Original Article

Evaluation of effects of morphine and ionizing radiation in cancer cell lines

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ABSTRACT

Purpose: Breast and cervical cancers are the two most common cancers among women worldwide. Morphine is a potent analgesic for cancer pain, and radiation therapy is a conventional treatment for cancer. Unfortunately, the combined adjuvant cellular effects of morphine and ionizing radiation in cancer cells are largely unknown.

Materials and Methods: In this study, we examined the effects of morphine and single radiation dose of 2 Gy on viability and survival fraction of human breast cancer cell line MDA-MB 231 and human cervical cancer cell line HeLa, by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and colony formation assays. We were also interested in evaluating these effects in human umbilical vein endothelial cells as well.

Results: We found that morphine did not have a dose- and time-dependent manner in endothelial, breast, and cervical cancer cells *in vitro*. It seems that pretreatment of breast and cervical cancer cells with morphine at some doses before irradiation reduces the cytotoxic effect of radiation. We also observed that endothelial cells were less sensitive than breast and cervical cancer cells to radiation or morphine + radiation. Based on the results of endothelial cells, morphine or radiation might not have a selective effect on the viability and clonogenic survival of different cell lines.

Conclusions: Our data may suggest that morphine and radiotherapy could not be administered together to breast and cervical cancer patients if additional and *in vivo* studies confirm our results.

KEY WORDS: Breast cancer, cervical cancer, morphine, opioids, radiation

INTRODUCTION

Breast and cervical cancers are the two most frequent cancers in women worldwide.[1] These two cancers contributed the two highest numbers of cancer-related deaths in women[2] and remain two significant public health concerns.[3,4] Three main therapeutic strategies are used today to treat or control breast cancer: surgery, radiation therapy, and anticancer drugs. However, surgery or radiation therapy still requires chemotherapy to eradicate remaining cancer cells and impede relapses.[5] The management of early stage cervical cancer often includes radical surgery[6] and radiotherapy,[4] whereas the standard of care for patients with advanced stage cervical cancer includes concurrent chemoradiotherapy.[7]

Morphine, an opiate-based drug, has been one of the oldest and most effective available analgesic drugs to treat moderate-to-severe pain in cancer patients.[8-10] Morphine reduces pain by the direct effect on central nervous system.[10-12] Morphine may also contribute to modulation of oxidative stress.[13] Oxidative stress is the result of the accumulation of free radicals inadequately neutralized by antioxidant agents.[14] Morphine has shown strong total antioxidant activity.[15]

Apart from the items specified above, the role of morphine in both growth promoting and growth inhibiting of various tumors is still not fully understood.[9] Several studies have shown that morphine may promote the growth of cancer cells and cause endothelial and tumor cells proliferation, migration, and angiogenesis.[9,10] On the contrary, other studies demonstrated that morphine increases tumor cell death in different human cancer cell lines and *in vivo* models.[11,16] Therefore, there is a controversy over the use of morphine in cancer patients. Moreover, potential therapeutic

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interactions of morphine with conventional cancer therapies, such as radiotherapy, remain largely unknown. In this study, we evaluated effects of different concentrations of morphine on the viability and clonogenic survival of two different cancer cell lines of MDA-MB 231 and HeLa. We particularly aimed to investigate whether pretreatment of these cancer cells with varying doses of morphine before irradiation may alter the efficacy of radiation therapy. We were also interested in evaluating these effects in human umbilical vein endothelial cells (HUVECs), as a normal and endothelial cell model, to find whether these effects are selective.

MATERIALS AND METHODS

Chemicals and cells

Morphine was purchased from Tehran Darou (Iran) and administered to the cells with the following doses of 0.3, 0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml. MDA-MB-231, HeLa cells, and HUVECs were obtained from the Pasteur Institute of Iran. Fetal bovine serum (FBS), penicillin, trypsin with ethylenediaminetetraacetic acid, Dulbecco's modified Eagle's medium, and streptomycin were from Gibco (UK). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), phosphate-buffered saline, and Giemsa were obtained from Sigma (USA). Dimethyl sulfoxide and methanol were purchased from Merck (Germany).

Cell culture

Cancer cell lines and HUVECs were cultured in DMEM containing 10% FBS and a 1% penicillin/streptomycin mixture. Cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air.[4] Cells were cultivated for 3–4 days to reach almost 80% confluence. Morphine was added to the cells once in DMEM/10% FBS.

Radiation therapy

Fraction size of 2 Gy or less is the standard of care in radiotherapy.[17] We employed 2 Gy fraction size to evaluate the effect of radiation. The single absorbed dose (2 Gy) ionizing radiation (IR) was from 6 MV photons at a source-to-surface distance of 100 cm. The IR was delivered by a radiation therapy machine (Siemens ONCOR linear accelerator) at room temperature.

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay

MDA-MB-231, HeLa cells, and HUVECs following nearly 80% confluence were cultured in separate 96-well plates (5000 cells/well) and allowed to adhere overnight. The first group of each cell line was then treated with different concentrations of morphine (0.3, 0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml) for 24 and 72 h. The second group of each cell line was exposed to a single radiation dose of 2 Gy and incubated for 24 and 72 h. The third group was pretreated with all indicated doses of morphine for 22 h before exposure to radiation, and then the cells were incubated for next 2 and 50 h. The control group was not treated with morphine, radiation, or both. Following the procedure specified above, MTT was added to the each well according to the instruction of the supplier company and incubated for 3 h. The absorbance was measured by a microplate reader (BioTek, OD 570 nm with 630 nm correction).

