EFFECT OF DIFFERENT DOSES AND DURATIONS OF MELATONIN ON THE GROWTH INHIBITION EHRlich ASCITES TUMOUR (EAT) IN MICE

Hüsiye DOĞRUMAN* Nuray UZUNÖREN** Tuncay ALTUĞ*
Harun CERİT*** Ayhan ÜNSAL**

Farklı Doz ve Sürelerde Uygulanan Melatoninin Farelerde Ehrlich Ascites Tümörü (EAT) Gelişimi Üzerine Etkileri

Özet: Fotoperiodik olarak salgılanan ve antioksidan özelliği fazla olan melatonin doğal bir onkostatik etkili madde olarak kabul edilmektedir. Bu çalışmada Ehrlich Ascites Tumour (EAT) uygulanan farelere farklı doz ve aralıklarla deri altı melatonin verilerek yaşam süreleri incelemiştir. Melatonin verilmeyen kontrol grubundaki tümörlü fareler 5 hafta içinde öldüler. Haftada üç kez sabahları 500 µg/kg melatonin verilen tümörlü farelerin (grup.1) sayıca azalarak 7. haftaya ulaşıkları izlendi. Her gün deri altı 500 µg/kg melatonin verilen tümörlü fareler (grup.2) daha istikrarlı azalarak 9. haftaya kadar yaşamışlardır. Haftada üç kez 1 mg/kg melatonin verilen tümörlü fareler (grup.3) yedi hafta yaşamışlardır. Her gün 1 mg/kg melatonin verilen tümörlü fareler (grup.4) azalarak sezik hafta yaşamışlardır.

Farelerde melatonin sabah uygulamasının EAT sıvı formunun oluşumunu engellemediği, ancak yaşam sürelerini uzattığı sonucuna varıldı.

Anahtar kelimeler: Melatonin, Ehrlich ascites tumour, fare.

Summary: Photoperiodically released melatonin is a well known antioxidant which have been shown to act as a natural oncostatic agent. The effect of subcutaneously...
(s.c.) injected of different doses of melatonin on the survive of EAT inoculated mice was examined in the present study. Animals with Ehrlich tumor that untreated melatonin in control group were all died within 5 week. Mice (group .1) injected (s. c.) 500 μg/kg melatonin in the morning for 3 times in a week were survived up to 7th week with the decreasing number of animal. The second group injected 500 μg/kg melatonin subcutaneously daily were achieved to survive up to 9th week with the more gradually decreasing number of animal .1mg/kg melatonin injected 3 times in a week (group 3) tumoral mice were survived 7 weeks. Same dose melatonin injected daily humoral mice (group 4) were achieved to survive 8 weeks.

The findings suggests that the mice treated with melatonin in the daily light period does not inhibit liquid form of EAT formation but it can be concluded that melatonin may help to increase the lifetime of mice.

**Key words:** Melatonin, Ehrlich ascites tumour, mice.

**Introduction**

Melatonin (N-acetyl-5-methoxytryptamine) was isolated from the mammalian pineal gland by Lerner and colleagues in 1959 and it was a photoperiodically released hormone (3). Secretion of melatonin levels in the daily light period was minimum, whereas during the dark period of day was maximum (circadian rhythm) (7). The major and well known effect of melatonin was that directly development of gonad or indirectly secretion of gonadotropins changing physiological reproduction mechanism (4). The role of melatonin in the defence against free radicals, which were highly dangerous for organisms, was investigated in the last years (7, 3).

Because of its antioxidant properties, immunostimulatory actions (especially increase in T-Lymphocyte), the abnormal levels of melatonin in cancer patients and results of clinical trials, it is concluded that melatonin could be considered as a natural oncostatic agent (4, 1, 9, 6). Blask (1) reported that exogenously melatonin administration inhibits the growth of transplantable tumour, including leukaemia, fibrosarcoma and Ehrlich tumour in mice.

Melatonin is soluble both in lipid and aqueous solutions (7). Because of this properties it can easily penetrate in the cytoplasm and can cross the nuclear membranes and than effects cycle. Also melatonin specific cytoplasmic membrane receptors were identified in variety of animal tissues (sperma, prostate, granulosa cells, cerebellum and malignant melanoma cells) (1, 9, 6).

