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Combination of Doxorubicin and Berberine Generated Synergistic Anticancer Effect on Breast Cancer Cells Through Down-regulation of Nanog and miRNA-21 Gene Expression

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Abstract

Background: Our purpose was to investigate the effect of berberine (BER) and doxorubicin (DOX) on the expression of stem cell markers Nanog and microRNA-21 in MCF-7 cells.

Methods: The study was an in vitro study employing the human breast cancer cell line MCF-7 that was divided into four groups: Group I: MCF-7 cell line maintained in drug-free environment as untreated control, Group II: MCF-7 cell line treated with different concentrations of DOX, Group III: MCF-7 cell line treated with various concentrations of BER. Group IV: MCF-7 cell line treated with different concentrations of combined DOX and BER. MTT assay determined the metabolic activity and viability of MCF-7 cells for all groups. We further extracted total RNA from MCF-7 cells, and RT-PCR assayed the expression of Nanog and miRNA-21.

Results: The results revealed that DOX and/or BER decreased the percentage of viable MCF-7 monolayer and mammospheres breast cancer cells in a concentration-dependent manner. Moreover, the combination of DOX and BER generated synergistic anticancer effect on MCF-7 monolayer cells and mammospheres. In addition, DOX alone, BER alone, and their combination significantly reduced Nanog and miRNA-21 gene expression in MCF-7 mammospheres compared with untreated mammospheres.

Conclusions: BER may affect the viability of breast cancer cells through down-regulation of Nanog and miRNA-21 gene expression, ultimately enhancing the sensitivity of breast cancer cell line to DOX. BER may be an effective chemotherapeutic agent against breast cancer where the combination of DOX and BER generates synergistic anticancer effects.

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Introduction

Breast cancer is a very common malignant tumor among female patients. It is estimated that breast cancer inflicts 1 in 10 women worldwide.[1] Recently, vaccines against BC have gained special scientific attention.[2,3] Chemo- and radiotherapy are still the best treatment options for metastatic breast cancer. However, while these therapies are very effective in reducing tumor size, resistance invariably is a major issue which has to be considered. It is now well established that cancer stem cells (CSCs) are among the main causes of tumor recurrence and resistance to both chemo- and radiotherapy.[4] CSCs are small population of cells in tumor that have unique characteristics such as self-renewal and ability to generate heterogenic lineages of cancer cells.[5] These characteristics make CSCs a likely source of tumor initiation, heterogeneity, progression, metastasis, and recurrence.[6]

In numerous solid tumors, including those in brain, pancreatic, ovarian, and breast cancers, CSCs show resistance to chemotherapy and radiotherapy. Furthermore, CSCs exhibit the characteristics of epithelial-to-mesenchymal transition, a known metastasis mechanism.[7]

Nanog is a transcription factor required to maintain the pluripotency of embryonic stem cells; this factor is not expressed in most normal adult tissues. However, recent studies have indicated that Nanog is overexpressed in many types of human cancers, including breast cancer.[8]

MicroRNA-21, also known as hsa-mir-21, is encoded by the miRNA-21 gene located on chromosome 17q23.2.[9] MiRNA-21 is one of the most common microRNAs frequently up-regulated in a variety of cancers including breast cancer.[10] MiRNA-21 is an oncogenic miRNA able to modulate the expression of multiple tumor suppressor genes such as phosphatase and tensin homolog (PTEN), Serpini1, and programmed cell death 4 (PDCD4).[11]

Berberine (BER) is an isoquinoline quaternary alkaloid (a 5, 6-dihydrodibenzo [a, g] quinolizinium derivative) employed in traditional Chinese and Indian medicine for centuries.[12] We can find BER in the roots and stems of numerous plants such as Berberisaetnensis C. Presl., Berberisaristata, Berberis vulgaris, Coptischinensis, and Tinosoracordifolia.[13] It has anti inflammatory and antimicrobial properties, anti diabetic and antioxidant effects and multiple pharmacological properties. BER has further been shown to have antitumor effects on many cancer cell lines, including leucocytes, liver, lung, stomach, colon, skin, oral, esophageal, brain, bone, breast, and genital cancer cells.[14]