Colony formation assay

The three cell lines following almost 80% confluence were seeded separately into six-well plates (1200 cells/well) and allowed to adhere overnight. The first group of each cell line was then treated with all indicated concentrations of morphine for 24 h. The second group of each cell line was exposed to a single radiation dose of 2 Gy and incubated for 24 h. The third group was pretreated with all indicated doses of morphine for 22 h before radiation, and then the cells were incubated for next 2 h. The control group was not treated with morphine, radiation, or both. The culture medium was then removed, and fresh DMEM supplemented with 10% FBS was added back. The cells were maintained in fresh culture medium for next 13 days at 37°C in a humidified 5% CO_2 incubator to form colonies. The colonies were then stained with Giemsa and counted (colonies of >50 cells) manually.

Statistical analysis

Experiments were performed in triplicate from three independent experiments. All data are shown as a mean ± standard deviation. Differences between variable and control groups were determined by a two-tailed independent *t*-test and one-way ANOVA. Statistical analysis was performed using SPSS Statistics, version 17.0. (SPSS Inc., Chicago, IL, USA). $P \leq 0.05$ was considered statistically significant.

RESULTS

Effects of morphine, radiation, and a combination of both on the viability of HeLa cells

We performed MTT assays to determine effects of morphine and single radiation dose of 2 Gy on the viability of HeLa, MDA-MB-231 cells, and HUVECs following 24 and 72 h. Actively proliferating HeLa cells were exposed to different doses of morphine alone or in combination with radiation for 24 and 72 h, then MTT was added and metabolized for 3 h. Following 24 and 72 h of treatment, all different doses of morphine alone did not have any significant effects on the viability of HeLa cells [Figure 1a and b]. Following 24 h of exposure, radiation did not affect the viability of HeLa cells [Figure 1a]. However, following 72 h, radiation significantly decreased the viability of cells by 46.73% ($n = 3$; $P \leq 0.05$) [Figure 1b].

Following 24 h of treatment, morphine at concentrations of 0.3, 0.3×10^{-4}, and 0.3×10^{-6} mg/ml in combination with radiation significantly impaired the viability of HeLa cells by 46.06%, 43.22%, and 41.43%, respectively, relative to control group. Other doses of morphine (0.3×10^{-2} and 0.3×10^{-8} mg/ml) in combination with radiation did not have any significant effects on the viability of cells compared

with control group. Morphine only at concentrations of 0.3, 0.3×10^{-4}, and 0.3×10^{-6} mg/ml in combination with radiation significantly reduced the viability of HeLa cells compared with radiation alone (2 Gy). There is no significant difference between the viability of treated cells by other doses of morphine (0.3×10^{-2} and 0.3×10^{-8} mg/ml) + radiation and radiation alone [Figure 1a]. Following 72 h, only 0.3×10^{-8} mg/ml morphine in combination with radiation significantly decreased the viability of HeLa cells by 47.53% relative to control group. Other doses of morphine (0.3, 0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6} mg/ml) in combination with radiation did not have any significant effects on viability of cells compared with control group. There is no significant difference between the viability of treated cells by varying doses of morphine + radiation and radiation alone [Figure 1b].

Effects of morphine, radiation, and a combination of both on the viability of MDA-MB 231 cells

Following 24 h, morphine at concentrations of 0.3, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml significantly reduced viability of MDA-MB 231 cells by 13.93%, 15.57%, 19.67%, and 27.87%, respectively. Morphine only at a dose of 0.3×10^{-2} mg/ml did not affect the viability of cells [Figure 2a]. Following 72 h, all indicated doses of morphine (0.3, 0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml) significantly increased the viability of MDA-MB 231 cells (by 29.53%, 38.26%, 31.54%, 34.90%, and 29.53%, respectively) [Figure 2b]. Following 24

