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Original Article

The combination of rosuvastatin and meloxicam enhances the radiotherapy efficacy of MCF7, T-47D, and MDA-MB-231 breast cancer cell lines

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Abstract

Background and purpose: Radiotherapy is an essential treatment for breast cancer, but radioresistance remains a major obstacle. Studies suggest that statins and cyclooxygenase-2 (COX-2) inhibitors can enhance radiotherapy, yet few have examined their combined effects on breast cancer radiosensitivity. This study investigates the impact of meloxicam and rosuvastatin pretreatment on the radiosensitivity of MCF-7, T-47D, and MDA-MB-231 breast cancer cell lines.

Experimental approach: MCF-7, T-47D, and MDA-MB-231 cells were pretreated with varying concentrations of meloxicam, rosuvastatin, or both. Their response to radiation was evaluated using micronucleus, clonogenic, catalase, and superoxide dismutase (SOD) assays to assess chromosomal damage, cell survival, oxidative stress (via hydrogen peroxide degradation), and SOD antioxidant enzyme activity, respectively.

Findings/Results: Pretreatment with combined rosuvastatin (R) and meloxicam (M) at R2+M10 µM, R10+M50 µM, and R20+M100 µM increased genotoxicity and reduced colony formation across all irradiated cell lines compared to radiation alone. R10 µM, R10+M50 µM, and R20+M100 µM decreased catalase activity across irradiated cell lines compared to radiation alone, whereas R2+M10 µM decreased catalase activity significantly only in T-47D cells. Pretreatment with R10 µM, R2+M10 µM, R10+M50 µM, and R20+M100 µM reduced SOD activity in all irradiated cell lines compared to radiation alone.

Conclusion and implications: The combination of rosuvastatin and meloxicam at specific concentrations increased the radiation sensitivity of MCF-7, T-47D, and MDA-MB-231 cells. Combined pretreatment with rosuvastatin 10 µM and meloxicam 50 µM notably enhanced genotoxicity while reducing colony formation, catalase activity, and SOD activity compared to radiotherapy alone in MCF-7, T-47D, and MDA-MB-231 cell lines.

Keywords: Breast cancer; MB-231; MCF7; Radiosensitizer; Radiosensitization; Radiotherapy; T47D.

INTRODUCTION

Breast cancer is the most prevalent type of cancer in women, with approximately 2.3 million new cases worldwide annually (1). The prognosis of breast cancer has considerably improved over time (2). To date, numerous treatments, including surgical resections, chemotherapy, radiotherapy, systemic therapy including endocrine/hormone therapy, targeted therapy, or a combination thereof, have been employed to treat breast cancer (1).

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Nevertheless, the majority of breast cancer patients are initially diagnosed at late stages, correlating with a less favorable prognosis and decreased survival time (3). Adjuvant therapy has recently been shown to play a substantial role in modern breast cancer treatment, leading to reduced loco-regional recurrence rates and improved overall survival (4-6). Hence, the majority of patients opting for breast-conserving surgery are now receiving radiotherapy as part of their adjuvant treatment (6). It is estimated that approximately 80% of cancer patients undergo radiation therapy either alone or in conjunction with chemotherapy or hormonal therapy (7).

Radiation therapy eliminates cancer cells through direct DNA damage and indirect damage from water radiolysis and free radical generation, preventing cancerous cells from dividing and multiplying further (8,9). Despite this, ionizing radiation also affects healthy cells, causing DNA damage, free radicals, and potential radiation poisoning (10-13). Thus, the goal of radiation therapy is to deliver the highest possible radiation dose to cancerous cells while minimizing damage to adjacent normal cells (10,11).

A major challenge in radiation therapy is radioresistance, where cancer cells develop mechanisms that reduce their sensitivity to radiation. Radioresistance hinders the ability to reach a therapeutic radiation dose, diminishing treatment effectiveness and increasing the risk of local failure (14). Overcoming radioresistance to ionizing radiation in malignant cells is, therefore, a primary challenge in cancer therapy (15). There are various methods to overcome the resistance of cancer cells to ionizing radiation, like elevating the dose of ionizing radiation or using radiation sensitizers (16). Nevertheless, increased doses of ionizing radiation may induce adverse effects on healthy tissue, potentially resulting in mismanagement of patient care during therapy. One way to overcome this obstacle is to use a radiation sensitizer, which can sensitize cancer cells to ionizing radiation (17). Radiosensitizers enhance the efficacy of cancer treatment by impeding cell repair processes (16,18).

As inflammation is a hallmark of cancer (19), nonsteroidal anti-inflammatory drugs (NSAIDs) have been suggested to possess anticancer properties (20). Numerous studies have shown that NSAIDs are correlated with reduced cancer risk in a variety of types of cancer, including breast cancer (21). Moreover, multiple cyclooxygenase-2 (COX-2) inhibitors have been investigated for their potential to enhance radiation and chemotherapy efficacy, as COX-2 inhibition reduces prostaglandin production, a key factor in tumor resistance to these treatments (22-25). Meloxicam, a selective COX-2 inhibitor, has been demonstrated to inhibit the proliferation of various cancer cell lines and animal neoplasms in a concentration-dependent manner. Under specific experimental conditions, meloxicam has exhibited radiosensitizing properties (22,23,26). However, further research is needed to explore these combined effects in different cancer models. While the molecular mechanisms underlying COX-2-mediated growth inhibition remain unclear, studies indicate that *in vitro* exposure to COX-2 inhibitors, including meloxicam, can induce cell cycle arrest and apoptosis (27).