Doxorubicin (DOX) is a potent chemotherapeutic agent employed in the treatment of solid tumors and malignant hematological diseases.[15] However, the clinical use of DOX has been largely restricted due to its cardiotoxicity, which may lead to the development of cardiomyopathy and ultimately congestive heart failure.[16] BER was effective in the inhibition of cell proliferation and promotion of apoptosis in different cancerous cells.[17] Based on these findings, we hypothesize that combining DOX with BER may be a novel strategy for tumor therapy.

The main target of this study was to investigate the effect of BER and DOX on the expression of stem cell markers Nanog and microRNA-21 in human breast cancer cell line MCF-7.

Materials and Methods

The present experimental in vitro study employed the human breast cancer cell line MCF-7 that was divided into four groups:

Group I: MCF-7 cells maintained in drug-free environment as untreated control.

Group II: MCF-7 cells treated with different concentrations of DOX as conventional anticancer chemotherapeutic agent (1, 10, 20, and 30 µg/mL) for monolayer culture and (1, 25, 50, and 100 µg/mL) for mammosphere culture.

Group III: MCF-7 cells treated with various concentrations of BER (5, 15, 25, and 50 µM) for monolayer culture and (5, 25, 50, and 100 µM) for mammosphere culture.

Group IV: MCF-7 cell line treated with different concentrations of DOX and BER (1 µg/mL + 5 µM, 1 µg/mL + 50 µM, 30 µg/mL +

5 µM and 30 µg/mL + 50 µM, respectively) for monolayer culture and (1 µg/mL + 5 µM, 1 µg/mL + 100 µM, 100 µg/mlL + 5 µM and 100 µg/mL + 100 µM) for mammosphere culture.

Cell culture

Throughout the study, we maintained human breast cancer cell line MCF-7 cells in high glucose Dulbecco's modified Eagle's Medium (DMEM, Lonza, Belgium) with 2 mM L-glutamine supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS, Sigma, USA), 100 IU/mL penicillin and 100 µg/mL streptomycin (Lonza, Belgium). All culture systems were basically carried out in a humidified CO_2 incubator at 37°C with a permanent atmosphere of 5% CO_2 and 95% air.[18]

Cell viability assay

We specified human breast cancer cell line MCF-7 metabolic activity and viability (reflecting their survival and growth) for all groups using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay according to the method described by Mosmann (1983).[19] This is a colorimetric assay measuring the reduction of yellow MTT by mitochondrial succinate dehydrogenase. MTT enters the cells and passes into the mitochondria. There, it is reduced to an insoluble dark purple formazan product. The cells