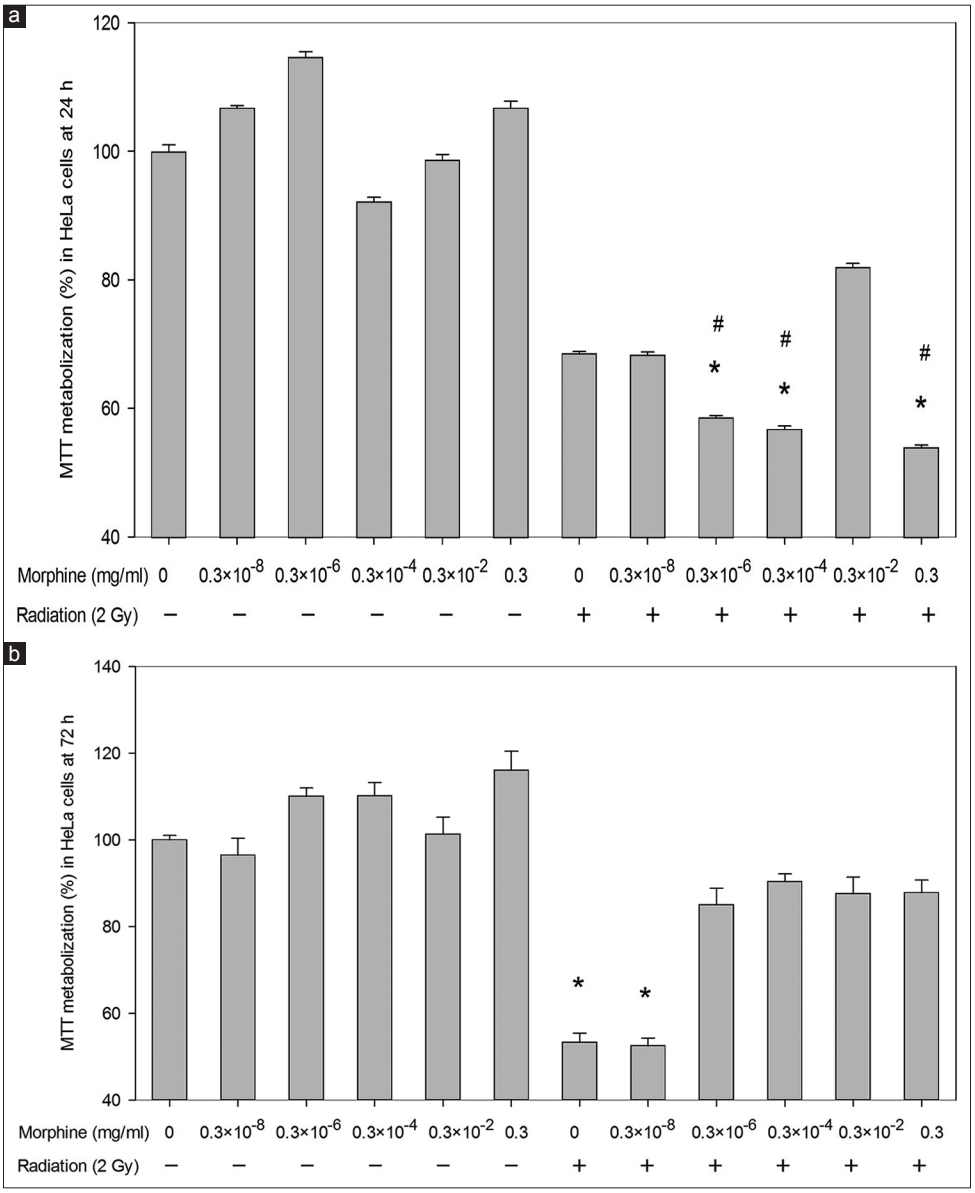

Figure 1: Evaluation of the viability of HeLa cells in response to morphine, single radiation dose of 2 Gy, and a combination of both (a) at 24 h and (b) 72 h using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. HeLa cells were treated as indicated. Data are mean ± standard deviation where control is set at 100% ($n = 3$). The asterisks indicate a statistically significant difference versus control, and the hashtags indicate a statistically significant difference of specific dose of morphine + radiation (2 Gy) versus radiation alone (2 Gy) ($P \leq 0.05$)

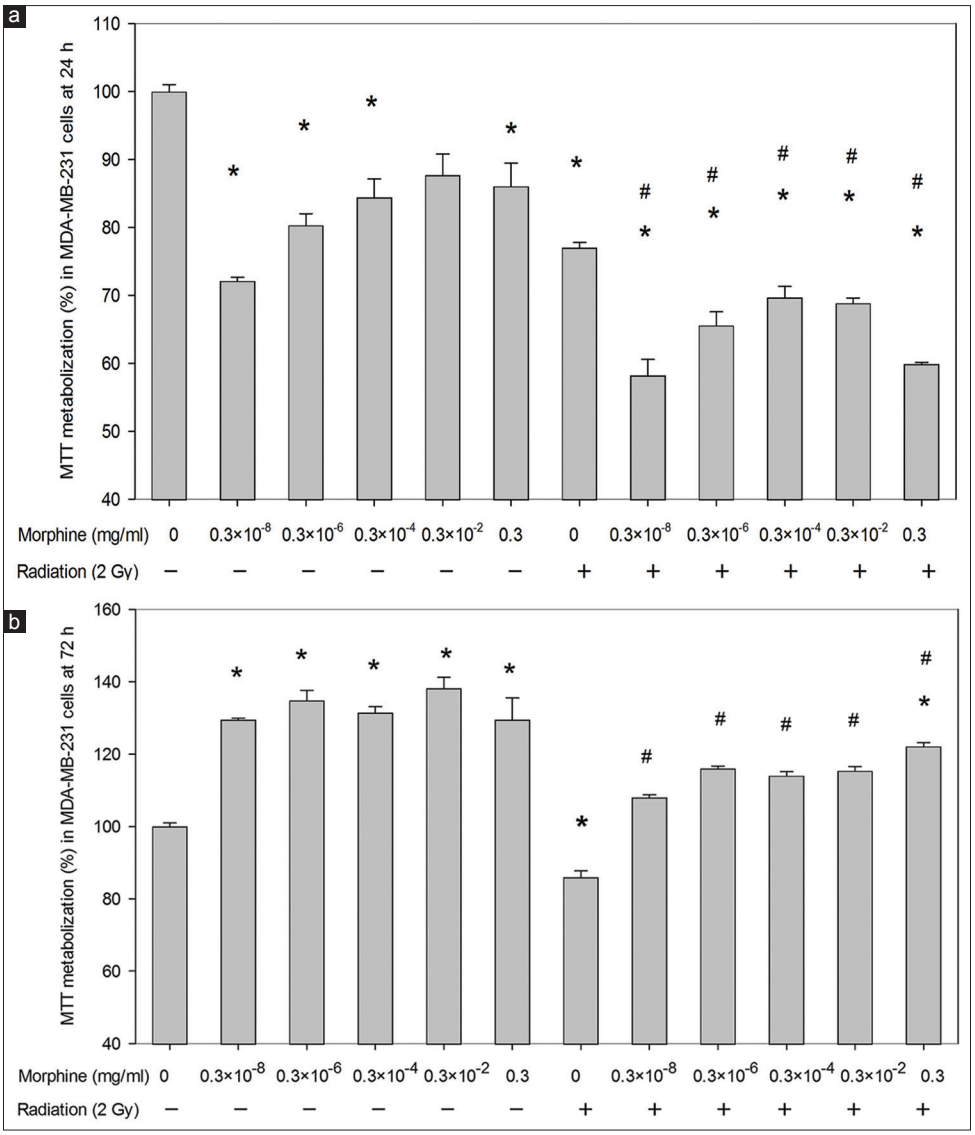

Figure 2: Evaluation of the viability of MDA-MB-231 cells in response to morphine, single radiation dose of 2 Gy, and a combination of both at (a) 24 h and (b) 72 h, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. MDA-MB-231 cells were treated as indicated. Data are mean ± standard deviation where control is set at 100% ($n = 3$). The asterisks indicate a statistically significant difference versus control, and the hashtags indicate a statistically significant difference of specific dose of morphine + radiation (2 Gy) versus radiation alone (2 Gy) ($P \leq 0.05$)

