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Comparative Effect of Melatonin and Night Light Application in Ehrlich Solid Tumor Mice

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Abstract:

Background: In this study, it was aimed to test the anti-tumoral effects of melatonin in vivo. In the literature review, no study was found that examined the anti-tumoral effect of melatonin at night on an experimental tumor model in vivo. Therefore, in the study, the anti-tumoral effect of melatonin was tested in solid tumor tissue formed in Swiss albino male mice.

Methods: Swiss albino male mice formed Swiss albino male mice in vivo and 80 Swiss albino male mice weighing 25-30 g and aged 10-12 weeks were used. The subjects were divided into 10 groups in total (n=8). These groups are divided into two main groups as light and dark. All animals except the control group were injected intramuscularly (i.m.) with EAT to create EST in the right leg scapula of the subjects. Different doses of melatonin were injected intraperitoneally (i.p.) daily to the experimental groups. EST was created by inoculating EAT 2 days after administration of melatonin. Melatonin application was made between 18:00 and 20:00 in the evening. In addition, light groups were exposed to 580 µW fluorescent light between 02:00 and 04:00 at night. Daily food and water consumption and weight gains of all animals were followed and recorded, and the application was continued for 14 days. Cardiac blood was collected from all animals with heparinized syringes on the 15th day of the experiment and tumor tissues were removed ambuloc. At the end of the experiment, all animals were sacrificed under ether anesthesia.

Results: The MDA and GSH parameters in the cardiac blood taken from the subjects were examined and the diameters of the tumor tissues were measured and examined under a light microscope. Weight change in all groups was not statistically significant ($p>0.05$). It was found that the increases and decreases in the MDA and GSH values of the light and dark groups were not statistically significant ($p>0.05$). It was determined that there was no statistically significant difference between the tumor diameters of the control light groups and the tumor diameters of the other light groups ($p> 0.05$). While the difference between the control of dark groups and the tumor diameters of Group K2 was not statistically significant ($p>0.05$), the increase in tumor diameter of the subjects of Group K3 and Group K4 was found to be statistically significant ($p<0.05$).

Conclusion: As a result; in this study, which investigated the effects of melatonin on experimental EST in dark and light environments, it was revealed that it did not show any positive effect on MDA and GSH levels.

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Keywords: Melatonin, Ehrlich solid tumor, Mice, Antioxidant Effect, MDA, GSH

1. Introduction

Many scientists continue their studies to develop solution plans against cancer. One of the alternatives that come to the fore among such plans is melatonin. Since then, a substantial number of studies have been conducted, and it has been evaluated that melatonin may have direct anti-cancer activity on the one hand, and that its chronobiological regulatory, antioxidant and immune system-supporting properties may also be associated with cancer.

Changes in the lifestyle and nutrition of today's people and industrialization have led to an intense increase in various diseases. Night shift has emerged as an additional health risk affecting more than 100 million people worldwide. Studies have revealed that sleep disorders, fatigue, cardiovascular diseases and certain types of cancer are more common in night shift workers than in day workers. One of the pathophysiological mechanisms of night shift-related diseases is impaired neurological and hormonal regulation, the most important of which is the disruption of the autonomic nervous system and melatonin release from the pineal gland [1].

In addition to demonstrating the effectiveness of melatonin on many types of cancer, studies have shown that its use with classical cancer treatments improves the general condition, increases the clinical response and life expectancy. These data suggest that melatonin, which is an easy and inexpensive molecule to obtain, should be evaluated as a supplement in cancer patients, since no serious side effects have been observed in general and its toxic dose safety limits are high [2]. The purpose of this study is to better understand the use of melatonin. Therefore, in this study, it was aimed to test the anti-tumoral effects of melatonin on Ehrlich solid tumor (EST) formed in Swiss albino male mice in vivo. In the literature review, no study was found that examined the anti-tumoral effect of melatonin at night on an experimental tumor model in vivo. Therefore, in the study, the anti-tumoral effect of melatonin was tested in solid tumor tissue formed in Swiss albino male mice.