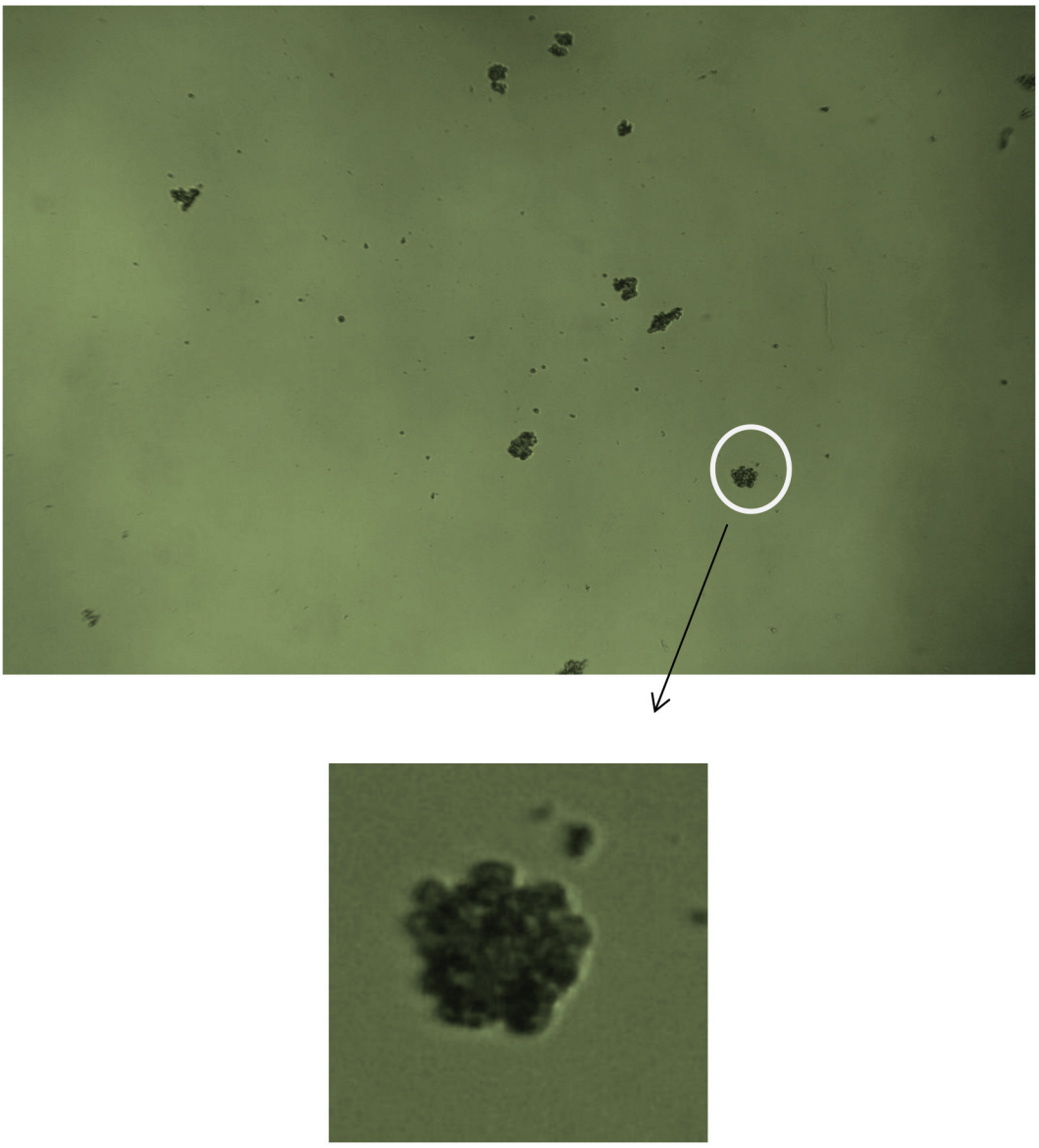

Figure 1. MCF-7 mammospheres in a six-well plate under inverted microscope.

are then solubilized with DMSO and the released solubilized formazan reagent is measured spectrophotometrically as optical density (OD). Since reduction in MTT can only occur in metabolically active cells, the OD can be considered as a measure of target cell viability.

Mammosphere assay

We seeded single cell suspension of MCF-7 cells in non-adherent 96-well tissue culture plate at a cell density of 5×104/well in a total volume of 200 µl of complete mammosphere medium. We further incubated the plate at 37°C in a humidified 5% CO_2 incubator for four days. At the end of the incubation period, we checked mammosphere formation both by naked eye and under an inverted microscope (Figure 1).[18]

Real-time PCR

We extracted the total RNA from dissociated mammospheres using a highly denaturating guanidine-thiocyanate–containing buffer. This buffer immediately inactivates RNases to ensure the purification of intact RNA (RNeasy Mini Kit, Qiagen, Germany). We synthesized cDNA by use of miScript II RT kit (Qiagen, USA). To detect microRNA-21 and other non-coding RNAs, cDNA prepared in the reverse transcription reaction served as the template for real-time PCR analysis using specific miScript Primer Assay (forward primer), miScript SYBR Green PCR Kit, containing the miScript Universal Primer (reverse primer), and QuantiTect SYBR Green PCR Master Mix. Regarding Nanog mRNA detection, we replaced the miScript Primer Assay and the miScript Universal Primer by Nanog QuantiTect Primer assay (containing forward and reverse primers of Nanog). To check the amplification specificity, we routinely performed a melting curve analysis. We employed GAPDH as an internal control and calculated the relative expression level of each transcript by the $2^{-\Delta\Delta ct}$ method.

