

Melatonin Does Not Alter Cell Proliferation in Metastatic Breast Cancer Cells [†]

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Abstract: Breast cancer treatments continue to be investigated with supported by new treatment methods. Melatonin is a hormone that can be effective in the treatment of breast cancer due to its anti-oxidant effect. Melatonin had previously shown to inhibit proliferation of cancer cells. In this study, we aimed to determine the effect of melatonin on the proliferation of metastatic breast cancer cells in comparison to doxorubicin, a well-known chemotherapeutic agent. Doxorubicin inhibited proliferation of metastatic breast cancer cells while melatonin has no effect. We are currently examining the effects of melatonin and doxorubicin combination therapy on metastatic breast cancer cells.

Keywords: breast cancer; melatonin; doxorubicin; WST

1. Introduction

Breast cancer is the common type of female cancer in the world. According to literature one-tenth of women have risk of developing breast cancer and one-third of these women who have developed breast cancer lost their life for this reason. Most common cause of breast cancer dependent deaths is metastasis. Moreover, breast cancer the second leading cause of cancer deaths in women worldwide. The most important challenge in the treatment of breast cancer is its inter tumoral and intra tumoral heterogeneity [1]. Breast cancer can be divided into different subtypes, according to the expression of molecular markers and hormone receptor such as; luminal A, luminal B, HER2+, and triple-negative (estrogen receptor, progesterone receptor, and HER2) [2]. Depends on to the subtypes, there are different therapeutic strategies in clinic. Most well-known treatments for breast cancer include surgery, radiotherapy, chemotherapy, endocrine therapy and targeted therapy [3]. Melatonin is a hormone which plays pleiotropic roles and is widely distributed in most organisms, and has different physiological functions. Melatonin is synthesized in the pineal gland during the night and is regulated by the circadian rhythm via the suprachiasmatic nucleus. Moreover, melatonin is known that anti-oxidant and anti-apoptotic signaling function. Melatonin is remove free radicals and up-regulates several antioxidant enzymes [4]. For these functions of the melatonin suggest that having a role of tumorigenesis. Melatonin exerts oncostatic effects on breast cancer via immunomodulation, antioxidation, enzyme regulation, regulation of various kinases and transcription factors. It is now accepted that melatonin has antioxidative effect, providing protection against damage from carcinogenic substances, acting as a free radical scavenger. Studies suggested that protective role of melatonin is related with both hormone-dependent and hormone-independent cancers [5]. Doxorubicin is well-known used anthracycline and has demonstrated significant therapeutic activity in many cancer types especially breast cancer. It works with interfering with the function of DNA.

Doxorubicin is commonly used chemotherapeutic for the treatment of patients whose breast tumors are resistant to endocrine therapy. In spite of that, its use is limited by a highly toxicity profile, especially cardio toxicity [6].

In this study, we aimed to determine the effect of melatonin on the proliferation of metastatic breast cancer cells in comparison to doxorubicin, a well-known chemotherapeutic agent.

2. Materials and Methods

In this study we used 4T1 derived 4TBM (4T1-Brain Metastatic Tumor) cells. 4TBM cell line were cultured at 37 °C in Dulbecco's modified Eagle's medium (DMEM-F12) (#10-090-CV, Corning, NewYork, NY, USA) supplemented with 10% fetal bovine serum (#35-016-CV, Corning, NewYork, NY, USA) and 1% Penicillin-Streptomycin (#30-002-CI, Corning, NewYork, NY, USA) in a humid incubator with 5% CO₂. 4TBM cells were seeded at a density of 1×10^4 cells per well in 96-well culture plates and allowed to be attached for plates for 24 h. We prepared doxorubicin (Janssen, NJ, USA) in 4 different concentration, 0.1 μ M, 1 μ M, 5 μ M, 10 μ M. Melatonin (#M5250-10 g, Sigma Aldrich, St. Louis, MO, USA) were used 5 different doses; 0.01 μ M, 0.1 μ M, 1 μ M, 10 μ M, 30 μ M. With these concentrations, the cells were incubated for 72 h. Cell proliferation was tested using WST-1-based colorimetry (Cell Proliferation Reagent WST-1) (5015944001, Roche Applied Science, Mannheim, Germany). After the incubation 10 mL per well Cell Proliferation Reagent WST-1 added into the cells and incubated the cells for 1 h. Measure the absorbance of the cells using a microplate reader at 420–480 nm. Cell viability results were presented in comparison to the Control using GraphPad Prism software.3.

3. Results

First step in this study we performed that cell proliferation rates in 4TBM cells with Melatonin and Doxorubicin treatment. Cell proliferation inhibited 4TBM cells in treated with Doxorubicin 1 μ M, 5 μ M and 10 μ M concentration (Figure 1). Cell proliferation decreased in treated with Melatonin groups, but this reduction not significantly difference.

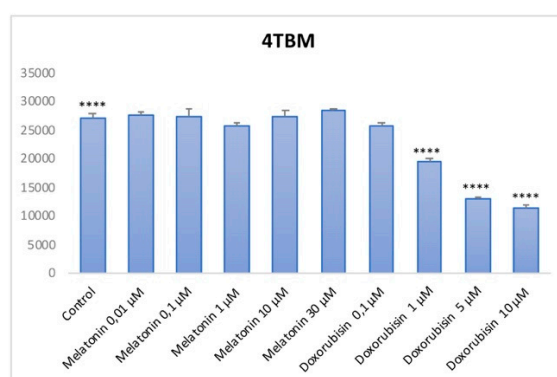


Figure 1. WST-1 cell proliferation assay after 72 h of incubation in normal medium (control), in treated with Melatonin and Doxorubicin (**** $p < 0.0001$).

4. Discussion

Efficient therapeutic approaches are lacking in metastatic breast carcinoma. The most effective treatment method is still carried out via chemotherapeutics. Therefore, recent studies emphasize that traditional therapies with combined therapies are more effective [7]. Recent studies showed that melatonin plays an important role in the progression of breast cancer [8]. Melatonin effect tumor progression via anti-estrogenic, anti-proliferative, anti-metastatic, pro-apoptotic, anti-angiogenic pathways [8]. We showed that melatonin decreases primary tumor growth and metastasis under in vivo conditions. We however did not observe any direct effect of melatonin on proliferation of brain metastatic subset of breast carcinoma (4TBM) although Doxorubicin effectively inhibited cell proliferation. These results suggest that in vivo anti-tumoral effects of melatonin are likely to be

mediated by immune system. We are currently examining whether doxorubicin and melatonin co-treatment alters the behavior of the cells under in vitro conditions.

Author Contributions: G.T and S.D. conceived and coordinated the study and wrote the abstract. N.E. is involved in planning of the studies and arranged and decided the treatment concentration. S.D. performed the WST-1 analysis. G.T., S.D. and N.E. made statistical analyze and interpreted the results.

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Conflicts of Interest: The authors declare that they have no conflicts of interest with the contents of this article.

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