and 72 h, radiation significantly impaired the viability of cells by 22.95% and 14.09%, respectively [Figure 2a and b].

Following 24 h, all indicated doses of morphine (0.3, 0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml) in combination with radiation significantly attenuated the viability of MDA-MB 231 cells (by 40.16%, 31.15%, 30.33%, 34.43%, and 41.80%, respectively, relative to control group). All indicated doses of morphine in combination with radiation significantly reduced the cell viability compared with radiation alone [Figure 2a]. Following 72 h, 0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml morphine + radiation did not have any significant effect on viability of cells compared with control group. Morphine at the concentration of 0.3 mg/ml + radiation significantly increased the viability of MDA-MB 231 cells by 22.15% relative to control group. All different doses of morphine in combination with radiation significantly increased the cell viability compared with radiation alone (2 Gy) [Figure 2b].

Effects of morphine, radiation, and a combination of both on the viability of human umbilical vein endothelial cells

Following 24 h, morphine at concentrations of 0.3×10^{-2}, 0.3×10^{-4}, and 0.3×10^{-6} mg/ml did not affect the viability of HUVECs. Morphine at the concentration of 0.3 mg/ml significantly decreased the viability of HUVECs by 22.62%, but 0.3×10^{-8} mg/ml morphine significantly increased the viability of the cells by 20.36% [Figure 3a]. Following 72 h, all different doses of morphine alone did not affect the viability of cells [Figure 3b]. Following 24 and 72 h, single radiation dose did not have any significant effect on the viability of the cells [Figure 3a and b].

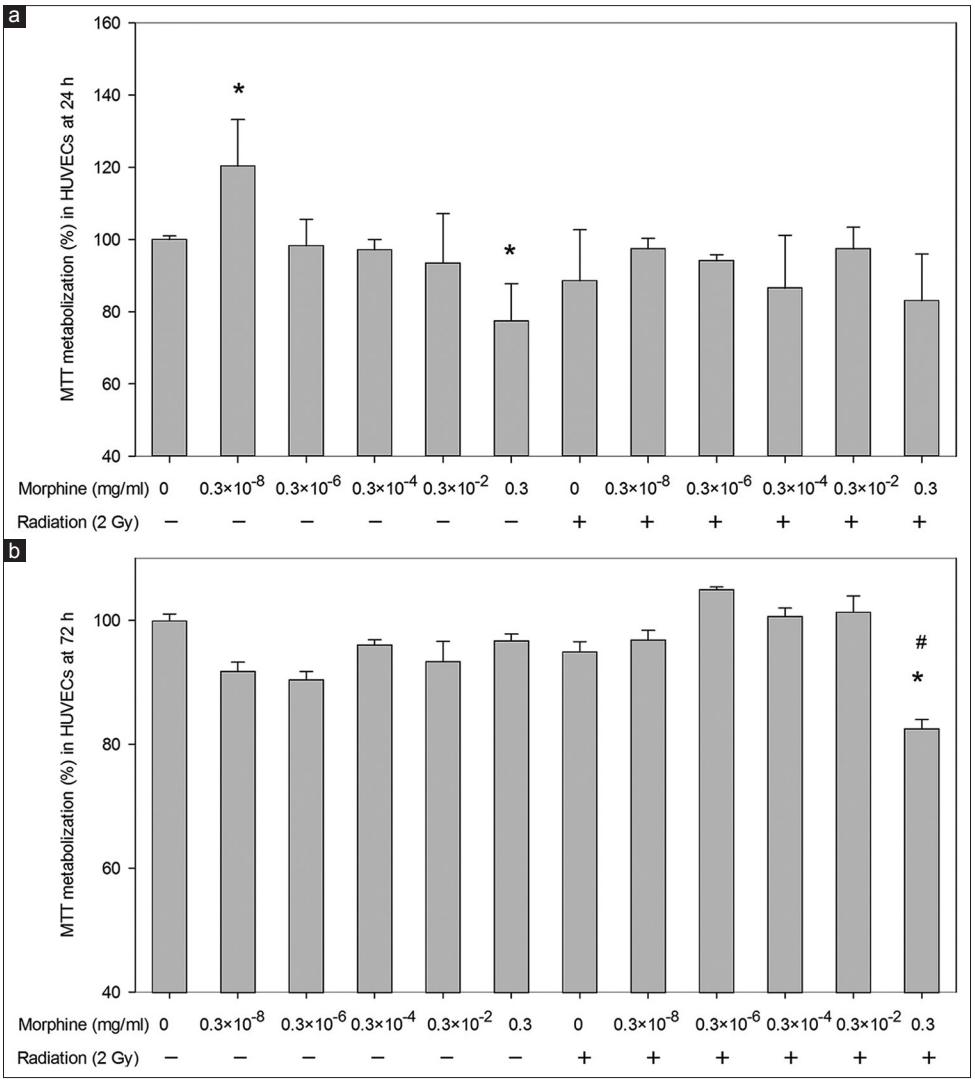

Figure 3: Evaluation of the viability of human umbilical vein endothelial cells in response to morphine, single radiation dose of 2 Gy, and a combination of both at (a) 24 h and (b) 72 h, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Human umbilical vein endothelial cells were treated as indicated. Data are mean ± standard deviation where control is set at 100% (n = 3). The asterisks indicate a statistically significant difference versus control, and the hashtags indicate a statistically significant difference of specific dose of morphine + radiation (2 Gy) versus radiation alone (2 Gy) ($P \leq 0.05$)