Statistical analyses

The statistical software package of SPSS version 20 (SPSS Inc, Chicago, USA) performed the statistical analyses. Student's t-test specified the difference between groups. We presented the data as mean ± standard error (SE) of triplicate data. $P \leq 0.05$ showed statistical significance.

Results

Effect of DOX and BER treatment on viability of MCF-7 monolayer cells

Tables 1 and 2, and figures 2-4 show the mean values of absorbance ± SE of the MTT assay concerning untreated MCF-7 monolayer cells (control) and cells treated with different

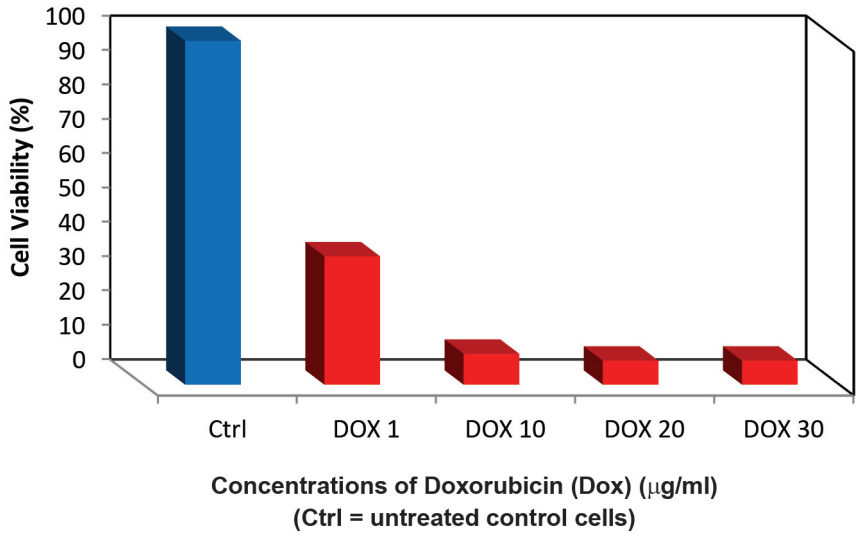

Figure 2. Bar chart representing the viability (%) of MCF-7 monolayer cells of untreated control and cells treated with various concentrations of doxorubicin.

Table 1. MCF-7 monolayer cell viability (%) without and with treatment with variable concentrations of DOX and/or BER

Group		Mean Abs ± SE	Cell viability (%)
Untreated cells		1.5 ± 0.01	100
DOX (µg/ml) treated cells	1	0.56 ± 0.02	37.2
	10	0.14 ± 0.05	9.1
	20	0.1 ± 0.01	6.8
	30	0.1 ± 0.04	6.8
BER (µM) treated cells	5	0.99 ± 0.06	66.1
	15	0.86 ±0.02	56.9
	25	0.7 ± 0.09	46.5
	50	0.69 ±0.01	46
DOX (µg/ml) + BER (µM) treated cells	1 + 5	0.48 ± 0.01	32
	1 + 50	0.44 ±0.02	29.3
	30 + 5	0.099 ± 0.001	6.5
	30 + 50	0.08 ±0.001	5.5

DOX=Doxorubicin, BER= Berberine, Abs: absorbance, SE: standard error

concentrations of DOX and/or BER .

