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Influences of Morphine on Cannabinoid Receptor 1, 2 Expression in Breast Cancer Cell Lines

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Abstract

Objectives: This study aimed to investigate the regulatory effect of morphine on the expression of the cannabinoid receptors in the cancer progression. The application of morphine to relieve pain in the patients with cancer may facilitate identifying the risk of tumor growth and metastasis.

Methods: The MDA-MB-231 and MCF-7 cells were treated with different concentrations (1.6, 0.16, 0.016, and 0.0016 μM) of morphine for 24 and 48 hours. The qPCR was performed to evaluate the effect of morphine treatment on the cannabinoid receptors type 1 and 2 gene expression.

Results: Our study results showed that the expression of cannabinoid receptors 1 (*CB1*) and 2 (*CB2*) was regulated by morphine in a dose-dependent manner. We observed in the expression of *CB1* and *CB2* in the cell lines in which the treated dose was 1.6 μM. However, administration of other doses of morphine reduced the expression of the cannabinoid receptors.

Conclusion: In general, the results of this research show that different doses of morphine have different effects on the expression of cannabinoid receptors, and the general purpose of this research is to gain experience to find the best dose of morphine in treatment. Also, to find out which dose is more useful for a cancerous patient.

Keywords: Breast cancer, Morphine, Cannabinoid receptors 1 and 2, MCF-7, MDA-MB-231

Introduction

Breast tumor is one of the most common forms of cancer, especially amongst females. Despite the recent advances in the strategies towards medical diagnosis and treatments, majority of the cancer patients are only diagnosed at the final stages of the disease (1,2).

One of the major problems faced by clinicians is the management of pain in the cancer patients. Severity of the pain depends on the type of tumor, stage of the disease, and therapy that the patient receives. Pain in the cancer patients may result from the chemotherapy, radiotherapy, and surgery treatments or the tumor tissue itself. A significant number of individuals with metastatic tumor develop chronic pain before the terminal stage of the disease (3,4).

Currently, Opioids – morphine, in particular – is used to relieve pain. However, some side effects have been reported for morphine including addiction, breathing problems, constipation, nausea, and the suppression of the immune system (5,6).

Recent studies have revealed that using a combination of cannabinoids and opioids leads to a synergistic act with improved analgesic effects. Apparently, lower drug dosage with fewer side effects can be used. Also, the cannabinoids and opioids receptors share a similar signaling mechanism. The interaction between these two drugs may help that run signal transduction and act through the similar signaling pathways to exert their pharmacological effects. Moreover, some studies have demonstrated that the cannabinoids increase the production and secretion of endogenous opioids (7,8).

The endocannabinoid system is composed of endogenous cannabinoids (neuromodulator lipids) and cannabinoid receptors. They have been defined as the suitable molecules for the therapy of various complications (e.g., cardiovascular diseases, obesity, multiple sclerosis, and neurodegenerative disorders) (9). In addition, cannabinoids are regarded as the potential compounds with anti-cancer effects. They have been reported to inhibit the tumor progression and growth by regulating the cell survival, proliferation, angiogenesis, and metastasis. The cannabinoid receptor 1(*CB1*) is highly expressed in the central nervous system as well as in the other tissues including the adipocytes, uterus, spleen, and vascular endothelium. The cannabinoid receptor 2(*CB2*) expression is more restricted to the immune system cells (10, 11).

The increased *CB1* and *CB2* expressions have been documented in different types of cancers. These expressions are closely associated with the progression stages of tumor, and determine the indicator of tumor invasion (12,13). The studies have shown that the higher

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Key Messages

- The expression of CB1 was significantly associated with morphine treatment in the MCF-7 and MDA-MB-213 cell lines. Moreover, the expression analysis of CB2 in the MCF-7 and MDA-MB-213 cell lines revealed a significant change after morphine treatment.

the expression of cannabinoid receptors in cancer cells, the greater the specific binding of cannabinoids to receptors. When the expression of cannabinoid receptors is lower in the cancer cells, for instance, cannabinoids mostly bind to the most frequent *CB2* receptors in the inflammatory cells of the immune system. This activates the Th2 response and immune suppression, which, in turn, increases the tumor growth and invasion (14,15).

