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 (Tuerxun 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 (Tuerxun 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 cells 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 achive 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 plate 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

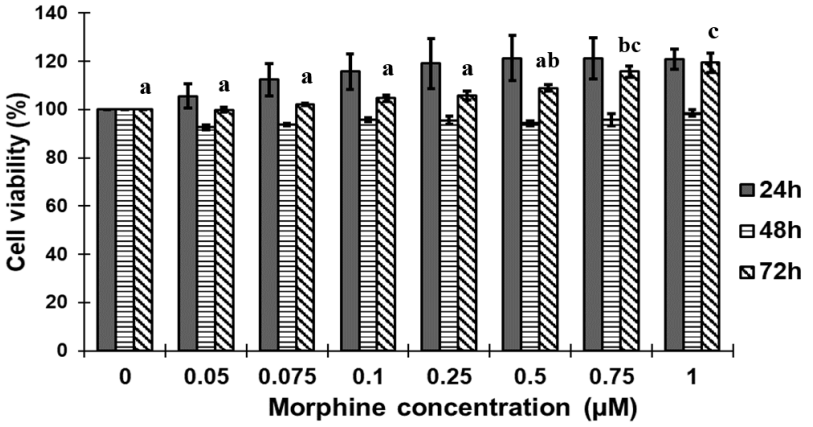

Figure 1. Effect of morphine on MCF7 cells at 24, 48 and 72 hours. Different letters between means from different groups denote significant differences ($p < 0.05$).

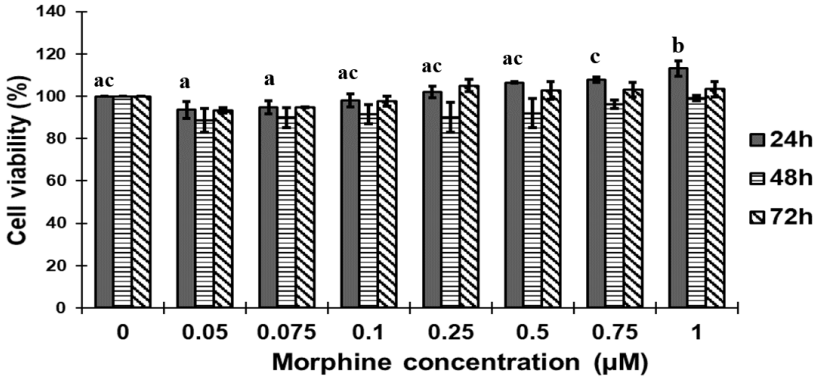

Figure 2. Effect of morphine on T47D cells at 24, 48 and 72 hours. Different letters between means from different groups denote significant differences ($p < 0.05$).

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 diffent studies could result

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 (Trescot *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.

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