Impact of Morphine on Viability of MCF-7 and T47D Breast Cancer Cells

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Abstract

Morphine, a highly potent analgesic, is prescribed for the treatment of severe pain associated with cancer. Several in vitro and animal studies suggest that morphine is involved both in promoting and inhibiting tumor growth. Our aim was to test the outcome of adding morphine to the culture media of cells from two of the most widely used breast cancer cell lines. MCF-7 and T47D cells were seeded into 96-well microplates and cultured for 24 hours in MEM and RPMI-1640 media respectively. Afterwards, cells were exposed for 24, 48 or 72 hours to media containing morphine at the following concentrations: 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1 µM. Cell viability was assessed by the MTT colorimetric method. After exposure of MCF-7 cells to morphine for 24 and 48 hours, viability was similar to the control while, after 72 hours, this parameter was significantly enhanced at 0.75 µM and 1 µM. Survival of T47D cells in the first 24 hours was significantly (p<0.05) increased by the presence of 1 µM morphine, while an increased exposure time did not improve the outcome. Our results show that morphine can increase viability of breast cancer cells, depending on concentration, exposure time and cell origin.

Keywords: cell viability, MCF-7, morphine, T47D

Introduction

Breast cancer is the most common cancer in women (Miller et al., 2019), death being usually caused by recurrence and metastasis (Ecimovic et al., 2011). One of the most common symptoms of cancer is pain, with 50-80% of cancer patients experiencing some degree of pain (Tuaxun and Cui, 2018).

Morphine was isolated in the 19th century by Sertürner from opium (Huxtable and Schwarz, 2001), the dried latex of the opium poppy, Papaver somniferum which has been cultivated for over 3000 years (Perea-Sasiaín, 2008). Opioids have a long history as treatment for diarrhea and pain (Zhang et al., 2008). Their potent analgesic and sedative effects make them widely used in clinical practice (Doornebal et al., 2015). Morphine is used to manage pain but also for anesthetic procedures in cancer patients undergoing surgery (Zhang et al., 2018).

The way morphine influences tumors, either by stimulating or inhibiting growth has been under debate, with in vivo and in vitro studies weighing on either side (Tuaxun and Cui, 2018). Retrospective studies reported that patients who received general anesthesia with large amounts of opioids showed more cancer progression or recurrence than patients who received regional anesthesia or a lower amount of opioids (Biki et al., 2008; Exadaktylos et al., 2006; Maher et al., 2014;
Scavonetto et al., 2014). This caused significant alarm for the use of morphine especially in lung, breast and prostate cancers.

Opioids have been shown to promote angiogenesis and increase breast cancer progression (Bimonte et al., 2015; Gupta et al., 2002), to accelerate the growth of tumour cells and induce metastasis in lung (Wang et al., 2015) and nasopharyngeal tumors (Cao et al., 2016). Morphine can also inhibit immune cell functions and cause immunosuppression (Gach et al., 2011). On the other hand, Kim et al. (2016) found that morphine may suppress progression of several lung cancer cell lines. Additional studies have reported that opioids can inhibit gastric cancer progression in vitro (Qin et al., 2012), may induce apoptosis in colon cancer (Palma et al., 2015; Zhang et al., 2014) and do not enhance breast cancer expansion (Chen et al., 2017; Doornebal et al., 2015).

Studies of morphine effects in breast cancer have often yielded contradictory results (Juneja, 2014), though it has become apparent that it plays a dual role in the regulation of tumors (Tuerxun and Cui, 2018).

Given the conflicting nature of the currently available evidence, our aim was to test the outcome of adding morphine to the culture media of cells from two of the most widely used breast cancer cell lines.

**Materials and Methods**

**Materials**

Culture media MEM and RPMI-1640 were acquired from Biochrom (Merck, Darmstadt, Germany). Morphine hydrochloride (20 mg/ml) solution was obtained from Zentiva (Prague, Czech Republic). Human recombinant insulin and the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent were obtained from Sigma-Aldrich (Taufkirchen, Germany).

**Cell lines and cell culture**

Both cell lines were obtained from The Global Bioresource Center (American Type Culture Collection – ATCC). MCF7 cells maintained in MEM supplemented with 0.01 mg/ml insulin while T47D were cultured in RPMI-1640.

**MTT assay for cell viability**

The effects of morphine on cell viability were assessed by the MTT colorimetric method (Mosmann, 1983). MCF-7 and T47D cells were seeded into 96-well microplates at a density of 3.5-4×10^3 cells/well and kept in their respective media for 24 hours. Morphine hydrochloride was added to the culture medium for each cell type to achieve the following final concentrations: 0.05, 0.075, 0.1, 0.25, 0.5, 0.75, 1 µM and the cells were incubated for 24, 48 or 72 hours. At the end of the these intervals cells were washed once with phosphate buffered saline (PBS) which was then replaced with a MTT solution in PBS (0.5 mg/ml) and incubated for 1 hour. Cells were lysed and formazan particles were solubilized with 150 µl dimethyl sulfoxide. The absorbance was read with the HT Synergy microplate reader (BioTek Instruments, USA) at 550 nm and expressed relative to the background absorbance read at 630 nm. Hydrogen peroxide was used as a positive control at a concentration of 100 µM and experiments were repeated 3 times. Results from the assay were reported as percentages of an untreated control. One-way ANOVA followed by Tukey multiple comparisons test was performed using Graph Pad Prism version 8.0.0 for Windows (GraphPad Software, San Diego, California USA).