Following 24 h, all different doses of morphine + radiation did not affect the viability of HUVECs compared with control group. There is no significant difference between the viability of treated cells by different doses of morphine + radiation and radiation alone [Figure 3a]. Following 72 h, morphine at the doses of 0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml in combination with radiation did not affect the viability of cells, but 0.3 mg/ml morphine + radiation significantly impaired the viability of the cells by 17.45% relative to control group. Morphine only at the concentration of 0.3 mg/ml in combination with radiation significantly reduced the cell viability compared with radiation alone (2 Gy). There is no significant difference between the viability of treated cells by other doses of morphine (0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml) + radiation and radiation alone [Figure 3b].

Effects of morphine, radiation, and a combination of both on the survival fraction of HeLa, MDA-MB 231 cells, and human umbilical vein endothelial cells

We performed colony formation assays to determine cell reproductive death[18] after treatment with morphine and IR. Cells were treated for 24 h with varying doses of morphine alone or in combination with radiation and maintained in fresh culture medium for next 13 days. The colonies were then counted (colonies of >50 cells) manually. All indicated doses of morphine (0.3, 0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml) alone significantly increased colony numbers of HeLa cell line (by 127.47%, 184.18%, 131.74%, 83.56%, and 38.43%, respectively) ($n = 3$; $P \leq 0.05$). Radiation alone significantly decreased colony numbers by 29.87%. 0.3, 0.3×10^{-2}, 0.3×10^{-4}, and 0.3×10^{-6} mg/ml morphine + radiation significantly increased colony numbers by 80.51%, 118.32%,