Alternatively, statins, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR) inhibitors, have been shown to reduce cancer-related mortality (28) and the incidence of breast cancer (29,30). Several studies have explored the role of various statins as radiosensitizers in different cancer cell lines (31-33). Potential mechanisms contributing to radiosensitization by statins include impairing DNA damage repair, increasing ferroptosis *via* mevalonate pathway inhibition (34), EGFR-RAS-ERK1/2 pathway (32), and modulating autophagy (35). As a member of the statin family, rosuvastatin has demonstrated anticancer properties, including preventing tumor growth, decreasing angiogenesis, and reducing metastasis. However, evidence of rosuvastatin's potential as a radiosensitizer remains scarce.

Although limited investigations have assessed the effect of COX-2 inhibitors or statins pretreatment to enhance the radiosensitivity of breast cancer cell lines (30,36), no studies, to the best of our knowledge, have evaluated the combined effects of meloxicam and rosuvastatin for

radiosensitizing breast cancer cell lines. Therefore, the objective of this study is to determine the impact of the COX-2 inhibitor meloxicam and the statin rosuvastatin, both individually and in combination, on the acute damage induced by ionizing radiation on the MCF-7, MDA-MB-231, and T-47D breast cancer cell lines.

MATERIALS AND METHODS

Chemicals, drugs, and reagents

Rosuvastatin and meloxicam (manufactured by Dana Pharmaceutical Co., Iran) were dissolved in dimethyl sulfoxide (DMSO) to create a stock solution, which was subsequently diluted in a culture medium to the desired concentration. Cytochalasin-B was acquired from Sigma Chemicals Company (St. Louis, USA). Superoxide dismutase (SOD) assay kit (Nasdox™) and catalase (CAT) activity assay kit (Nactaz™) were obtained from Navand Salamat Company (Iran). Methanol, acetic acid, and Giemsa stain were sourced from Merck (Germany).

Cell lines

MCF-7 and T-47D are luminal A breast cancer cell lines known for being estrogen receptor-positive (ER+), progesterone receptor-positive (PR+), human epidermal growth factor receptor 2 (HER2-), and low in invasiveness (37,38). In contrast, MDA-MB-231 represents the triple-negative (ER-, PR-, HER2-) basal-like subtype (39,40), characterized by high invasiveness and metastatic potential (41). These cell lines were chosen to model different molecular subtypes of breast cancer in our study. Human breast cancer cell lines (MCF-7, T-47D, and MDA-MB-231) were obtained from the Iranian Biological Resource Center (IBRC). All cell lines were confirmed to be mycoplasma-free and were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Invitrogen, Paisley, UK) supplemented with 10% fetal bovine serum (FBS; Gibco, Invitrogen, Paisley, UK) and 100 µg/mL penicillin-streptomycin (Gibco, Invitrogen, Paisley, UK). All cell lines were incubated in an incubator with 5% CO_2 and 95% humidity at 37 °C. The culture medium was replaced every three days. After attaining 80% confluence, cells were sub-cultivated using 0.25% trypsin (Gibco, UK). All experiments were done after 3-4 passages of established cell lines.

Rosuvastatin and meloxicam pretreatment and ionizing radiation

After 24 h of plating the cells, the medium was replaced with either fresh medium for untreated cells or medium containing various concentrations of rosuvastatin and/or meloxicam for treated cells. For pretreatment, cells were treated with different concentrations of rosuvastatin and/or meloxicam alone, radiation alone, or a combination of both, followed by a 4-h incubation period before radiation exposure (42,43). Rosuvastatin and meloxicam were applied individually or in combination at concentrations of 2 µM, 10 µM (42,44), and 20 µM (45) for rosuvastatin (R2, R10, and R20) and 10 µM (46,47), 50 µM (46), and 100 µM (48) for meloxicam (M10, M50, and M100) in 12-well plates. In the control groups, equivalent volumes of medium were added instead of rosuvastatin and meloxicam. For irradiation, cells were exposed to ionizing radiation at 3 Gy (33,43). The irradiation was carried out using a 6 MV X-ray beam generated by a Linear Accelerator (Shinva, China) at 1.96 Gy/min and a source-to-sample distance of 60 cm (42). Following irradiation, the plates were transferred to the incubator at 37 °C under 5% CO_2 and 95% humidity.