**Results and Discussion**

Contradictory effects of morphine on the viability and multiplication of cancer cells have been reported both in vivo and in vitro. In this context we sought to investigate the outcome of its addition to the culture media of breast cancer cell lines MCF7 and T47D. The opioid concentrations were close to those reported in the plasma of cancer patients receiving chronic morphine treatment for pain relief (0.9-3.4 µM) (Tegeder et al., 2003) and to the baseline concentration of morphine in cerebrospinal fluid of approximately 0.4 µM detected by Smith et al. (1999).

We found that morphine increased the viability of MCF7 cells in a dose dependent manner beyond what was observed for untreated cells (Fig. 1). This effect was visible at 24 hours starting from the lowest concentrations. However, it became statistically significant at 72 hours, particularly for 0.75 µM and 1 µM. On the other hand, morphine seemed to have little effect on cell viability at 48 hours.

In T47D cells low morphine concentrations (0.05, 0.075 and 0.1 µM) resulted in percentages similar to the control for all time frames (Fig. 2). However, starting from 0.25 µM morphine and an exposure of 24 hours, cell viability reached a
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statistically significant level for 1 µM. At 72 hours the overall trend was similar but differences between treatments were not so marked. At 48 hours, the situation was comparable to the one observed for MCF7 cells.

The similar situation observed for both cell types at 48 hours could be caused by morphine which may inhibit their growth at this exposure interval before cells can recuperate at 72 hours.

Our results regarding MCF7 cells are similar to those of Ecimovic et al. (2011) who proved that clinical concentrations of morphine directly stimulate migration and proliferation of breast adenocarcinoma cells (MCF7 and MDA-MB-231 cell lines) by increasing expression of the NET1 gene in a mechanism that does not involve opioid receptors. It has also been shown that morphine can induce chemoresistance and promote tumor growth in MCF7 cells (Niu et al., 2015) and that clinically relevant concentrations of morphine can increase breast cancer progression (Bimonte et al., 2015). In vivo this effect would be supported by the proangiogenic activity of morphine found by Gupta et al. (2002).

Hatsukari et al. (2007) found that clinical concentrations of morphine similar to ours induced apoptosis and necrosis in MCF7 cells after a much shorter exposure time of only 4 hours while cytotoxic activity was visible in the mM range. However, the concentrations we employed increased MCF7 proliferation during long exposure such as 72 hours.

In the work of Chen et al. (2017) morphine inhibited cell growth by blocking the cell cycle and promoting apoptosis in MCF-7 cells. Concentrations over 10 µM inhibited proliferation of MCF7 cells while concentrations over 0.5 mM caused cell death, via a p53-dependent mechanism (Tegeder et al., 2003). The contrasting effects of morphine observed in different studies could result

**Figure 1.** Effect of morphine on MCF7 cells at 24, 48 and 72 hours. Different letters between means from different groups denote significant differences (p < 0.05).

**Figure 2.** Effect of morphine on T47D cells at 24, 48 and 72 hours. Different letters between means from different groups denote significant differences (p < 0.05).
from different mechanisms (with or without opioid receptors) or from the combination of dose and exposure time. Interestingly, the work of Gonzalez-Nunez et al. (2014) in the neuroblastoma SH-SY5Y cell line proposes a dual effect of morphine, with low doses (0.01 µM) promoting cell proliferation, while higher doses (1µM) would inhibit cell proliferation.

The different effects of morphine in the two cell lines could be ascribed to the presence of different receptors on their surface. These would induce contrasting reactions depending on cell type. Opioid receptors contain three subtypes, µ, κ, and δ which modulate a variety of physiological functions such as pain regulation, emotional tone, and cognitive functions (Trescort et al., 2008). They can be found both in neural tissues and extraneural tissues (spleen, stomach, lung, pancreas, liver, heart, blood, and blood vessels) (Chaturvedi et al., 2000).

Hatzoglou et al. (1996) showed that opioids inhibit T47D cell growth, a mechanism mediated through κ- and δ-opioid receptors. Their research also showed that T47D cell have no µ-receptors which probably accounts for their different behavior when exposed to morphine. In contrast µ-opioid sites have been identified in MCF7 cells (Maneckjee et al., 1990) and their presence has been shown to promote tumor growth and metastasis (Mathew et al., 2011). This difference in receptors could explain the contrasting responses of the two cell lines, even if both are estrogen receptor-positive.

**Conclusion**

Our experiment shows that morphine acts on breast cancer cell viability depending on concentration, exposure time and cell origin, even if both cell lines belong to the same molecular subtype of breast cancer. The mechanism used by opioids to influence cancer cell development is still unclear and more studies should be undertaken to clarify it.

**References**


