Growth-inhibitory action of melatonin and thiazolidinedione derivative CGP 52608 on murine 16/C breast cancer cells

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Submitted: February 22, 2006 Accepted: February 26, 2006

Key words: melatonin; CGP 52608; RZR/ROR receptors; cell proliferation; breast cancer

Abstract

OBJECTIVES: Melatonin may influence directly tumor cells through the specific binding sites. The best known melatonin binding sites are membrane receptors. Recently, the participation of nuclear signalling via estrogen as well as RZR/ROR receptors in oncostatic action of melatonin on the breast cancer has been widely discussed. The aim of present study was to investigate effects of melatonin, the selective ligand for nuclear RZR/ROR receptors – CGP 52608, and methotrexate on growth of murine 16/C breast cancer cells.

MATERIAL AND METHODS: The experiment was performed in vitro. The breast cancer cells were incubated for 2 days in the presence of melatonin, CGP 52608 (at concentrations of 10⁻⁵M, 10⁻⁷M, 10⁻⁹M, 10⁻¹¹M) and methotrexate (at concentrations of 0.25 and 0.125 μg/ml). The growth of cells was measured using the modified Mossman method.

RESULTS: All examined compounds significantly inhibited the growth of cancer cells. The effects of MLT and CGP 52 608 were comparable with suppression caused by methotrexate. The significant differences of efficacy between two examined concentrations of methotrexate were not observed.

CONCLUSION: The obtained data together with our previous results indicate that nuclear receptors RZR/ROR play an important, although not sufficiently recognized role in the oncostatic action of melatonin.

Introduction

Different therapeutic models are applied in the treatment of breast cancer, but their effectiveness is still not satisfying. Therefore, the new drugs increasing anticancer effect for this pathology are investigated. Melatonin (MLT), the main hormone of pineal gland is a new candidate as oncostatic agent. Several of experimental study conducted on animal and human cell lines, and also on experimentally induced animal tumors have confirmed the oncostatic properties of pineal hormone [22].
The mechanism by which MLT inhibits tumor growth is very complex. Among different ways such as the modulation of endocrine [27] and immune system [16], and the antioxidative action [14]. MLT influences target cells directly via the specific receptors [12]. At present, it is believed that MLT may act not only through MT₁ and MT₂ membrane receptors [6], but also via nuclear RZR/ROR receptors [29]. The involvement of nuclear receptor in oncostatic action of MLT is suggested by our and other studies assessing effect of CGP 52608 (selective ligand for nuclear RZR/ROR receptors) [11, 21, 23]. We have found that MLT and thiazolidinedione CGP 52608 inhibited, in similar manner, the cell growth of murine colonic cancer [11] and diethylstilbestrol-induced rat pituitary tumor [21]. Additionally, the other authors have shown the similar antiproliferative effects of both compounds on human ovarian adenocarcinoma cell line BG-1 [23] and on human androgen-dependent (LNCaP) [18, 19] as well as on androgen-independent (DU 145) prostate cancers [17, 20]. There is an evidence, coming mainly from the experimental studies, indicating the oncostatic action of MLT on breast cancer [3, 7, 9], but data on CGP 52608 effect are rather rare [4]. Thus, we decided to examine the influence of both agents on murine adenocarcinoma cell line 16/C and to compare their effects with action of methotrexate, one of the drugs applied in the therapy of breast cancer.

Material and methods

Cell culture

The murine adenocarcinoma cell line 16/C, kindly obtained from Hirszfeld Institute of Immunology and Experimental Therapy, Wroclaw, was used in the experiment. The continuous culture of the cells was maintained in culture flasks (Nunc Easy flask 25 cm², NUNC). The cells were cultured in RPMI 1640 medium (Sigma) supplemented with 25 mM Hepes buffer (Sigma), 100 U/mL penicillin and 100 μg/mL streptomycin solution (Sigma) and 10% heat-inactivated bovine fetal serum (FBS, Biochrom) at 37°C in the humidified atmosphere of 95% air and 5% CO₂. Before confluence (twice a week) the cells were harvested after a 10-min incubation at 37°C the presence of trypsin-EDTA (0.05 and 0.02% respectively) in Hanks-balanced salt solution (Sigma). The cells were washed three times in complete RPMI and after last centrifugation seeded at 1x10⁶ cells in 5 mL of fresh medium.

Experiment

The cells were subjected to the trypsinization process as described above and suspended at 1x10⁶/mL cells in complete RPMI. 5x10⁴ cells (50 μl of cell suspension) were placed in the wells of cell culture plates (96 Cell Culture Cluster Dish, Nunclon MicroWell Plates, NUNC) containing 130 μL of complete RPMI. After 24 hrs of incubation (5%CO₂, 37°C, 95% humidity), the 20 μL of investigated compounds: methotrexate (Ebeve) in final concentrations 0.25 μg/mL and 0.125 μg/mL, melatonin (Sigma, USA) and CGP 52608 (Novartis Pharma Inc., Basel, Switzerland) in final concentrations 10⁻⁵M, 10⁻⁷M, 10⁻⁹M, 10⁻¹¹M were added to the appropriate wells. Melatonin and CGP 52608 were both dissolved firstly in 0.9% NaCl with 10% of 95% ethanol and then in RPMI-1640. The highest concentration of 95% ethanol in wells was 0.13% (in samples with 10⁻⁵M of melatonin and CGP 52608). The equal volume of culture medium (20 μL) and 95% ethanol (in final concentration 0.13%) was added to the control samples. After 48hrs of incubation the cell proliferation was measured using EZ4Y system (EZ4Y, Easy for You, The 4th Generation Non Radioactive Cell Proliferation & Cytotoxicity Assay, Biomedica Gruppe, Austria, Bellco Biomedica, Poland). The assay is based on the transformation of tetrazolium salt into colored soluble formazans as a result of the mitochondrial activity of the viable cells. The red soluble formazans, released to the culture medium, were determined by the extinction measurement using the enzyme-linked immunosorbent assay reader.