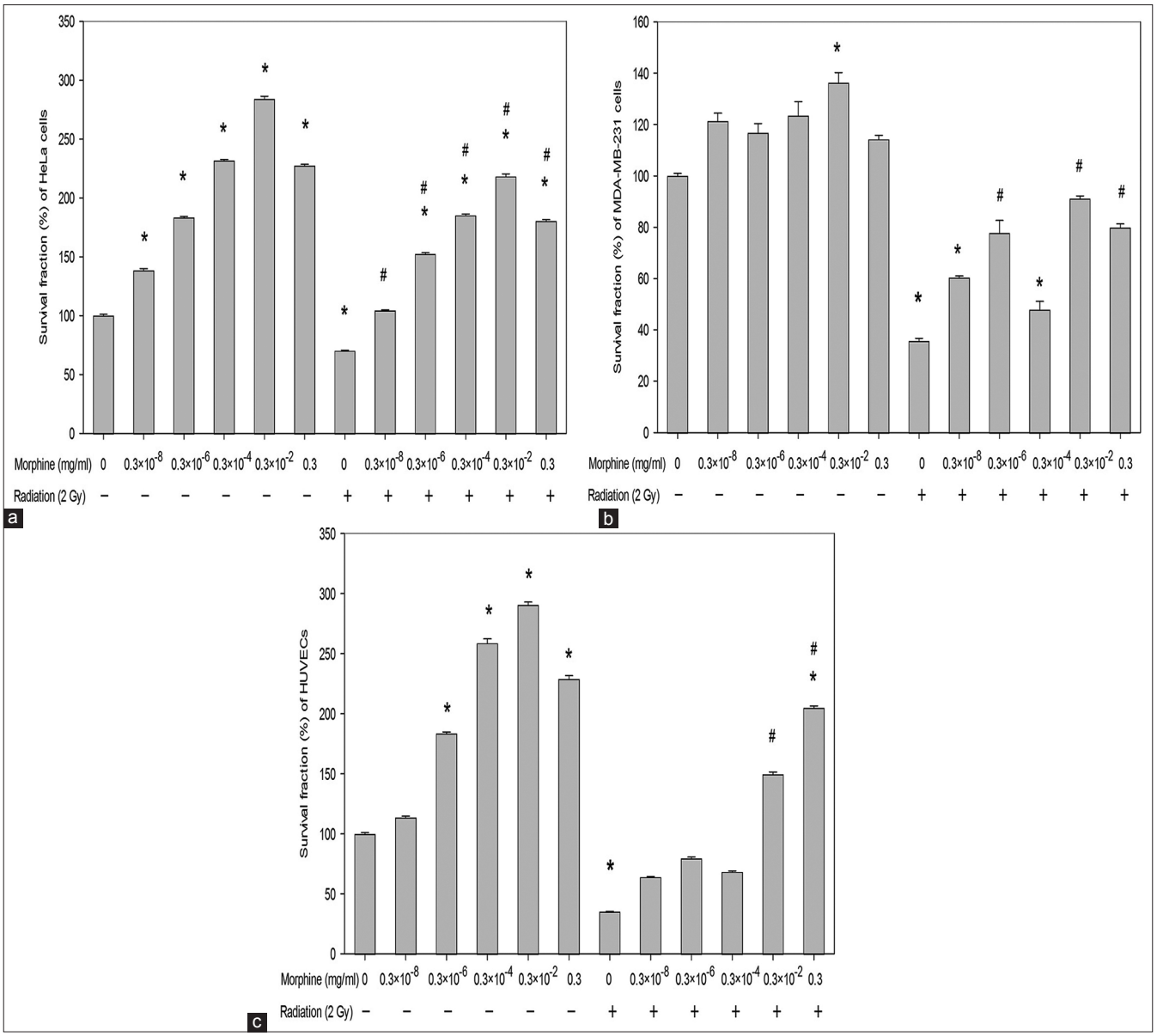

Figure 4: Evaluation of the survival fraction of (a) HeLa, (b) MDA-MB-231 cells, and (c) human umbilical vein endothelial cells in response to morphine, single radiation dose of 2 Gy, and a combination of both following 14 days, using colony formation assay. Cells were treated as indicated. Data are mean ± standard deviation where control is set at 100% ($n = 3$). The asterisks indicate a statistically significant difference versus control, and the hashtags indicate a statistically significant difference of specific dose of morphine + radiation (2 Gy) versus radiation alone (2 Gy) ($P \leq 0.05$)

85.39%, and 52.46%, respectively, relative to control group. Morphine at the dose of 0.3×10^{-8} mg/ml + radiation did not have any significant effect on the survival fraction of HeLa cells compared with control group. All different doses of morphine in combination with radiation significantly increased the survival fraction compared with radiation alone [Figure 4a].

Morphine at concentrations of 0.3, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml did not have any significant effect on the clonogenic survival of MDA-MB 231 cells. The treatment of MDA-MB 231 cells with 0.3×10^{-2} mg/ml morphine induced a significant increase in the survival fraction by 36.28%. Radiation significantly attenuated the survival fraction of MDA-MB 231 cells by 64.33%. Morphine at concentrations of 0.3×10^{-4} and 0.3×10^{-8} mg/ml in combination with radiation significantly reduced the survival fraction of the cells by 52.13% and 39.63%, respectively, relative to control group. Other concentrations of morphine (0.3, 0.3×10^{-2}, and 0.3×10^{-6} mg/ml) in combination with radiation did not affect the survival fraction of MDA-MB 231 cells compared with control group. Morphine only at the concentrations of 0.3, 0.3×10^{-2}, and 0.3×10^{-6} mg/ml in combination with radiation significantly increased the survival fraction compared with radiation alone. There is no significant

difference between the survival fraction of treated cells by other doses of morphine (0.3×10^{-4} and 0.3×10^{-8} mg/ml) + radiation and radiation alone [Figure 4b].

The treatment of HUVECs with morphine at concentrations of 0.3, 0.3×10^{-2}, 0.3×10^{-4}, and 0.3×10^{-6} mg/ml significantly increased the clonogenic survival of the cells by 129.06%, 190.60%, 158.97%, and 83.76%, respectively. Morphine only at the concentration of 0.3×10^{-8} mg/ml did not have any significant effect on the clonogenic survival of HUVECs. Radiation significantly decreased the survival fraction of HUVECs by 64.96%. Morphine at different concentrations of 0.3×10^{-2}, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml in combination with radiation did not affect the survival fraction of HUVECs compared with control group. The treatment of HUVECs with 0.3 mg/ml morphine + radiation significantly increased the survival fraction by 105.13% relative to control group. Morphine only at the concentrations of 0.3 and 0.3×10^{-2} mg/ml in combination with radiation significantly increased the survival fraction of HUVECs compared with radiation alone. There is no significant difference between the survival fraction of treated cells by other doses of morphine (0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml) + radiation and radiation alone [Figure 4c].

DISCUSSION

MTT and colony formation assays show that morphine does not have a concentration- and time-dependent manner in endothelial, breast, and cervical cancer cells *in vitro*. These assays also demonstrate that the single radiation dose of 2 Gy has the cytotoxic effect in endothelial, breast, and cervical cancer cells. It seems that pretreatment of breast and cervical cancer cells with morphine at some doses before radiation decreases the efficacy of radiation.

Morphine has long been considered as one of the most effective opioid analgesic drugs that was given in clinic to manage chronic pain.[9,19] The World Health Organization classified the opioid drugs as the second step in the analgesic ladder for the management of long-term pain such as chronic cancer pain.[19-21] Although morphine has a strong effect to relieve the pain in cancer patients, its effects on tumor growth are still contradictory, as both growth-promoting and growth-inhibiting effects have been reported.[9,22,23] On the one hand, morphine has been demonstrated to promote proliferation and migration of cancer cells.[9,23] On the other hand, pro-apoptotic properties of morphine were also shown.[9,19,23] Tegeder *et al*.[11] have reported that morphine alone reduces the proliferation of MCF-7 breast cancer cells at concentrations of ≥10 μM. Part of our results is in agreement with the study of Tegeder *et al*.[11] and shows that morphine at doses of 0.3, 0.3×10^{-4}, 0.3×10^{-6}, and 0.3×10^{-8} mg/ml significantly reduces the viability of MDA-MB 231 cells following 24 h. Bimonte *et al*.[10] have shown that morphine *in vitro* at three different doses (1, 10, and 100 μM) promotes the proliferation and inhibits the apoptosis of MDA-MB 231 cells following 2, 4, and 6 days. Ecimovic *et al*.[23] have reported that 10–100 ng/ml morphine alone increases proliferation of MDA-MB 231 cells, in a serum-free medium. Another part of our results is consistent with two studies specified above and shows that all different doses of morphine significantly increase the viability of MDA-MB 231 cells following 72 h. However, following 14 days, morphine only at a concentration of 0.3×10^{-2} mg/ml significantly increases the survival fraction, and morphine at other concentrations does not affect the survival fraction of MDA-MB 231 cells. The present study is the first research that investigated the potential effect of morphine on the viability and clonogenic survival of HeLa cells. Our data suggest that morphine does not have dose- and time-dependent cytotoxic or cytoprotective effects on breast and cervical cancer cells *in vitro*.