2. Materials and Methods

80 Swiss albino male mice, 25-30 gr, 10-12 weeks old, obtained from Çukurova University Medical Sciences Experimental Research and Application Center (TIPDAM) used in the study were used. Standard pellet feed brought from Gaziantep Feed Factory was used and tap water was given ad libitum to the subjects. Mice were divided into 10 groups, 8 in each group (n=8). (+) All animals except the control group prepared 1/1 volume in 0.2 ml NaCl solution and injected 1x106 EAT intramuscularly (i.m.) to create EST. The experimental groups were given 1ml volume of 8 mg/kg/day, 12 mg/kg/day, 16 mg/kg/day melatonin i.m. injected daily. EAT was inoculated 2 days after the start of melatonin administration, and EST was created and the application was continued for 14 days. Melatonin application was made between 18:00 and 20:00 in the evening. In addition, some groups were exposed to 580 µW fluorescent light between 02:00 and 04:00 at night. Two different applications were made in our experimental groups as light and dark groups. From the first day of the experiment, 580 µW/cm2 light was applied to the bright groups between 02:00 and 04:00 every night. On the 2nd day of the experiment, 0.2 ml of 1x106 EAT cells i.m. were injected as an injection, and there were two control groups in the light groups. Group A1(n=8) and Group A5(n=8) were determined as control groups. Group A1 was treated with night light and tumor formation, but melatonin was not applied. On the other hand, only night light was applied to Group A5. Groups A2, A3 and A4 were administered 8, 12 ve 16 mg/kg/day melatonin by i.p injection every day between 18:00 and 20:00, respectively, and night light was applied. Tumor formation was also done. In dark

groups, on the other hand, melatonin was administered to the subjects from the 1st day of the experiment and tumor formation was made on the 2nd day of the experiment. There were two control groups in our dark groups as well as in our light groups. In Group K1, tumors were formed in the subjects on the 2nd day of the experiment, but melatonin and light were not applied. Group K5 was administered 8 mg/kg melatonin by i.p injection every day between 18:00 and 20:00 from the first day of the trial. Groups K2, K3 and K4 were administered 8 mg/kg/day, 12 mg/kg/day, 16 mg/kg/day melatonin, respectively, by i.p injection every day between 18:00 and 20:00.

Cardiac blood was collected from all animals via heparinized syringes on the 15th day of the experiment, and then all animals were sacrificed under ether anesthesia. Daily food and water consumption and weight gain of all animals were followed and recorded.

2.1. Biochemical Analysis

Packs of erythrocytes were prepared from cardiac blood collected from all animals and stored at -70 °C. Then, the GSH and MDA parameters of these samples were studied in accordance with the specified procedures [3].

2.2. Histopathological Evaluation

The tumor tissues of all animals were removed from the ambulok and placed in 10% buffered formaldehyde for 24 hours. It was then passed through different concentrations of alcohols and xylol and determined within 24 hours. Thus, tissue sections of 4 micron thickness were taken from tissue samples prepared with paraffin blocks and deparaffinization process was applied. After the samples stained with hematoxylin were cleared in xylol, they were evaluated under Nikon brand light microscope.

2.3. Statistical Analysis

The variance analysis of the data obtained at the end of the research was made using the SAS package program [4], and the Duncan Multiple Comparison test was used to compare the means [5].

3. RESULTS

3.1. Findings Related to Monitoring of Weight Change of Subjects

The animals were weighed on a sensitive balance every day from the start of the experiment and the weighing results were recorded. The weight change of each group was calculated by subtracting the initial weights from the weights of the experimental animals at the end of 15 days.

3.2. Biochemical Findings

MDA and GSH parameter values of experimental animals are given in Figures 1 and 2. The highest GSH value in the light experiment groups was found in Group A1 (5758 nmol/ml), while the lowest GSH value was obtained in Group A5 (1949 nmol/ml). It was determined that the difference between the GSH values of the light experiment groups was not statistically significant ($p>0.05$). When the GSH values of the dark groups were examined, it was determined that there were decreases in the GSH amounts of all groups. Compared to the control group (Group K1), GSH levels of Group K2, Group K3, Group K4 and Group K5 decreased by 30.3%, 35.2%, 38.6% and 8.4%, respectively.

The highest MDA value in the light experiment groups was found in Group A1 (4,735 nmol/ml), while the lowest MDA value was found in Group A5 (0.897 nmol/ml). It was determined that the difference between the MDA values of the light experiment groups was not statistically significant ($p>0.05$). When the control of dark groups and other dark groups were compared, decreases and increases in MDA amounts were determined. Compared to the control group (Group 1), it was determined that there was an increase of 34% and 10% in Group 2 and Group 5, respectively, and a decrease of 52.8% and 36% in Group 3 and Group 4, respectively.

3.3. Immunohistopathological Findings

As a result of histopathological examination of solid tumor tissues removed as ambuloc from experimental animals, it was determined that tumor involvement was present in all groups that underwent tumor inoculation.

The diameters of the tumor tissues removed from the experimental animals were measured. It was determined that there was no statistically significant difference between the tumor diameters of the control (Group A1) light groups and the tumor diameters of the other light groups ($p> 0.05$). While it was determined that the difference between the tumor diameters of the dark groups (Group K1) and Group K2 was not statistically significant ($p>0.05$), the increase in tumor diameter of the subjects of Group K3 and Group K4 was found to be statistically significant ($p<0.05$).