As presented in table 1, the mean value of absorbance was 1.5 for untreated cells; 0.56, 0.14, 0.1, and 0.1 for cells treated with different concentrations of DOX (1, 10, 20, and 30 µg/ml, respectively); 0.99, 0.86, 0.7, and 0.69 for cells treated with different concentrations of BER (5, 15, 25, and 50 µM, respectively); and 0.48, 0.44, 0.099, and 0.08 for cells treated with different concentrations of combined DOX and BER (1 µg/mL+5 µM, 1 µg/mL+50 µM, 30 µg/mL+5 µM, and 30 µg/mL+50 µM, respectively). We further calculated the combination index (CI) to analyze the synergistic effects of the two drugs on the MCF-7 monolayer cells. The combination index was 0.73, indicating that combination of DOX and BER generated synergistic antitumor effects on MCF-7 monolayer cells.

The statistical analyses of these results showed that the percentage (%) of MCF-7 cell viability treated with the highest concentration of DOX (30 µg/mL), BER (50 µM) and DOX+BER (30 µg/mL+50 µM) was significantly lower than the control group ($P< 0.001$). (Table 2)

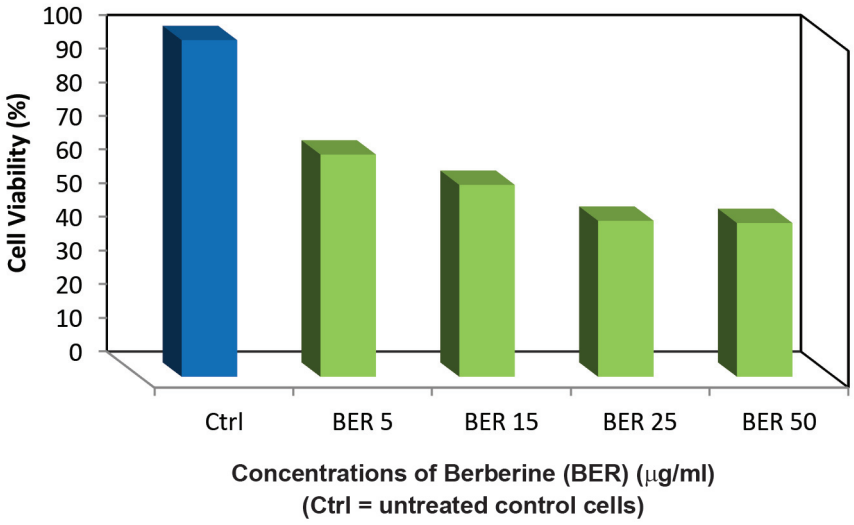

Figure 3. Bar chart representing the viability (%) of MCF-7 monolayer cells of untreated control and cells treated with various concentrations of berberine.

Table 2. Statistical analyses of MCF-7 monolayer cell viability (%) without and with treatment with the highest concentration of DOX (30 µg/ml), BER (50 µM) and DOX+BER (30 µg/ml+50 µM)

	Untreated cells	DOX treated cells (30 µg/ml)	BER treated cells (50 µM)	DOX+BER treated cells (30 µg/ml+50 µM)
Cell viability (%)	100	6.8	46	5.5
P		< 0.001*	<0.001*	<0.001*

P: *P* value comparing the untreated control group with each studied group, significance was considered at $P \leq 0.05$.: *: Significantly different from untreated control group. µM= Micro Molar, DOX=Doxorubicin, BER= Berberine

Effect of DOX and BER treatment on viability of MCF-7 mammospheres

Tables 3 and 4, and figures 5-7 show the mean values of absorbance ± SE of the MTT assay regarding untreated MCF-7 mammospheres (control) and mammospheres treated with different concentrations of DOX and/or BER .

As presented in table 3, the mean value of absorbance was 0.36 for untreated mammospheres; 0.22, 0.15, 0.14, and 0.1 for mammospheres treated with different concentrations of DOX (1, 25, 50, and 100 µg/mL, respectively); 0.21, 0.2, 0.19, and 0.18 in regard to mammospheres treated with different concentrations of BER (5, 25, 50, and 100 µM, respectively) and 0.19, 0.16, 0.09, and 0.06 for mammospheres treated with different concentrations of DOX and BER in combination (1 µg/mL+5 µM, 1 µg/mL+100 µM, 100 µg/mL+5 µM, and 100 µg/mL+100 µM, respectively). We calculated the CI in order to analyze the synergistic effects of the two drugs. The CI was 0.63, meaning the combination of DOX and BER generated synergistic antitumor effects on MCF-7 mammospheres.