Cytokinesis block micronucleus assay

To quantify DNA damage, cells were seeded at a cell density of 1×10^5 cells per well in 12-well plates and incubated at 37 °C for 24 h in a humidified atmosphere with 5% CO_2. The pretreatment and irradiation were conducted under the conditions described previously. After 48-h irradiation, cells were treated with 100 µL of cytochalasin B at 6 µg/mL to halt proliferation and induce binucleation, facilitating the identification of micronuclei. The execution of the cytokinesis block micronucleus assay followed established procedures from prior publications (49,50). Afterward, the medium with cytochalasin B was removed, and cells were washed twice with PBS and harvested with trypsin 0.05%

(incubated for 3 min at 37 °C). Trypsin was then inactivated with a complete culture medium, and cells were transferred to a 15-mL tube. Next, cells were centrifuged at 1500 RPM for 5 min; the supernatant was removed, and cells were resuspended in 5 mL of cold fixative solution (methanol-acetic acid, 6:1). Following another 5 min of centrifugation at 1500 RPM, pellets were resuspended in 500 µL of fixative solution and dropped onto clean and cold slides, which were air-dried overnight. Subsequently, slides were immersed in 10% (v/v) Giemsa solution for staining. Finally, the micronuclei and binucleate cells were counted under a light microscope, and the micronucleus frequency was specified as the ratio of the total number of micronuclei in binucleate cells.

Clonogenic assay

The clonogenic assay was conducted to assess cell survival. Cells were plated at a density of 2×10^3 cells per well in 6-well plates and incubated for 24 h at 37 °C in a humidified atmosphere with 5% CO_2. After rosuvastatin and/or meloxicam pretreatment and exposure to ionizing radiation, MCF-7 cells were incubated in a complete culture medium for up to 14 days to form colonies. This time was 7 days for MDA-MB-231 cells and 12 days for T-47D cells. Afterward, colonies were washed with PBS, fixed with fixative solution (methanol/acetic acid, 6:1), and stained with 10% Giemsa (v/v) in water. The colonies containing a minimum of 50 cells were counted and considered viable cells. The surviving fraction was determined as the ratio of the number of colonies formed to the product of the initial number of cells plated and the plating efficiency (51).

SOD evaluation

The Nasdox™ SOD Activity Assay Kit (Navand Salamat Co., Iran) was used to measure SOD activity as an index of oxidative-stress responses. All procedures were performed according to the manufacturer's instructions. The assay is based on the inhibition of pyrogallol autoxidation by SOD. Pyrogallol rapidly oxidizes in air, and its autoxidation half-life is established at a defined concentration. Samples containing unknown SOD concentrations were added, and the degree of inhibition of pyrogallol autoxidation was quantified at a fixed time point. Absorbance was measured at 405 nm using an EPOCH microplate reader (BioTek, USA).

CAT evaluation

Catalase activity was measured using the Nactaz™ Catalase Activity Assay Kit (Navand Salamat Co., Iran) as an index of oxidative stress. All procedures were performed according to the manufacturer's instructions. Briefly, 1×10^6 cells were homogenized in 1000 µL of lysis buffer on ice and centrifuged at 8,000 × g for 10 min at 4 °C. Next, 20 µL of the supernatant was sequentially mixed with 30 µL of Reagent 1, 20 µL of Reagent 2, and 100 µL of assay buffer, then incubated for 20 min at < 20 °C with gentle shaking using a Gyromax incubator shaker. Subsequently, 30 µL of Reagent 3 and 30 µL of Reagent 4 were added and incubated for an additional 10 min. Finally, 10 µL of Reagent 5 was added. The absorbance of the developed color was measured at 550 nm using an EPOCH plate reader. CAT activity was calculated according to the kit protocol and expressed in U/mL.

Statistical analysis

Statistical analysis involved conducting a one-way analysis of variance (ANOVA) using GraphPad Prism software (version 9.0, GraphPad Inc., USA), followed by post-hoc comparisons for irradiated groups that showed significance relative to the radiation-only (Hv) group. The data were expressed as mean ± SD. *P*-values ≤ 0.05 were considered statistically significant.

RESULTS

Micronucleus

According to the micronucleus assay conducted on T-47D, MCF-7, and MDA-MB-231 cell lines, the number of micronuclei in the radiation-only (hv) groups significantly increased in comparison with the control groups. In contrast, the number of micronuclei in all irradiated cell lines pretreated with either rosuvastatin or meloxicam did not differ significantly from the radiation-only groups. Pretreatment with a combination of

rosuvastatin and meloxicam significantly increased micronuclei in all irradiated MCF-7, MDA-MB-231, and T-47D cells compared with the radiation-only group (Fig. 1A-C). Moreover, no significant differences among the MCF-7 and MDA-MB-231 cells treated with R2+M10 µM, R10+M50 µM, and R20+M100 µM groups were observed. Notably, T-47D cells treated with R2+M10 µM demonstrated significantly higher micronuclei counts than those treated with R20+M100 µM.

Clonogenic

According to the clonogenic assay, the number of colonies decreased significantly in the radiation-only group compared to the controls for T-47D, MDA-MB-231, and MCF7 cell lines, indicating increased toxicity in these groups. Additionally, pretreatment with combined rosuvastatin and meloxicam further significantly reduced colony formation in all irradiated cell lines compared with the radiation-only groups (Fig. 2A-C). Moreover, significant differences between R2+M10 µM and R10+M50 µM, as well as between R2+M10 µM and R20+M100 µM in the T-47D cells, were observed. In contrast, no significant differences were found among these groups in the MCF-7 or MDA-MB-231 cells that received the combination of rosuvastatin and meloxicam.