Calculation of necrosis areas seen in the tissues of tumors removed from the subjects was determined by the (+,-) scoring system. It was determined that the change between the control of the light groups (Group A1) and the necrosis areas of the other light groups was not statistically significant ($p>0.05$). However, when the control of dark groups (Group K2) and the necrosis areas of other dark groups were compared, it was found to be statistically significant ($p <0.05$).

After tumor inoculation, the area of necrosis was determined as 62.5% as a result of histopathological evaluations of Group A1, which consisted of animals that were only exposed to light. The mean tumor diameter was calculated as 0.7 cm. (Figure 3). Histopathological evaluations of Group A2, which was given 8 mg/kg melatonin (MLT) after tumor inoculation and applied light, revealed that the areas of necrosis were 77.5%. Tumor diameter was calculated as 0.96 (Figure 4). As a result of the histopathological evaluations of 12 mg/kg MLT and light-treated Group A3, it was determined that the necrosis areas were 77% and the tumor diameter was 0.8 cm. (Fig. 5). As a result of the histopathological evaluations of 16 mg/kg MLT and light-treated Group A4, it was determined that the necrosis areas were 75% and the tumor diameter was 0.96 cm. (Figure 6) As a result of the histopathological evaluations of Group K1, where only darkness was applied to the animals with tumors, it was determined that the necrosis areas were 65% and the tumor diameter was 0.85 cm. (Figure 7). As a result of the histopathological evaluations of 8 mg/kg MLT and dark application of Group K2, it was determined that the necrosis areas were 100% and the tumor diameter was 1.14 cm. (Figure 8). As a result of the histopathological evaluations of 12 mg/kg MLT and dark application of Group K3, it was determined that the necrosis areas were 100% and the tumor diameter was 1.4 cm. (Figure 9). As a result of the histopathological evaluations of 16mg/kg MLT and dark application of Group K4, it was determined that the necrosis areas were 100% and the tumor diameter was 1.44 cm. (Figure 10).

RESULTS

The pineal gland, which has been known for thousands of years and whose biological importance is known, and the possible effects of melatonin released from this region on cancer have been systematically discussed for the first time since the beginning of the 20th century. In addition to the knowledge that melatonin may have direct anti-cancer activity, there are studies showing that it is a chronobiological regulator and that its antioxidant and immune system-supporting properties may be associated with cancer [6,7].

MDA, which is one of the most frequently measured parameters as an in vivo indicator of oxidative damage, is one of the degradation products of lipid peroxidation. In a study by Skrzydlewska et al., they showed that plasma and tissue MDA concentrations increased in colorectal cancer patients [8]. In a study by Erata et al., they compared the MDA levels between normal and malignant tissues of patients with colorectal cancer and showed that MDA levels were 111% higher in malignant tissue [9]. In the study of Bayraktar et al., a significant increase was observed in serum MDA levels in patients with colon cancer when compared to the control group [10]. Otamiri et al. found a significant increase in MDA levels in colorectal cancer patients compared to normal tissue in their study [11]. However, in addition to studies showing an increase in MDA levels in cancer cases, there are also studies in which decreases in MDA levels are observed and these results are not statistically significant. It is emphasized that changes in MDA levels are insignificant, especially in studies where melatonin is applied. Mei et al. investigated the effects on in vivo and in vitro by stimulating the inflamed colon from rats with colitis with lipopolysaccharide [12]. In vitro experiments showed that 1 mM dose of melatonin application in cells stimulated with lipopolysaccharide caused a significant decrease in MDA and NO levels, while there was a decrease in NO levels at 10 µM and 100 µM doses, but this decrease was not significant. Yerer et al. showed in their study that changes in plasma melatonin levels cause changes in erythrocyte MDA levels, but these changes are not statistically significant [13]. Kavak et al. also found that the effect of melatonin on matrix metalloproteinase-9 in colorectal tumors did not differ significantly in tissue MDA levels [14].