One of the challenging aspects of the cancer pain management is the relationship between opioids and cancer progression (16). Studies have indicated that the application of morphine to deal with cancer patients may facilitate the tumor growth and metastasis mainly by immunomodulation and angiogenesis promotion. In contrast, morphine usage reduces the pain and stress which significantly contribute to the immune system function. The produced hormones in response to the stress conditions (e.g., cortisol) suppress the immune system and stimulate the production of the cancer mediators (17).

The implication of the endocannabinoid system in the cancer treatment has recently received research attention. Since the functional interaction between opioids and endocannabinoid system was reported in the previous studies, this study aimed to evaluate the possible regulatory effects of morphine on the expression of *CB1* and *CB2* in two breast cancer cell lines, as well as to understand the morphine dosage influences on the tumor cells to clear the way for conducting future studies in the clinical setting.

Materials and Methods

Cell Culture

Two breast cancer cell lines including MCF-7(Estrogen positive [+ER] and progesterone positive [+PR]) and MDA-MB-213 (Triple negative) were purchased from Pasteur Institute, Tehran, Iran. MDA-MB-213 cells cultured in Roswell Park Memorial Institute (RPMI 1640) and MCF-7 cells cultured in Dulbecco's Modified Eagle Medium (DMEM) were supplemented with 1% antibiotics (100 µg/mL streptomycin and 100 units/mL penicillin), 10% fetal calf serum, and Sodium bicarbonate (1.5 g/L).

Cell Treatment

The MCF-7 and MDA-MB-213 cells were seeded into 12-well plates and grown for 24 and 48 hours. The cells were treated with specific doses of Morphine (1.6, 0.16, 0.016, and 0.0016 µM) and were incubated. The cells were detached with trypsin-EDTA after 24 and 48 hours, and then were suspended in DMEM with 10% FBS. Eventually, the cells were washed with phosphate-buffered saline (PBS) and prepared for RNA extraction.

RNA Extraction and CDNA Synthesize

Total RNA was extracted from breast cancer cells after 24/48 hours using RNA extraction kit (Yata, Iran) and according to the manufacturer's instruction. The RNA quality was approved by NanoDrop spectrophotometer. CDNA was synthesized using kit (Fermentas International, Canada) with oligo dT primers and random hexamers.

Primer Design and Real-Time PCR

The specific primers were designed for *CB1*, *CB2*, and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Internal control) using the beacon designer software (PREMIER Biosoft, Inc, USA). The sequence of the designed primers was as follow. CGCCCTAACCCTGGATTGCC forward and TGATGGTGCGGAAGGTGGTA reverse for *CB1*; CCACAACACAACCCAAAGCC forward and TCTGTCACCCAGCATTCCTC reverse for CB2. Quantitative real time PCR was carried out using specific primers for the selected genes with the QuantiTect SYBR Green PCR Master Mix (Qiagen, Hilden, Germany) and the Rotor-gene 6000 instrument (Qiagen, Hilden, Germany). The PCR conditions included an initial denaturation at 95°C for 10 minutes, followed by 40 amplification cycles including denaturation at 95°C for 15 seconds, annealing at 60°C for 20 seconds and an extension at 72°C for 20 seconds.

Data Analysis

The data analysis was performed using SPSS (SPSS, Chicago, IL, USA). The gene expression was analyzed using one sample -test and one-way ANOVA, and $P<0.05$ was used as the cut-off for statistical significance.

Results

The expression of *CB1* is significantly associated with morphine treatment in the MCF-7 and MDA-MB-213 cell lines.

Herein, a significant change was observed in the gene expression of the cannabinoid receptors type 1 in response to morphine treatment after the 24 and 48 hours in the MCF-7 cell line. According to our study results, the expression of *CB1* in the MCF-7 cells treated with 0.0016 µM morphine returned to the normal range (i.e., without treatment) after the 48 hours in comparison to 0.016,0.16 and 1.6 µM (Figure 1). The gene expression analysis in the MDA-MB-213 cells showed the same significance association ($P<0.05$) (Figure 2).