Statistical analysis

The data was presented as the means ± SEM. Statistical comparisons between experimental groups were determined with nonparametric Mann-Whitney’s test. Differences were considered significant if p<0.05.

Results

All examined compounds significantly inhibited the growth of murine 16C breast cancer cells. As can be seen in Fig. 1, melatonin was the most effective at concentration of 10⁻⁷M. Action of CGP was the strongest at concentration of 10⁻⁵M. The effects of MLT and CGP were comparable with suppression caused by methotrexate. No significant differences between two examined concentrations of methotrexate were observed.

Discussion

On the basis of the previous reports, it can be stated that a direct action of melatonin on breast cancer may depend on the membrane receptors or and the nuclear RZR/ROR receptors and also depends on the presence of estrogen receptors (ER) in tumor cells. Immunohistochemical examination showed MT₁ membrane receptors in normal and malignant human breast tissues, however, in tumor cells high receptors levels occurred [5]. Expression of MT₁ but not MT₂ receptors has been also found in MCF-7 human breast cancer [25]. Furthermore, overexpression of the MT₁ receptor in MCF-7 cancer cells reduces tumor incidence in mice receiving MLT [1] and enhances the antiproliferative effect of MLT on breast cancer cells [32]. Ram et al. [25] have shown that pineal hormone inhibits the cell proliferation by activation of MT₁ receptor and melatonin’s growth-inhibitory effect occurs only in breast cancer cells having ER. The relationship between the expression of ER and the antitumor action of MLT has been the object of extensive investiga-
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In recent years [28], it has been proved that there is a correlation between the level of ER in tumor cells and the oncostatic effect of MLT on breast and other types of cancers [10, 24]. It was suggested that MLT acts through destabilization of the binding of the estradiol-estrogen receptor complex to the estrogen responsive element on DNA [26]. In the present study, we observed the inhibitory-growth effect of MLT on 16/C breast cancer cells. 16/C mouse mammary adenocarcinoma was derived from lung metastasis of mammary cancer spontaneously arisen in female C3H mouse [2] and was used for anticancer drug screening [15]. It has been known that these cancer cells possess ER, but the expressions of membrane melatonin and nuclear RZR/ROR receptors have not been examined. However, the involvement of the estrogen receptors cannot be excluded. Similar inhibitory effect of CGP 52608 and MLT observed in our experiment suggests the participation of nuclear signalling in the oncostatic action of MLT. The contribution of RZR/ROR receptors in action of MLT has been lately investigated by our and other groups [4, 11, 21, 23]. We have found that MLT and RZR/ROR receptor ligand CGP 52608 exerted similar antiproliferative effects on colonic cancer cells having nuclear RORα receptors [11, 13]. Moreover, the study conducted in our laboratory on murine Colon 38 cancer has proved that MLT and CGP as well, cause not only the inhibition of tumor proliferation, but also induce apoptosis [30]. In the next, our investigations showed that thiazolidinedione CGP 55644 (an antagonist of nuclear RZR/ROR receptor) blocked the proapoptotic effect of pineal hormone [31]. These results suggest that the induction of apoptosis by MLT depends mainly on its action via nuclear RZR/RORα receptors. Transcripts of RORα receptors have been identified in the ER-positive MCF-7 cancer cells as well as in the breast cancers, which do not possess ER [4, 8, 25]. It was found also that melatonin inhibits the transcriptional activity of RZR/ROR receptors in MCF-7 breast cancer [4]. Besides, both MLT and CGP 52608, RZR/RORα agonist, at a concentration of 10^{-9} M, similarly decrease MCF-7 cell proliferation in a time-dependent manner [25]. However, the data concerning the effects of MLT in dependence of RZR/ROR expression are controversial. Ram et al. [25] have observed, that MLT, and also ligand RZR/ROR did not inhibit the growth of breast cancer cells, which are ER-negative and express only trace amount MT_1. On the other hand, Girgert et al. [8] have shown that in the breast cancers cells possessing higher expression of RZR/ROR but not expressing MT_1 receptors, MLT was most effective at lower concentrations.

In summary, results of the present study showed that melatonin and the selective ligand of RZR/ROR receptors have similar antiproliferative effect on 16/C breast adenocarcinoma cells and action of both compounds are comparable with suppression caused by methotrexate. Above data, together with earlier findings coming from our and other laboratories indicate that nuclear receptors RZR/ROR play an important, although not sufficiently recognized role in the oncostatic action of melatonin.

Fig. 1. Effects of melatonin (MLT), the ligand of RZR/ROR receptor – CGP 52608 and methotrexate (Mtx) on optical density of sample in 16/C cancer cells in vitro; C-control group. Bars represent means ± SEM.

REFERENCES

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