CAT activity assay

According to the CAT activity assay, no significant difference was noted in CAT activity levels in cell lines pretreated with meloxicam or rosuvastatin alone, without radiation exposure, compared to the control groups. However, CAT activity levels declined significantly in the radiation-only (hv) groups compared to the control groups. In the MCF7 cell line, irradiated groups R10 µM, R10+M50 µM, and R20+M100 µM exhibited significantly lower CAT activity than the radiation-only group (Fig. 3A). Moreover, no statistically significant differences were found among the R10 µM, R10+M50 µM, and R20+M100 µM groups in MCF-7 cells.

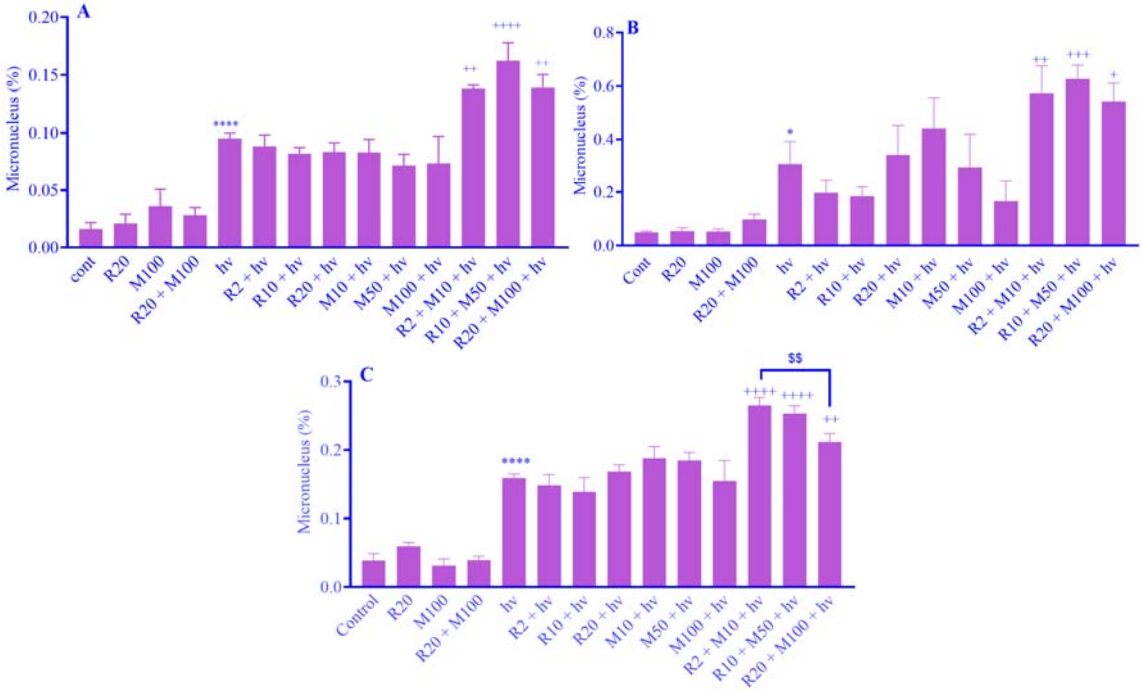

Fig. 1. Cytokinesis block micronucleus assay. The micronuclei percentage in irradiated (3 Gy) and non-irradiated (A) MCF-7, (B) MDA-MB-231, (C) and T-47D cells pretreated with meloxicam (M10, M50, and M100 µM) and rosuvastatin (R2, R10, and R20 µM) was assessed. In comparison to the radiation-only group, combined rosuvastatin and meloxicam treatment (R2+M10, R10+M50, and R20+M100 µM) resulted in increased genotoxicity in all irradiated cell lines. $^{*}P < 0.05$ and $^{****}P < 0.0001$ indicate significant differences compared to the control group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$, and $^{++++}P < 0.0001$ versus the hv group; $^{\$\$}P < 0.01$ between designated groups. Hv, irradiated cells.