The expression analysis of *CB2* in the MCF-7 and MDA-MB-213 cell lines revealed a significant change after implementation of the morphine treatment

Our study results also showed that the expression of *CB2*

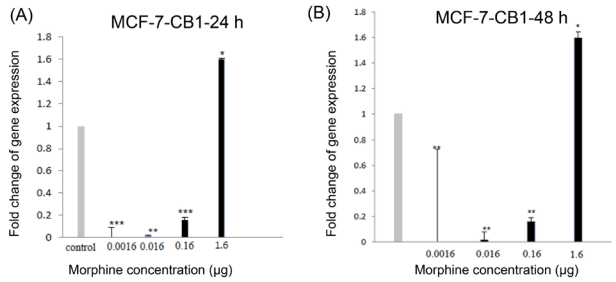

Figure 1. Expression Analysis of *CB1* in the MCF-7 Breast Cancer Cell Line After Treatment With Different Doses of Morphine (1.6, 0.16, 0.016, and 0.0016 µM). Data obtained after both 24 and 48 hours are presented. Gene expression is reported as mean ± SEM (* P<0.05; ** P<0. 01; *** P<0.001).

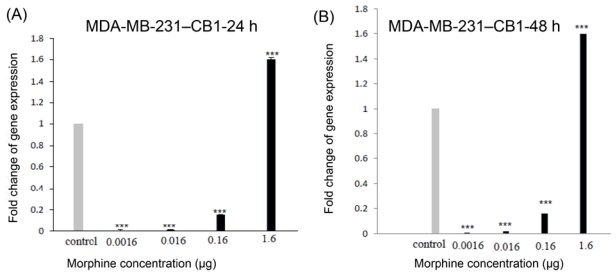

Figure 2. Expression Analysis of *CB1* in MDA-MB-213 Breast Cancer Cell Line after Treatment with Different Doses of Morphine (1.6, 0.16, 0.016, and 0.0016 µM). Data obtained after both 24 and 48 hours are presented. Gene expression is reported as mean ± SEM (* P<0.05; ** P<0. 01; *** P<0.001).

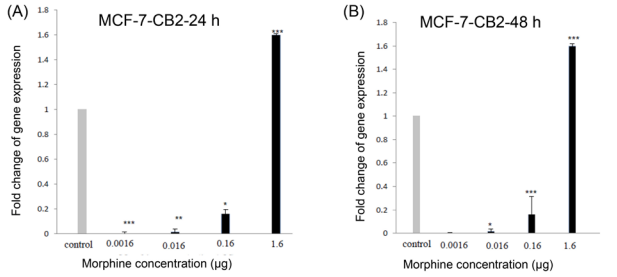

Figure 3. Expression Analysis of *CB2* in the MCF-7 Breast Cancer Cell Line after Treatment With Different Doses of Morphine (1.6, 0.16, 0.016, and 0.0016 µM).

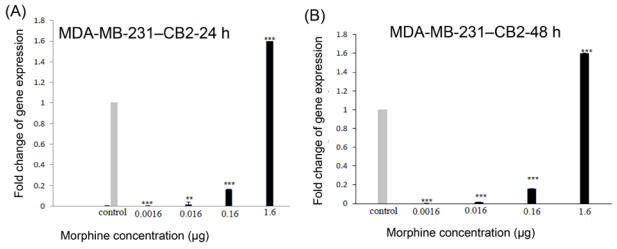

Figure 4. Expression Analysis of *CB2* in MDA-MB-213 Breast Cancer Cell Line after Treatment with Different Doses of Morphine (1.6, 0.16, 0.016, and 0.0016 µM). Data obtained after both 24 and 48 hours are presented. Gene expression is reported as mean ± SEM (* P<0.05; ** P<0. 01; *** P<0.001).

was regulated by morphine in MCF-7 (Figure 3) and MDA-MB-213 cells (Figure 4) after both 24 and 48 hours.

Discussion

Herein, a dose-dependent pattern of the cannabinoid receptors gene expression was recognized in the breast cancer cells treated with specific doses of morphine, which confirmed the potential regulatory effect of morphine on the cannabinoid receptors gene expression. In addition, different doses of morphine were associated with different responses, so that the highest dose (1.6 µM) led to the increased expression of the *CB1* and *CB2*; however, other doses of morphine resulted in the reduced the expression of both receptors. This finding has been particularly highlighted in the translational research and the medical management of the cancer patients. The expression analysis showed a significant increase in the expression of *CB1* and *CB2* in the cells treated with 1.6 µM morphine. On the contrary, other doses of morphine (0.16, 0.016, and 0.0016 µM) were accompanied by the reduced expression of the cannabinoid receptors. This pattern of gene expression was observed in both of the treated cell lines (Figures 1 and 2).

