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ORIGINAL ARTICLE

Melatonin Shortens the Survival Rate of Ehrlich Ascites-inoculated Mice

Sergiu-Bogdan Catrina, Elena Curca, Anca Irinel Catrina, Corina Radu & Mihai Coculescu

Department of Endocrinology II, University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania.

Correspondence to: Sergiu-Bogdan Catrina, MD. PhD
Dept. Endocrinology II, University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania.
PO Box 41-31, TEL/FAX +40-1-2307705,
E-MAIL: sergiu-bogdan.catrina@molmed.ki.se

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Abstract

OBJECTIVES Pineal gland may have a role in organism's protection against cancer. Melatonin as well as still unidentified low-weight molecular pineal substance(s) have been reported to have growth inhibitory effect on different tumor cells. We tested the influence of melatonin and of a bovine pineal extract on the survival rate of AKR mice inoculated with Ehrlich ascites. The tumor is known to have an accelerated development after pinealectomy.

MATERIAL AND METHODS: Male AKR mice, kept under a 14/10 hours - Light /Dark cycle, were inoculated intraperitoneally (*i.p.*) with 1.5x 10^6 Ehrlich ascites cells. On day three after inoculation the animals were divided in three groups (n=10). Each animal received *i.p.* daily (20.00H), until their death, 250 µl of solution containing melatonin (250 µg), pineal extract (equivalent of 1 bovine pineal gland) or saline.

RESULTS: The average survival rate of the animals treated with melatonin was shorter (14.8+/–2.23 days) compared to control animals (21.9+/–2.21 days)(p=0.01). The animals treated with the pineal extract had a longer survival rate (22.6 +/– 1.8 days) but not statistically significant. The pineal extract was not available for testing at higher doses.

CONCLUSION: In our model, melatonin had a deleterious effect on the survival rate raising the question whether it is correct to assume that the hormone shows lack of adverse reactions.

Introduction

The pineal gland possesses a proven role in signaling the changes of the day/night length to the neuroendocrine system. Apart from its role in seasonal reproductive behavior, pineal gland may have oncostatic properties. In animal models, pinealectomy accelerates growth of a variety of experimental tumors [1], but the mechanism by which the pineal gland protects against malignancy is yet unknown. In this respect, melatonin has been reported to have growth inhibiting properties for some malignant tumors, *in vitro* as well as *in vivo* (reviewed by [2]). However, still unidentified low-weight molecular pineal substance(s) has (have) been shown to have a very potent proapoptotic, antitumoral activity *in vitro* [3, 4].

We tested the influence of melatonin and of a pineal extract on the survival rate of AKR mice inoculated with Ehrlich ascites. The tumor is known to have an accelerated development after pinealectomy [5, 6].

Materials and methods

Melatonin was purchased from Sigma (Stockholm, Sweden). The bovine pineal extract was prepared as previously described [4]. Briefly, the glands were boiled in water (10 min) and frozen until extraction with 0.5 M acetic acid (20 h at 4°C). After filtration, the extracted material was adsorbed to alginic acid and eluted with 0.2 M HCl. The chloride was exchanged by acetate using a DEAE–column (Sephadex 15x7.5 cm) pre-equilibrated with 0.2M acetic acid. The eluate was lyophilized and stored at –20°C.

The experiment was carried out in May on 3 groups of 10 male AKR mice each, aged 50+/–5 days, weight 22+/– 3g. The animals were kept at 25+/– 2°C under a 14/10 hours – Light /Dark cycle (light on at 6.00 a.m.). Food and water were available *ad libidum*. The ethics committee of the University of Medicine and Pharmacy "Carol Davila", Bucharest approved the study protocol.

$1.5x10^6$ Ehrlich ascites cells (provided generously by Dr. M. Terbea from Oncology Institute of Bucharest) were inoculated intraperitoneally (*i.p.*) to each mouse. On day three after inoculation the treatment was started and continued until death. In the melatonin group, each mouse received (*i.p*) 250 µg melatonin every evening (20.00hrs). The other two groups of animals received at the same hour the pineal extract (equivalent of one bovine pineal gland) or saline (as control). The vehicle volume was 250µl/mouse for all three groups.

Significance of the difference between survival rate of melatonin, pineal extract and control treated animals was tested by Student's two sample tests. $P<0.05$ was considered significant.

Results

All inoculated animals developed ascites that did not differ between groups in terms of volume and time of appearance. However, the average survival rate of the animals was different. Compared to control animals (21.9+/–2.21 days) the pineal extract treated group showed a slightly longer survival rate (22.6+/–1.8 days) which was statistically non-significant. The melatonin treated group had a shorter survival rate (14.8+/–2.23 days) ($p=0.01$) (Figure).