The statistical analyses of these results showed that the % of MCF-7 mammosphere viability treated with the highest concentration of DOX (100 µg/mL), BER (100 µM), and DOX+BER (100 µg/mL+100 µM) was significantly lower compared to the control group ($P< 0.001$) (Table 4).

IC_{50} values of DOX (µg/mL) and BER (µM) in MCF-7 monolayer cells and MCF-7 mammospheres

IC_{50} values of DOX (µg/mL) and BER (µM) in MCF-7 mammospheres (16 µg/mL and 100 µM, respectively) were higher than that in MCF-

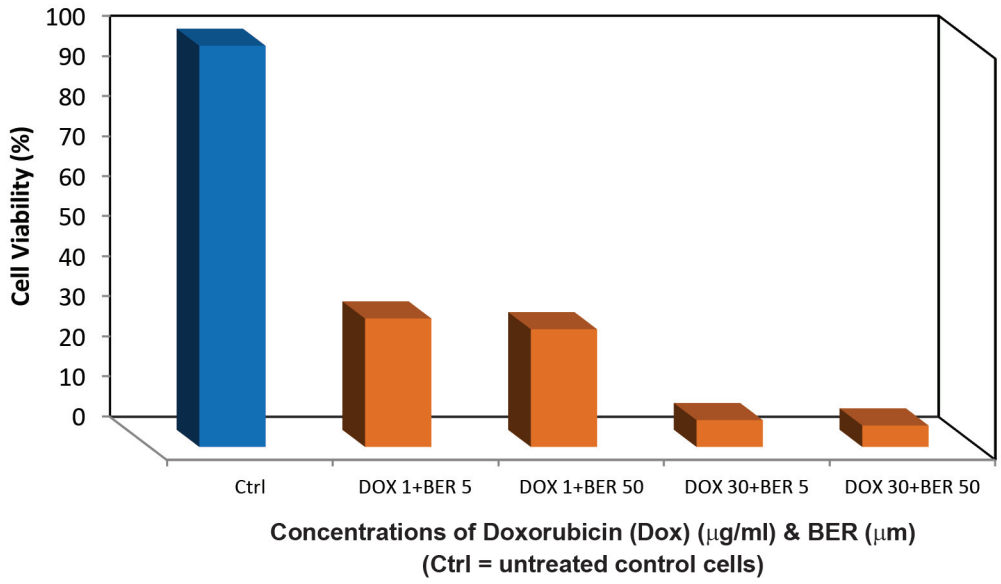

Figure 4. Bar chart representing the viability (%) of MCF-7 monolayer cells of untreated control and cells treated with combination of different concentrations of doxorubicin and berberine.

Table 3. MCF-7 mammosphere viability (%) without and with treatment with variable concentrations of DOX and/or BER

Group		Mean Abs ± SE	Cell viability (%)
Untreated mammospheres		0.36 ± 0.004	100
DOX (µg/ml) treated mammospheres	1	0.22 ±0.001	59.6
	25	0.15 ± 0.006	41.1
	50	0.14 ±0.005	39.8
	100	0.1 ±0.02	29.5
BER (µM) treated mammospheres	5	0.21 ±0.005	58.4
	25	0.2 ±0.02	55.7
	50	0.19 ±0.03	55.4
	100	0.18 ±0.001	49.8
DOX (µg/ml)	1+5	0.19 ±0.01	53.9
+	1+100	0.16 ±0.01	44.8
BER (µM) treated mammospheres	100+5	0.09 ±0.004	24.4
	100+100	0.06 ± 0.004	16.3

DOX=Doxorubicin, BER= Berberine, Abs: absorbance, SE: standard error

7 monolayer cells (0.2 µg/mL and 25 µM, respectively).