In our study, it was found that the effect of melatonin administration on the increase and decrease in MDA levels in Swiss albino mice inoculated with EST in dark and light environments was not statistically significant ($p>0.05$). While our results regarding MDA level are in line with the results of the studies of both Kavak and Mei et al., they are not compatible with the research results of Skrzydlewska, Özdemirler, Bayraktar, Otamiri et al. [8-11]. GSH is the most abundant non-enzymatic antioxidant in cells, which plays a critical role in the defense against oxidative stress caused by cell injury. Decreased GSH level may affect the development of the disease [15]. Cöl et al. investigated the effects of melatonin injection and pinelactomy on experimental acute pancreatitis in a study they conducted [16]. They found that MDA values, which reached a certain level in subjects with acute pancreatitis in the control group, increased statistically with the addition of pinealectomy, whereas GSH and SOD levels decreased. Kuş et al. investigated the protective effect of melatonin hormone against oxidative damage caused by formaldehyde exposure in the perifrontal cortex of rats [17]. They found that there was an increase in enzyme activities in SOD and GSH-Px, and a statistically significant decrease in MDA values in rats injected with melatonin with formaldehyde exposure. Baydaş et al. examined the daily changes of antioxidant enzymes such as lipid peroxidation levels, glutathione peroxidase activity and oxidized glutathione in the tissues of pinealectomized rats and found that both exogenous and endogenous melatonin increased antioxidant enzyme activity [18]. It has been stated that some of these enzymes show parallelism with the circadian rhythm of melatonin and this rhythm is affected in pinealectomized rats. In our

study; We found that the plasma change in MDA and GSH levels of mice carrying EST to which we applied melatonin did not make a significant difference. This result suggests that the antioxidant activity of melatonin has no effect on lipid peroxidation.

Studies on the anticarcinogenic effects of melatonin, which have many articles that show antioxidant and immune system-supportive properties, have also been reported. There are studies showing that melatonin can increase the antitumor activity of IL-2 by inhibiting tumor growth factor (TGF) production [19]. Melatonin acts as an oncostatic agent [20]. Melatonin has been shown to inhibit the development of melanoma and breast cancers in animal experiments [21,22]. On the other hand, it is stated that melatonin has negative effects in tumors that do not secrete hormones such as leukemia [23]. Venhuskin et al. demonstrated for the first time the inhibitory effect of melatonin in malignancies of mesenchymal origin [24]. In another study, the stimulating effects of melatonin in human prostate cancer cells were investigated and as a result, it was reported that melatonin has a strong support in the prevention of prostate and secondary development process [25]. It has been reported that melatonin inhibits tumor growth in rat breast cancer cells [26]. Many factors affect the synthesis and release of melatonin in the pineal gland and thus the plasma melatonin level [27,28]. The best known and most effective of these factors is the light condition of the environment, that is, whether it is light or dark. In the development of breast cancer, the hypothesis of intense exposure to light and therefore melatonin deficiency in developed countries is put forward [23]. Anna et al., in a study conducted on Chinese women in Singapore, found that women who slept for 9 hours had a lower risk of developing breast cancer than women who slept for 6 hours [29].

The concentration of plasma melatonin is 3-10 times higher at night than during the day. Melatonin secretion begins between 21:00 and 22:00 in the evening, reaches its maximum levels between 02:00 and 04:00, and begins to decrease between 07:00 and 09:00 in the morning. Due to the fact that the hormone melatonin works in a circadian rhythm, in our study, which consists of light and dark groups, we applied light to our subjects between 02:00 and 04:00, when melatonin peaks at night, to inhibit the physiologically secreted melatonin in the body. When the tumor development of our two different experimental groups was evaluated histopathologically, contrary to some other studies, it was found that tumor diameters and necrosis areas in our dark group were higher than in our light group. In parallel with our study, there are studies stating that melatonin has no effect on cancer: In a study evaluating the effects of melatonin on skin cancer in mice, they reported that melatonin suppressed MDA but did not affect tumor tissues [24]. No effect of melatonin on apoptosis in adenocancer cells was observed [14]. They investigated the effect of melatonin on MCF-7 cells and reported that melatonin triggered two different apoptotic processes.

However, as a result of literature review, no in vivo animal models were found in cancer studies in which the secretion of melatonin was inhibited by night light application, this effect was tried to be eliminated by applying additional melatonin and the antioxidant, anticarcinogen properties of melatonin were evaluated. In our study, we determined the triggering effect of EST tumor, which is an aggressive tumor, in the subjects in the dark group, depending on the dose and dark/light cycle of melatonin administration. We found that the tumor diameters and areas of necrosis in the subjects in the light group were smaller and less than the subjects in the dark group.

4. DISCUSSION

As a result, in our study investigating the effects of melatonin on experimental EST in dark and light environments, it was revealed that it did not show any positive effect on MDA and GSH levels. It was determined that melatonin had a triggering effect in the dark on tumor diameter and necrosis

areas. This suggests that melatonin could not inhibit this tumor, since EST is an aggressive tumor that grows quickly and rapidly in mice. Although there are studies in the literature showing that melatonin has inhibitory properties, there are no studies showing that it is a tumor trigger. The fact that no results suggesting that melatonin triggers tumors at night have been found in the literature so far, which is a remarkable result of our study. However, further studies are needed to prove the effects of melatonin on the tumor. We think that a final judgment can be made as a result of investigating the effects of melatonin not only on EST but also on other types of cancer.

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