control group (Ketamine) and the experimental group (Ketamine + Melatonin + L - Theanine) ($P < 0.05$)

Parameters	Group		P
	Ctrl (K)	Exp (K+ M + Th)	
Anesthesia Induction (min)	2.60±0.24	2.3±0.20	0.372
Anesthesia Duration (min)	26.20±2.64	27.00±1.76	0.809
Analgesia Duration (min)	0	0	--
Deep Anesthesia	0	0	--
SPO2(%)	81.46±0.90	85.28±1.34	0.051
Heart Rate (beat/min.)	225.46±6.58	241.11±6.84	0.138
Respiration Rate (Resp/min)	70.25±3.01	75.46±4.28	0.352
Temp. (^{o}C)	38.05±0.38	39.17±0.34	0.064

DISCUSSION

The effects of premedication of melatonin and L-theanin on ketamin induced anesthesia using various measurements and monitoring the conditions of the experiments were conducted and the data obtained and analyzed.

Melatonin or 5-Methoxy-N-acetyltryptamine is a methoxy-indole compound with a half-life of approximately 20-50 minutes whose plasma concentration is approximately 10-50 pg/ml. In the analgesic effect of melatonin, glutamate, GABA, and in particular opioid systems are involved. For example, melatonin can activate opioid receptors and reduce the formation of cAMP in neurons and also open potassium channels in neurons through the influence on opioid receptors and by hyper-polarization mechanism cause activation of the spinal cord analgesic system and inhibit the pain transmission fibers in this sector [19,20]. On the other hand, melatonin can reduce the expression of lipoxygenase and cyclooxygenase enzymes and prevent the generation of inflammatory precursors where this effect may lead to its ability for reduction. In addition, melatonin can reveal some of its effects by eliminating free radicals [21,22].

As already mentioned, melatonin is secreted by the pineal gland. In a study it was found that in people whose pineal gland has been removed, Flunitrazepam connection rate to its binding site in GABA receptors is reduced [23] and this reduction goes back to normal by administration of exogenous melatonin [24,25]. In a study it was shown that melatonin has anti-anxiety activity in mice [26] and the results of some studies suggest that melatonin may have analgesic effects in mice and rats [27,28]. Regarding the hypnotic and analgesic potential of melatonin, it has been used in dental surgery for induction of anesthesia and sleep [29]. Previous studies have shown that administration of melatonin in dosages of 0.5 to 2.5 mg/Kg before propofol administration could significantly reduce the reflex rate and accelerate the onset of action of propofol and anesthesia [30]. In another study, the use of melatonin at a dose of 0.2 milligrams per kilogram of body weight 50 minutes before surgery reduced anxiety and stress due to surgery. Melatonin also enhances the anesthetic effects of sodium thiopental [31]. The results of the present study regarding the onset time of anesthesia in the control group compared to the experimental group, suggest that melatonin reduced the onset time of anesthesia in the experimental group due to the hypnotic effects, however, this decrease in time has no significant difference compared to the control group with a significant level of 95%. This is probably due to the effect of melatonin on GABA, MT1 and MT2 receptors. In addition, as noted earlier, the effect of melatonin on increasing GABA levels in the brain may increase the inhibitory effect of GABA in the brain and cause sedation and faster induction of anesthesia.

Regarding the duration of anesthesia in the two control and experimental groups, the results suggest that the duration of anesthesia was greater in the experimental group compared to the control group but there was no significant difference with a confidence level 95%. Regarding the effect of melatonin on opioid receptors and also increasing the function of GABAergic receptors, a study was conducted on the performance of melatonin in formalin-induced pain indicating the analgesic effects of melatonin.

Probably higher doses of melatonin are able to enhance the duration of anesthesia significantly by increasing the analgesic effects; this requires future research.

Regarding the duration of analgesia in the two experimental and control groups, it is worth noting that the used dosage of melatonin was not able to increase the duration of analgesia in laboratory animals and in this case no significant differences were observed between the two groups.

Evaluation of hemoglobin saturation percentage is very important for anesthetic drugs because reduced oxygen intake or reduction of cardiac output during surgeries can be risky. Mean comparison of oxygen saturation percentage of hemoglobin in the experimental and control groups indicated a difference between the two groups; such that oxygen saturation percentage of hemoglobin in animals who received oral combination of melatonin and L-theanine before injection of ketamine was higher than oxygen saturation percentage of hemoglobin in animals who only received an injection of ketamine. Although this difference was not statistically significant at confidence level 95%, it was very close to the significance level ($p = 0.051$). Thus, the use of melatonin in combination with ketamine not only did not lead to a decrease in hemoglobin saturation percentage, but also increased the saturation percentage.

Regarding the mean comparison of heart rate, respiratory rate and rectal temperature in the control and experimental groups, no significant differences were observed which is also statistically not significant at confidence level 95%.

In this study, with respect to the effects of melatonin and L - theanine in the induction of sleep and also their analgesic and anti-stress effects, we wanted to study the effect of premedication with melatonin and L - theanine on ketamine-induced anesthesia. The results suggest in spite of the role of melatonin in analgesia and inducing sleep and the role of L - theanine in relaxatin, their administration before ketamine cannot make a significant difference on the onset, depth and duration of anesthesia.

CONCLUSION

As the results showed no significant differences in onset of anesthesia, duration of anesthesia, analgesia and anesthetic depth, It seems that oral administration of 3 mg melatonin and 25 mg L-theanine, 60 minutes prior to intramascular 35 mg/kg ketamine Hcl could not affect on in New Zealand white rabbits anesthesia. Also results indicated that this combination will not interfere with the vital signs as the heart rate, respiratory rate, body temperature and oxygen saturation of hemoglobin in experimental group were not differ from those results from contorl group.

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