Effect of DOX and BER treatment on Nanog and miRNA-21 genes expression in MCF-7 mammospheres

Table 5, and figures 8 and 9 show the mean±SE of the fold change related to Nanog and miRNA-21 genes expression in MCF-7 mammospheres without and with treatment with DOX and/or BER. The results showed that DOX, BER, and the combination of DOX and BER significantly decreased the expression of Nanog gene expression when compared to untreated mammospheres (*P1*=0.002, *P1*=0.007, and *P1*=0.001, respectively). Moreover, Nanog gene expression in mammospheres treated with BER alone was insignificantly higher than that treated

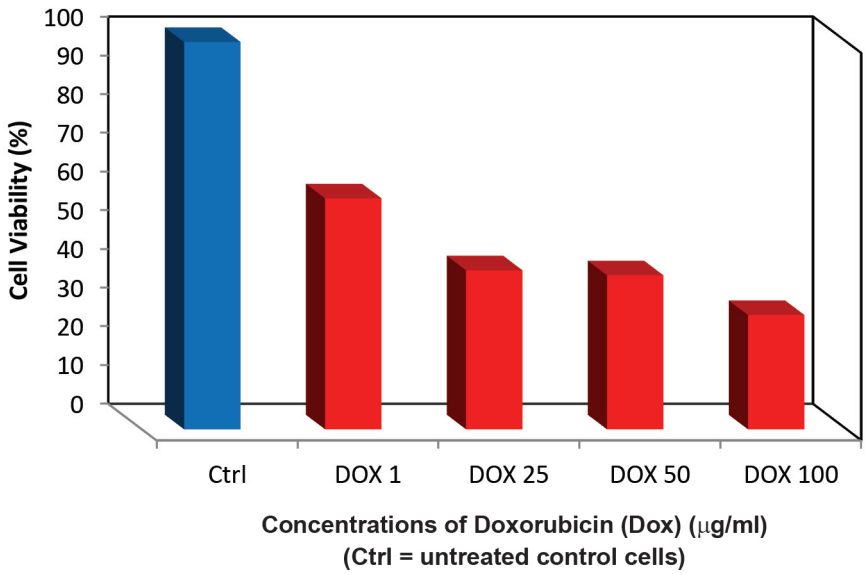

Figure 5. Bar chart representing the viability (%) of MCF-7 mammospheres of untreated control and cells treated with various concentrations of doxorubicin.

Table 4. Statistical analyses of MCF-7 mammosphere viability (%) without and with treatment with the highest concentration of DOX (100 µg/mL, BER (100 µM) and DOX+BER (100 µg/mL+100 µM)

	Untreated mammospheres	DOX treated mammospheres (100 µg/ml)	BER treated mammospheres (100µM)	DOX+BER treated mammospheres (100 µg/ml+100 µM)
Cell viability (%)	100	29.5	49.8	16.3
P		<0.001*	<0.001*	<0.001*

P: *P* value comparing between the untreated control group with each studied group, significance was considered at $P \leq 0.05$.; *: Significantly different from untreated control group.; DOX=Doxorubicin, BER= Berberine

with DOX alone (*P2* = 0.08). Furthermore, Nanog gene expression in mammospheres treated with the combination of DOX and BER was significantly lower compared to treatment with either DOX or BER alone (*P2*=0.03 and *P3*=0.04, respectively).

Regarding miRNA-21 gene expression, the results revealed that miRNA-21 gene expression in mammospheres treated with DOX, BER, and the combination of DOX and BER was significantly lower than in untreated mammospheres (*P1*=0.000, *P1*=0.02, and *P1*=0.003, respectively). Moreover, miRNA-21 gene expression in mammospheres treated with BER alone was insignificantly higher than that treated with DOX alone (*P2*=0.06). Moreover, miRNA-21 gene expression in mammospheres treated with combined DOX and BER, was significantly lower in comparison to treatment with either DOX or BER alone (*P2*=0.02 and *P3*=0.01, respectively).