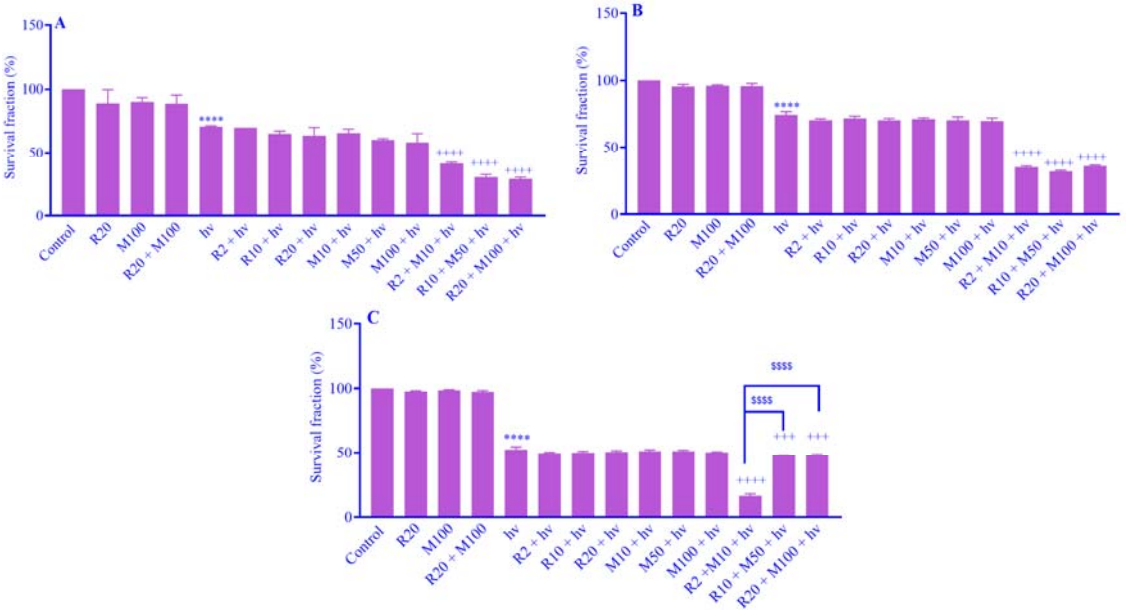

Fig. 2. Clonogenic assay. The colony formation in irradiated (3 Gy) and non-irradiated (A) MCF-7, (B) MDA-MB-231, (C) and T-47D cells pretreated with meloxicam (M10, M50, and M100 µM) and rosuvastatin (R2, R10, and R20 µM) was assessed. In comparison to the radiation-only group, combined rosuvastatin and meloxicam treatment (R2+M10, R10+M50, and R20+M100 µM) resulted in reduced colony formation in all irradiated cell lines. ****$P < 0.0001$ indicates significant differences compared to the control group; $^{+++}P < 0.001$ and $^{++++}P < 0.0001$ versus the hv group; $^{\$\$\$\$}P < 0.0001$ between designated groups. Hv, irradiated cells.

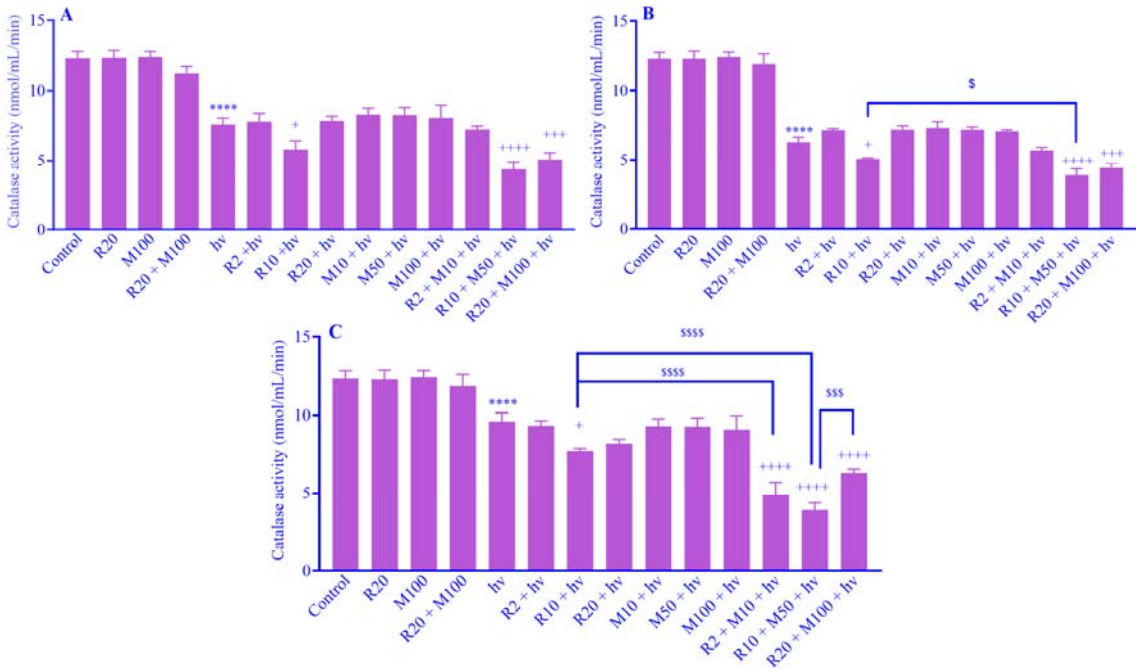

Fig. 3. Catalase activity assay. The catalase activity in irradiated (3 Gy) and non-irradiated (A) MCF-7, (B) MDA-MB-231, (C) and T-47D cells pretreated with meloxicam (M10, M50, and M100 µM) and rosuvastatin (R2, R10, and R20 µM) was investigated. Compared with the radiation-only (hv) group, CAT activity was significantly reduced in irradiated cells pretreated with R10 µM, R10+M50 µM, and R20+M100 µM across all three cell lines, whereas R2+M10 µM produced a significant reduction only in T-47D cells. ****$P < 0.0001$ indicates significant differences compared to the control group; $^{+}P < 0.05$, $^{+++}P < 0.001$, and $^{++++}P < 0.0001$ versus the hv group; $^{\$}P < 0.05$, $^{\$\$\$}P < 0.001$, and $^{\$\$\$\$}P < 0.0001$ between designated groups. Hv, irradiated cells.

Catalase activity in the MDA-MB-231 cell line was significantly reduced in irradiated groups R10 µM, R10+M50 µM, and R20+M100 µM compared to the radiation-only group (Fig. 3B). Additionally, a post-hoc analysis conducted across the R10 µM, R10+M50 µM, and R20+M100 µM groups showed statistically significant differences between R10 µM and R10+M50 µM.