The cellular effects of morphine in endothelial cells are crucial since morphine is often employed to relieve pain without having well-documented effects on nonneuronal tissue. Despite the use of morphine to relieve pain in cancer patients, little is known regarding the potential effects of morphine on vascular endothelium, a major player in angiogenesis and tumor growth. The clinical doses of morphine are 10–2450 mg/day, resulting in serum concentrations that are between 2 nM and 3.5 μM.[24] Leo *et al*.[24] have shown that morphine at clinically relevant doses (1 μM to 10 nM) significantly stimulates human umbilical arterial endothelial cells proliferation following 72 h. Chen *et al*.[25] have demonstrated that morphine promotes mouse retinal endothelial cells proliferation. However, Hsiao *et al*.[26] have reported that morphine enhances apoptosis of HUVECs. As can be seen from these previous studies, effects of morphine on the viability of endothelial cells are contradictory. Our results confirm these contradictory effects. Like breast and cervical cancer cells *in vitro*, endothelial cells respond to morphine but not in a concentration- and time course-dependent manner, as shown by our results.

It should be taken into consideration that opioid administration has been associated with changes in oxidative stress mechanisms.[13,14] The contradictory results specified the paragraphs above, regarding the effect of morphine on the viability and survival fraction of MDA-MB 231, HeLa cells, and HUVECs, may be associated with different cell types and/or concentration/time of morphine treatment.

Even though new therapeutic strategies such as targeted molecular therapy in cancer have been demonstrated, radiation therapy is one of the most common integral components of the therapy and effective nonsurgical and local treatments for many types of cancer.[27-29] Radiation therapy preserves as much as possible the normal adjacent tissues[30] and induces cytotoxicity through DNA damage, triggering cell cycle arrest, and apoptosis.[27-29] Our results support previous studies regarding the cytotoxic effects of IR in breast[30] and cervical cancer cells.[31]

Nubel *et al*.[32] have reported that IR dose of 10 Gy induces apoptosis in HUVECs after 48, 72, and 96 h. Another study has shown that radiation (gamma rays) doses of 1–8 Gy significantly decrease the viability of HUVECs following 36 h.[33] Part of our results is inconsistent with the results of these two studies. We found that single radiation dose of 2 Gy significantly reduces the clonogenic survival of HUVECs following 14 days although this dose does not have any significant effects on the viability of the cells after 24 and 72 h. It is important to highlight that a variety of factors may influence radiobiological effects such as cell type,[34] a dose of radiation, quality of the ionizing energy, single fraction, continued or fractioned exposure, and exposure time.[30]

The present study is the first research that investigated the combined adjuvant cellular effects of morphine and radiation in MDA-MB 231, HeLa cells, and HUVECs. In general, it seems that morphine at some doses reduces the cytotoxic effect of radiation in breast and cervical cancer cells when morphine combined with radiation. However, combined effect of morphine and radiation in these two cancer cell lines did not follow a dose- and time-dependent relationship. Further studies are required to elucidate underlying mechanisms. Our study also showed that breast and cervical cancer cells are more sensitive than endothelial cells to radiation or morphine + radiation. Based on the results of HUVECs, morphine or irradiation might not have a selective effect on the viability and survival fraction of different cell lines *in vitro*.