Narita and colleagues reported that melanoma cells inoculated mice treated with melatonin, (μg/body weight) in drinking water, inhibited tumour growth on 40th day (5).

EAT is a special tumour for mice which was used in this trial generally have two different forms; liquid and solid, also it has rapidly growing character and highly trans-
portable nature and 100% killing rate (2). EAT treated mice were all died exactly in three weeks. Liquid types of EAT are produced by intraperitonaly injected mice (2).

The aim of this study was to investigate the effect on tumour development and the lifetime by application of melatonin subcutaneously (s.c.) with different doses and durations in the morning, in the intraperitonaly (i. p.) EAT treated male mice.

**Material and Methods**

Male Balb C mice that have approximately 20 g body weight and 40-45 day old were used. One control and 4 experimental groups were designed in this research. 10 mice were randomly assigned to one of the groups. 0.4 ml Ehrlich Ascitic Tumour liquid was injected intraperitonaly each mouse.

Experimental and control groups were both treated with same volume of liquid. Experimental groups were injected melatonin (Sigma μ 5250) subcutaneously at the same day just before tumour treatment continued by the melatonin injection, until the death of animals. 500 μg/kg melatonin (s.c.) were injected to group 1 three times in a week. Group 2 was injected 500 μg/kg melatonin everyday, group 3; were injected 1mg/kg melatonin three times in a week and group 4, were injected same dose of melatonin everyday in the morning.

Number of death mice and tumour formation in the abdominal region was observed, and results were presented in graph.

**Results**

After two weeks, followed by the treatment, significant tumour formations were obtained in the abdominal regions for both experimental and control animals. For first 15 day after treatment, no mice died. In every experimental and control groups number of survived mice are presented in the graphic through periods of 15-20, 21-25, 26-30, 31-35, 36-40, 41-45 and 46-50 day (Graphic 1). During 15-20 day period, 6 mice survived in the group of control, 1 and 4. At the same period, 7 rats survived in group 2 and all animals survived in group 3. In the second period, 3 mice survived in groups of 1, 4 and control. In groups 2 and 3, 5 and 6 mice survived respectively. In the third period 26-30 day, only one mouse survived in groups of control and 1. Number of survival animals detected as 4, 3, 2 mice in groups 2, 3 and 4 respectively. During 31-35 day period, all animals were died in groups of control, although only one mouse in groups 1 and 2, 3 and 2 mice in groups 3 and 4 survived respectively. During 36-40 day, one mouse in groups 1, 2, 3 and 4, and during 41-45 day one mouse in groups 2 and 4, and during last period only one mouse in group 2 survived.
Graphic 1. Number of survived animal in control and experimental group

Discussion

Two weeks later than the EAT treatment, tumour development is obviously visualised in all mice, in experimental and control groups, confirmed that the rate of transplantation is 100%.

There was a tumour development in all melatonin treated mice in group 1,2,3 and 4, 2 weeks later than treatment with EAT which was not appropriate to reports by Blask (1) who reported that melatonin application cause inhibition in the transplanted tumours such as EAT of mice.

The differences between present study and Blask (1) observation might be explained by the formation of liquid form of EAT in all groups and the differences between way of melatonin injection or the number of tumour cells in EAT passage. In addition, the other investigators in this field do not confirm the inhibitory effect of melatonin yet.

Narita et al. (5) reported that melanoma cells inoculated mice got smaller on 40th day when compared to control animals. Presence of melatonin-specific cytoplasmic membrane receptor was identified in malignant melanoma cells by Ying et al. (9) This cytoplasmic membrane receptor seem to have a higher affinity for melatonin and resulting inhibitory effect on tumour (9). On the other hand, no melatonin receptors have been identified on EAT yet.
In the present study, as monitored by graphic tumour inoculated mice in control group were all died up to 5th week. There was a significant difference between death number of experimental and control groups of animals at the period of 15-20 day (p<0.01). The animal in the control groups could not survive up to 26-30 day period and survival number for the others groups gradually decreased (group 1 and 2, 36-40 day; group 4, 41-45 day; group 2 45-50 day).

The findings suggest that the mice treated with melatonin in the daytime, does not inhibit liquid form of EAT formation, but it can be concluded that melatonin may cause longer lifetime. The effect is probably due to highly antioxidative properties of melatonin.

References