In T-47D cells, catalase activity was significantly reduced in the irradiated groups R10 µM, R2+M10 µM, R10+M50 µM, and R20+M100 µM compared with the radiation-only group (Fig. 3C). Furthermore, a post-hoc analysis comparing the R10 µM, R2+M10 µM, R10+M50 µM, and R20+M100 µM groups in T-47D cells revealed significant differences between R10 µM and R2+M10 µM, R10 µM and R10+M50 µM, and R10+M50 µM and R20+M100 µM.

SOD activity assay

In cells treated with meloxicam or rosuvastatin without radiation exposure, no significant difference was observed in the SOD enzyme activity level compared to the control groups. However, the SOD activity level in the radiation-only groups of each cell line significantly decreased compared to the controls. Furthermore, across all cell lines, SOD activity was significantly lower in the R10 µM, R2+M10 µM, R10+M50 µM, and R20+M100 µM groups compared to their respective radiation-only (hv) groups, as depicted in Fig. 4A-C. Moreover, a post-hoc analysis comparing the R10 µM, R2+M10 µM, R10+M50 µM, and R20+M100 µM groups revealed significant differences between R10 µM and R10+M50 µM and between R10+M50 µM and R20+M100 µM in the T-47D cell line. Additionally, significant differences were observed between R10 µM and R10+M50 µM in the MDA-MB-231 cell line. However, no significant differences were found among these groups in the MCF-7 cell line.

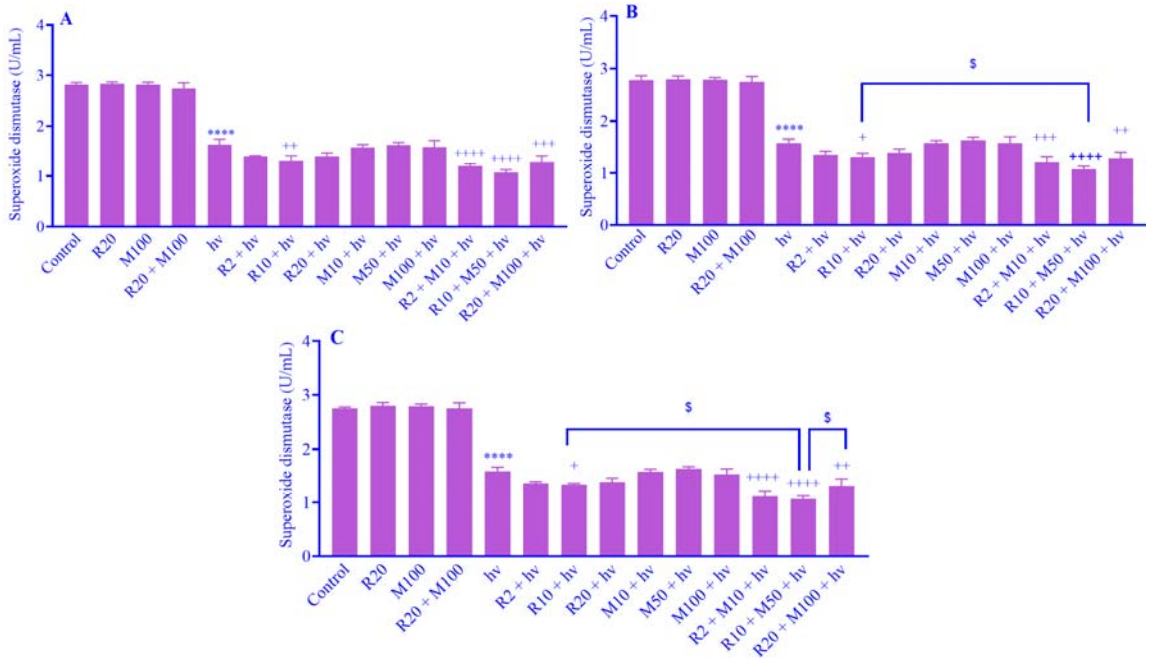

Fig. 4. Superoxide dismutase activity assay. The activity of superoxide dismutase in irradiated (3 Gy) and non-irradiated (A) MCF-7, (B) MDA-MB-231, (C) and T-47D cells pretreated with meloxicam (M10, M50, and M100 µM) and rosuvastatin (R2, R10, and R20 µM) was assessed. Compared with the radiation-only (hv) group, rosuvastatin and meloxicam treatment (R10 µM, R2+M10 µM, R10+M50 µM, and R20+M100 µM) significantly reduced SOD activity in all irradiated cell lines. ****$P < 0.0001$ indicates significant differences compared to the control group; $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$, and $^{++++}P < 0.0001$ versus the hv group; $^{\$}P < 0.05$ between designated groups. Hv, irradiated cells.