Discussion

One main finding of this study is that melatonin decreases the survival rate of the Ehrlich ascites inoculated mice by 32.5%. Melatonin has been reported to have growth promoting effect on other tumor types *in vivo* [7] as well as *in vitro [8]* but most of the reports on its effects on Ehrlich tumour evolution showed a benefic effect [9], [10]. However, *Bartsch and Bartsch* reported, for the first time, a dual effect of melatonin on Ehrlich tumor weight depending on the photoperiod and the time of day of administration [11]. They found an increased Ehrlich tumor weight when melatonin was given in the morning, in animals kept in a photoperiod similar to our conditions. The deleterious effect of melatonin observed in our study could be caused by the high dose of melatonin used (250 µg/mouse) compared to other reports. (100µg/mouse [11], 5 and 100µg/kg [10]). It could also reside in the particular time of the year the experiment was run, knowing that the activity of the pineal gland has a seasonal rhythm in melatonin secretion [12] as well as in its antitumoral activity [13]. We also cannot exclude that the difference in the strain of mice or a difference of Ehrlich cells, due to clonal selection under different laboratory conditions, may have led to the current results. Whatever would be the explanation, it is important to stress that although most of the evidence suggests a protective effect of melatonin in cancer disease, this is not so in general. The discrepancy between the tumor growth promoting effects of surgical extirpation of the pineal gland in most animal models and the dual or unclear effects of melatonin on different tumors could be explained, as well, by the existence of some yet unknown oncostatic substances in the pineal gland. In this respect the crude pineal extract that contains such substance(s) [4] increases, the survival rate, even though not statistically significant. It is conceivable that the pineal extract if given at higher doses may have shown more

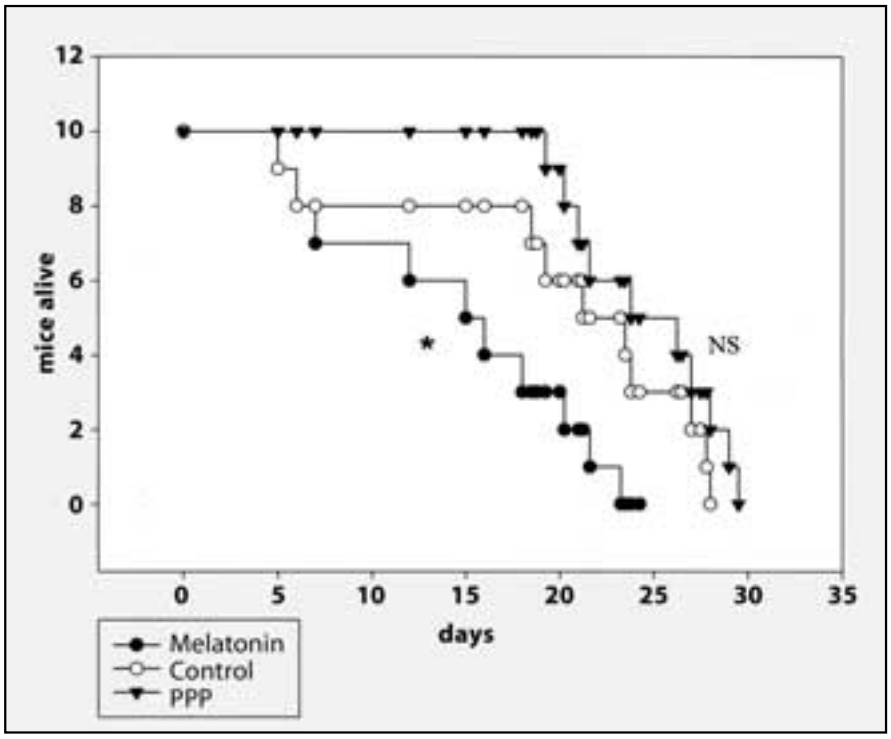

Figure. The survival rate of male AKR mice inoculated with Ehrlich ascites and treated every day with melatonin (black circle) (10 mice), pineal extract (black triangle) (10 mice) and saline (white circles) (10 mice). *-p<0.01 and NS- statistically non-significant

inhibiting effect. The pineal extract, however, was not available for testing at higher doses.

In conclusion, the deleterious effect of melatonin observed in our model raises the question whether it is correct to assume that the hormone shows lack of adverse reactions on tumor growth.

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