CONCLUSIONS

Our results may suggest that radiotherapy could not be administered alongside morphine to breast and cervical cancer patients if these *in vitro* data can be translated to *in vivo* studies in human subjects.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Barbieri M, Weatherly HL, Ara R, Basarir H, Sculpher M, Adams R, *et al.* What is the quality of economic evaluations of non-drug therapies? A systematic review and critical appraisal of economic evaluations of radiotherapy for cancer. Appl Health Econ Health Policy 2014;12:497-510.
2. Peng L, Yuan X, Jiang B, Tang Z, Li GC. LncRNAs: Key players and novel insights into cervical cancer. Tumour Biol 2016;37:2779-88.
3. Diaby V, Tawk R, Sanogo V, Xiao H, Montero AJ. A review of systematic reviews of the cost-effectiveness of hormone therapy, chemotherapy, and targeted therapy for breast cancer. Breast Cancer Res Treat 2015;151:27-40.
4. Koh WJ, Greer BE, Abu-Rustum NR, Apte SM, Campos SM, Cho KR, *et al.* Cervical Cancer, Version 2.2015. J Natl Compr Canc Netw 2015;13:395-404.
5. Nwabo Kamdje AH, Seke Etet PF, Vecchio L, Tagne RS, Amvene JM, Muller JM, *et al.* New targeted therapies for breast cancer: A focus on tumor microenvironmental signals and chemoresistant breast cancers. World J Clin Cases 2014;2:769-86.
6. Medlin EE, Kushner DM, Barroilhet L. Robotic surgery for early stage cervical cancer: Evolution and current trends. J Surg Oncol 2015;112:772-81.
7. Angioli R, Luvero D, Aloisi A, Capriglione S, Gennari P, Linciano F, *et al.* Adjuvant chemotherapy after primary treatments for cervical cancer: A critical point of view and review of the literature. Expert Rev Anticancer Ther 2014;14:431-9.
8. Nersesyan H, Slavin KV. Current aproach to cancer pain management: Availability and implications of different treatment options. Ther Clin Risk Manag 2007;3:381-400.
9. Gach K, Wyrebska A, Fichna J, Janecka A. The role of morphine in regulation of cancer cell growth. Naunyn Schmiedebergs Arch Pharmacol 2011;384:221-30.
10. Bimonte S, Barbieri A, Rea D, Palma G, Luciano A, Cuomo A, *et al.* Morphine promotes tumor angiogenesis and increases breast cancer progression. Biomed Res Int 2015;2015:161508.
11. Tegeder I, Grösch S, Schmidtko A, Häussler A, Schmidt H, Niederberger E, *et al.* G protein-independent G1 cell cycle block and apoptosis with morphine in adenocarcinoma cells: Involvement of p53 phosphorylation. Cancer Res 2003;63:1846-52.
12. Forster AB, Reeh PW, Messlinger K, Fischer MJ. High concentrations of morphine sensitize and activate mouse dorsal root ganglia via TRPV1 and TRPA1 receptors. Mol Pain 2009;5:17.
13. Almeida MB, Costa-Malaquias A, Nascimento JL, Oliveira KR, Herculano AM, Crespo-López ME. Therapeutic concentration of morphine reduces oxidative stress in glioma cell line. Braz J Med Biol Res 2014;47:398-402.
14. Mannelli P, Patkar A, Rozen S, Matson W, Krishnan R, Kaddurah-Daouk R. Opioid use affects antioxidant activity and purine metabolism: Preliminary results. Hum Psychopharmacol 2009;24:666-75.
15. Gülçin I, Beydemir S, Alici HA, Elmastas M, Büyükokuroglu ME. *In vitro* antioxidant properties of morphine. Pharmacol Res 2004;49:59-66.
16. Sueoka N, Sueoka E, Okabe S, Fujiki H. Anti-cancer effects of morphine through inhibition of tumour necrosis factor-alpha release and mRNA expression. Carcinogenesis 1996;17:2337-41.
17. Fletcher GH. Textbook of Radiotherapy. 2nd ed. Philadelphia: Lea and Febiger; 1973.
18. Franken NA, Rodermond HM, Stap J, Haveman J, van Bree C. Clonogenic assay of cells *in vitro*. Nat Protoc 2006;1:2315-9.
19. Olin MR, Roy S, Molitor T. *In vivo* morphine treatment synergistically increases LPS-induced caspase activity in immune organs. J Neuroimmune Pharmacol 2010;5:546-52.
20. Radbruch L, Grond S, Zech DJ, Bischoff A. High-dose oral morphine in cancer pain management: A report of twelve cases. J Clin Anesth 1996;8:144-50.
21. Portenoy RK, Lesage P. Management of cancer pain. Lancet 1999;353:1695-700.
22. He B, Tong X, Wang L, Wang Q, Ye H, Liu B, *et al.* Tramadol and flurbiprofen depress the cytotoxicity of cisplatin via their effects on gap junctions. Clin Cancer Res 2009;15:5803-10.
23. Ecimovic P, Murray D, Doran P, McDonald J, Lambert DG, Buggy DJ. Direct effect of morphine on breast cancer cell function *in vitro*: Role of the NET1 gene. Br J Anaesth 2011;107:916-23.
24. Leo S, Nuydens R, Meert TF. Opioid-induced proliferation of vascular endothelial cells. J Pain Res 2009;2:59-66.
25. Chen C, Farooqui M, Gupta K. Morphine stimulates vascular endothelial growth factor-like signaling in mouse retinal endothelial cells. Curr Neurovasc Res 2006;3:171-80.
26. Hsiao PN, Chang MC, Cheng WF, Chen CA, Lin HW, Hsieh CY, *et al.*

Morphine induces apoptosis of human endothelial cells through nitric oxide and reactive oxygen species pathways. Toxicology 2009;256:83-91.
27. Aghamohammadi A, Hosseinimehr SJ, Ghasemi A, Azadbakht M, Pourfallah TA. Radiosensitization effects of a zataria multiflora extract on human glioblastoma cells. Asian Pac J Cancer Prev 2015;16:7285-90.
28. Meng Z, Gan YH. Activating PTEN by COX-2 inhibitors antagonizes radiation-induced AKT activation contributing to radiosensitization. Biochem Biophys Res Commun 2015;460:198-204.
29. Merle P, Gueugneau M, Teulade-Fichou MP, Müller-Barthélémy M, Amiard S, Chautard E, *et al.* Highly efficient radiosensitization of human glioblastoma and lung cancer cells by a G-quadruplex DNA binding compound. Sci Rep 2015;5:16255.
30. Falcão PL, Motta BM, de Lima FC, Lima CV, Campos TP. Enhancement of viability of radiosensitive (PBMC) and resistant (MDA-MB-231) clones in low-dose-rate cobalt-60 radiation therapy. Radiol Bras 2015;48:158-65.
31. Samani F, Monfared AS, Zabihi E, Khafri S, Karimi M, Akhavan Niaki H. Evaluation of the effects of paederus beetle extract and gamma irradiation on HeLa cells. Iran J Basic Med Sci 2014;17:303-6.
32. Nübel T, Damrot J, Roos WP, Kaina B, Fritz G. Lovastatin protects human endothelial cells from killing by ionizing radiation without impairing induction and repair of DNA double-strand breaks. Clin Cancer Res 2006;12(3 Pt 1):933-9.
33. Soltani B, Bodaghabadi N, Mahpour G, Ghaemi N, Sadeghizadeh M. Nanoformulation of curcumin protects HUVEC endothelial cells against ionizing radiation and suppresses their adhesion to monocytes: Potential in prevention of radiation-induced atherosclerosis. Biotechnol Lett 2016;38:2081-2088.
34. Rofstad EK. Radiation biology of malignant melanoma. Acta Radiol Oncol 1986;25:1-10.