DISCUSSION

Drug resistance is commonly acknowledged as the primary reason for treatment failures in cancer therapies involving radiotherapy and chemotherapy (52-54). Consequently, combination therapies are frequently employed to mitigate the development of resistance. In this study, our objective was to increase the radiosensitivity of breast tumor cells by combining radiation with cytotoxic agents. To the best of our knowledge, this study is the first to investigate various concentrations of meloxicam and rosuvastatin pretreatment in irradiated MDA-MB-231, T-47D, and MCF-7 cell lines. Interestingly, our findings from the micronucleus and clonogenic assays demonstrated that pretreatment with a combination of rosuvastatin and meloxicam at varying concentrations (R2+M10 µM, R10+M50 µM, or R20+M100 µM) enhances radiation sensitivity in all irradiated cell lines, resulting in significantly higher genotoxicity and reduced cell survival compared to radiation-only (hv) groups. So far, various studies have investigated the radiosensitizing potential of various statins (17,32,55) or COX-2 inhibitors (22,56) on different cancer cells. In this regard, some have indicated that simvastatin exhibits a robust cytotoxic effect, leading to the death of human breast cancer MCF-7, MDA-MB-231 (57,58), and T-47D (59) cell lines. However, pretreatment with rosuvastatin alone in the current investigation did not significantly enhance radiation sensitivity or induce significant differences in genotoxicity or survival in the examined breast cancerous cell lines compared to the radiation-only groups. Consistent with our findings, some authors have shown that although lipophilic statins, including lovastatin, mevastatin, pitavastatin, and simvastatin, have anticancer activities, the hydrophilic rosuvastatin and pravastatin have minimal or no impact on neoplastic cells (60,61). Preclinical investigations have utilized animal models and various breast cancer cell lines to clarify the mechanisms linking mevalonate inhibition to anticancer effects. Among these investigations, lipophilic statins have consistently demonstrated antitumor properties. For instance, simvastatin impedes the DNA binding of the NFκB transcription factor, lowers the expression of the anti-apoptotic protein Bcl-xL, and enhances PTEN expression, thus impeding the oncogenic phosphatidylinositol 3-kinase pathway (62). Additionally, lipophilic statins, unlike hydrophilic statins like pravastatin, have been shown to lessen the proliferation of breast cancer cells by inhibiting NF-B and AP1 transcription factors, particularly in cells with HER2 overexpression or constitutively active RAS. Numerous studies have also indicated statin-mediated suppression of PI3K signaling as well as NFκB deactivation, including those directly implicating the delocalization of prenylated guanosine triphosphatase in the antitumor effect (63-65). Moreover, simvastatin has been reported to enhance cytostatic cell death by arresting cells at the G0/G1 and G2/M phases (66) and directly inducing apoptosis in breast cancer cells through the activation of the JNK/CHOP/DR5 pathway (67). Some studies have also highlighted the significant role of the EGFR-RAS-ERK1/2 pathway, through which statins enhance radiation sensitivity (32). Overall, apart from the need to further investigate the underlying pathways for different types of statins, it appears that one possible reason for lipid-soluble statins' greater efficacy may be their ability to better penetrate cell membranes, potentially leading to stronger effects on cellular metabolic processes.

In the present study, various concentrations of meloxicam (10, 50, and 100 µM) did not increase radiosensitivity in irradiated MCF-7, MDA-MB-231, and T-47D cell lines. Consistent with our results, Ayakawa *et al.* conducted a study examining the antitumor impacts of meloxicam, both individually and in combination with radiation and/or 5-fluorouracil, in cultured tumor cells (26). In their study, the authors found that although a concentration of 250 µM meloxicam in combination with radiation resulted in a higher antitumor effect compared to radiation alone, at lower concentrations, meloxicam had no radiosensitizing effect (26). In this regard, Bijnsdorp et al. studied the radiosensitizing potential of 250-750 µM meloxicam on human glioma cells after 24-72 h exposure and demonstrated that 750 µM meloxicam led to radiosensitization of D-384 and U-87 cells, but not U-251 cells (22). Although the precise molecular mechanisms behind COX-2-mediated

growth inhibition are not well understood, two potential mechanisms have been proposed, comprising the induction of apoptosis and cell cycle blockade. However, in their study, the concentration and exposure time of the meloxicam did not induce apoptosis but halted the cell cycle (22). Besides, other general potential mechanisms by which COX-2 inhibition impacts radiosensitization may include the inhibition of angiogenesis and metastasis (68-71), suppression of DNA repair mechanisms (47,72), tumor cell redistribution (26,46,73), induction of apoptosis (74-76), and increased tumor oxygenation (77-79). However, these outcomes may differ across studies due to variations in the type of drug, dosage, pretreatment duration, and experimental conditions.

The key discovery of the current investigation was the increased radiosensitization of MCF-7, MDA-MB-231, and T-47D cells pretreated with the combination of meloxicam and rosuvastatin. This phenomenon is likely attributed to the synergism between meloxicam and rosuvastatin in these processes. Studies have indicated that statins, HMG-CoA reductase inhibitors, and COX-2 inhibitors have a synergistic effect (80,81). Specifically, research has demonstrated that the combined use of statins and COX-2 inhibitors synergistically inhibits caveolin-1 and its related signaling pathways (82). Caveolin-1 plays a pivotal role in promoting breast tumorigenesis by contributing to cell proliferation, invasion, migration, apoptosis, autophagy, and metastasis, while also inhibiting apoptosis through cyclin D1 induction (82-84). Besides, there may be interactions between rosuvastatin and meloxicam due to their association with breast cancer resistance protein (BCRP). BCRP is a clinically significant ATP-binding cassette transporter involved in drug disposition, which limits the gastrointestinal absorption of various drug classes, encompassing tyrosine kinase inhibitors, the anti-inflammatory sulfasalazine, and lipid-lowering statins (such as fluvastatin, atorvastatin, and rosuvastatin) (85,86). Studies have shown that meloxicam is a potent inhibitor of BCRP. Therefore, given meloxicam's substantial bioavailability of 89%, it is likely to inhibit intestinal BCRP, potentially enhancing the absorption of other BCRP substrate drugs, such as rosuvastatin (86).