Morphine is a common analgesic used for the pain management of the terminally ill cancer patients. Furthermore, it is commonly used for achieving anesthetic purpose in the surgery of the cancer patients. The effect of morphine on cancer recurrence and metastasis in patients has been the subject of several studies; however, the results of the studies are contradictory (18-20).

Some animal studies have demonstrated that morphine treatment is accompanied by infection, cancer aggravation, and reduced survival (21). Other studies have also revealed that morphine inhibits apoptosis, increases cyclin D expression and cell cycle progression, as well as activates proangiogenic signaling in the cancer cell lines (22,23). Niu et al recently reported that morphine increased the cancer stem cells population and promoted the drug resistance in the breast cancer cells (24).

On the other hand, there are some reports on the anti-cancer effects of morphine. A recent study by Khabbazi et al indicated that morphine may have reduced tumor progression by inhibiting the macrophage proteases production in the tumor microenvironment (25). Another recent study also demonstrates that the *in vitro* treatment of morphine exerted an anti-angiogenic effect on the Lewis lung carcinoma cells (26).

The interaction between morphine and endocannabinoid system has been discovered by some studies. The cannabinoid receptors are attractive therapeutic targets in the cancer treatment. But some studies have indicated that they may produce tumor-promoting effect. For instance, it has been shown that the inhibition of CB1 signaling by using antagonists prevents the progression of some types of cancers (27). The differential expression of the cannabinoid receptors in the various cell lines may be responsible for these contradictory observations. In fact,

low or none expression of both receptors in the tumor cells is accompanied by immune suppression and the subsequent tumor cells proliferation (28).

Due to the implication of the cannabinoid system in the disease treatments, the mechanisms involved in the cannabinoid system regulation are of great attention. We previously referred to the studies on the interaction of morphine and cannabinoid receptors. There are studies showing that morphine treatment can regulate the expression of the cannabinoid receptors (29,30).

Our results were in line with findings of some previous investigations. In one study, the chronic treatment of morphine was found associated with the increased expression of CB1 in both protein and mRNA levels (30). In addition, recent research in the breast tumor cells determined that the chronic doses of morphine were associated with cell growth inhibition by modulation of the ErbB signaling (31).

It should be noted that higher doses of morphine, according to many studies, were found associated with tumor inhibition due to an unknown mechanism. For instance, studies showed that morphine treatment with the concentration of >10 μM inhibited the proliferation of MCF-7 and MDA-MB-213 cells. Also, the repeated administration of morphine in the mouse model may have exerted the anti-tumor effect (32).

The result of this study and the literature review indicated that the dual effects of morphine in the regulation of tumor growth may have been associated with the concentration of morphine and the origin of the tumor. However, it was recommended that further studies should be conducted to investigate the mechanism of this dose-dependent observation in greater details. The morphine effect on the cannabinoid receptors expression and function is presently one of the suggested mechanisms.

In this study, a dose-dependent pattern of *CB1* and *CB2* mRNA expression was identified in the breast cancer cells in response to varying doses of morphine. Therefore, it was suggested that future studies should be carried out in order to determine the best dosage of morphine for managing the pain in cancer patients and to reduce the burden of accompanying side effects of the palliative care. The current study faced few limitations. First, the related proteins in the pathway were not evaluated in this study. Moreover, there were problems regarding the preparation of materials as well limitations on the setup of methods.

Authors' Contribution

Conceptualization: Fariba Nabatchian.
Methodology: Golnaz Vaseghi, Zohreh Shafiezadeh.
Validation: Fariba Nabatchian.
Formal analysis: Golnaz Vaseghi, Zohreh Shafiezadeh.
Investigation: Homa HamLedari, Zohreh Shafiezadeh.
Resources: Golnaz Vaseghi.
Data curation: Golnaz Vaseghi, Zohreh Shafiezadeh
Writing–original draft preparation: Reza Afrisham.
Writing–review and editing: Reza Afrisham, Golnaz Vaseghi.
Visualization: Reza Afrisham, Homa Hamledari.
Supervision: Fariba Nabatchian.
Project administration: Fariba Nabatchian.
Funding acquisition: Fariba Nabatchian.

Conflict of Interests

Authors declare that they have no conflict of interests.

Ethical Issues

The study protocols were approved by the Medical Ethics Committee of Tehran University of Medical Sciences with the code IR.TUMS.VCR.REC.1395.233.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available upon reasonable request from the corresponding author.

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