In the present study, we also evaluated cellular oxidative stress through CAT and SOD enzyme assays. Compared to the cells that received only radiation, SOD enzyme activity decreased significantly in all irradiated cell lines pretreated with rosuvastatin (10 μM) or a combination of rosuvastatin and meloxicam in different concentrations (R2+M10 μM, R10+M50 μM, or R20+M100 μM), indicating greater oxidative stress in these cells. A similar pattern was observed for CAT activity in the T-47D cell line. Nevertheless, in the MDA-MB-231 and MCF-7 cell lines, CAT activity decreased significantly only in the irradiated groups pretreated with R10 alone or the higher-dose combinations (R10+M50 and R20+M100), compared with the radiation-only group. Although antioxidant enzymes such as SOD and CAT are commonly reported to increase following radiation exposure as part of an adaptive cellular defense response, in our experimental conditions, radiation alone significantly reduced SOD and CAT activity, suggesting impairment of the antioxidant defense system. The additional reduction observed after meloxicam and rosuvastatin pretreatment indicates a further disruption of cellular redox homeostasis, which may contribute to enhanced oxidative stress and increased radiosensitization of breast cancer cells. To date, limited studies have explored the effects of statins and COX-2 inhibitors on SOD and CAT activity in vitro. Ungureanu *et al*. reported that simvastatin therapy significantly reduces the activity of SOD, CAT, and glutathione peroxidase (GPx) (87). Furthermore, some studies have demonstrated that simvastatin disrupts the antioxidant defense system by suppressing the expression of reactive oxygen species (ROS) scavengers, particularly Mn-SOD, CAT, GPx1, and SESN3, leading to increased oxidative stress and apoptotic cell death (88). Based on these findings, the authors concluded that simvastatin induces colon cancer cell death, at least in part, by elevating intracellular oxidative stress and triggering apoptosis (88). In another study examining the impact of atorvastatin on the radiosensitivity of PC-3 prostate cancer cells, the authors found that atorvastatin enhances the cell-killing effect of irradiation. This effect was attributed to a reduction in endogenous ROS levels and a prolongation of radiation-induced ROS lifespan,

achieved through decreased NOX (NADPH oxidase) levels and SOD activity (44). Similarly, a study investigating the effects of nimesulide, a COX-2 inhibitor, on radiation treatment in non-small cell lung cancer (NSCLC) in vitro and in vivo evaluated the impact of nimesulide alone and in combination with radiation on the NF-κB target gene products, MnSOD and survivin. The results showed that nimesulide induced a dose-dependent reduction in MnSOD and survivin levels at concentrations of 100-300 μM after 24 h. Additionally, nimesulide suppressed the radiation-induced upregulation of MnSOD, further supporting its potential to enhance the efficacy of radiation therapy (89). In contrast, a study investigating the effects of nimesulide on oxidative stress and antioxidant enzyme activities in vivo found that nimesulide treatment decreased CAT activity, while SOD activity remained unchanged (54). Overall, due to the limited data available in this context, further research is necessary to fully elucidate the mechanisms by which statins and COX-2 inhibitors enhance radiosensitivity in breast tumors.

CONCLUSION

The combination of rosuvastatin and meloxicam in specific concentrations increased the radiation sensitivity of MCF-7, T-47D, and MDA-MB-231 breast cancer cell lines. Combined pretreatment with rosuvastatin (10 μM) and meloxicam (50 μM) notably enhanced genotoxicity while decreasing colony formation, SOD activity, and CAT activity compared to radiation therapy alone in MCF-7, T-47D, and MDA-MB-231 cell lines. These findings suggest that this combination therapy holds promise for enhancing the efficacy of radiotherapy. Future studies, including *in vivo* investigations and clinical trials, are recommended to further explore its therapeutic potential and facilitate the translation of these findings into clinical applications.

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Conflict of interest statement

The authors declared no conflict of interest in this study and also declared that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Authors' contributions

M. Haddad Zahmatkesh contributed to conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, software, resources, validation, supervision, visualization, and writing the original draft, review, and editing. A. Naeimi contributed to investigation, methodology, project administration, software, supervision, visualization, and writing the original draft, review, and editing. H. Saeidi Saedi, A.M. Bakhtiyari, K. Shabani, and Z. Babajani contributed to the investigation. All authors have read and approved the finalized article. Each author has fulfilled the authorship criteria and affirmed that this article represents honest and original work.

Ethics statement

The experimental protocols were approved by the Ethics Committee of Guilan University of Medical Sciences (IR.GUMS.REC.1400.380) and were conducted in accordance with the ethical standards established by national health and research institutes.

Data availability statement

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

AI declaration

The authors did not use any AI-assisted technologies in the preparation of this manuscript.

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