Discussion

In this study, our results revealed that DOX and/or BER reduced the percentage of viable MCF-7 monolayer or mamospheres breast cancer cells in a concentration-dependent manner. These results are in agreement with previous studies, [20-22] where BER or DOX alone or in combination, exhibited antiproliferative effects against human breast cancer MCF-7 cell line; this effect was mediated through interference with normal cell cycle distribution and induction of apoptosis. This suggests that BER may be an effective chemotherapeutic agent against breast cancer. Moreover, the present results revealed that the combination of DOX and BER generated synergistic anticancer effects on MCF-7

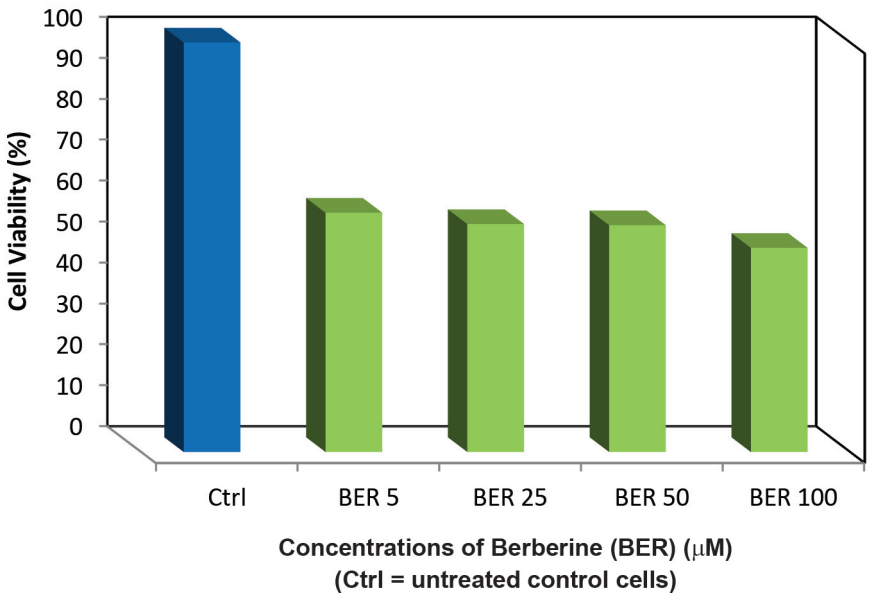

Figure 6. Bar chart representing the viability (%) of MCF-7 mammospheres of untreated control and cells treated with various concentrations of berberine.

Table 5. Statistical analyses of Nanog and miRNA-21 genes expression in MCF-7 mammospheres in different studied groups

Nanog gene	Untreated mammospheres	DOX treated mammospheres (16 µg/ml)	BER treated mammospheres (100 µM)	DOX+BER treated mammospheres (16 µg/ml+100 µM)
Mean ± SE	1.0 ± 0.0	0.043 ± 0.004	0.10 ± 0.01	0.006 ± 0.005
*P*1		0.002*	0.007*	0.001*
*P*2			0.08	0.03*
*P*3				0.04*
miRNA-21 gene				
Mean ± SE	1.0 ± 0.0	0.627 ± 0.003	0.710 ± 0.01	0.375±0.04
*P*1		0.000*	0.02*	0.003*
*P*2			0.06	0.02*
*P*3				0.01*

*P*1: *P* value comparing the untreated control group with each studied group.; *P*2: *P* value comparing between DOX treated group with BER treated group and DOX+BER treated group.; *P*3: *P* value comparing between BER treated group and DOX+BER treated group.; Significance was considered at $P \leq 0.05$.; DOX=Doxorubicin, BER= Berberine

monolayer cells (CI=0.73) and MCF-7 mammospheres (CI=0.63). The induction of apoptosis is one of the antitumor mechanisms of DOX and BER, which is in accordance with the theory of independent similar action. Therefore, DOX combined with BER probably results in a synergistic antitumor effect. In this regard, more studies should be conducted to detect the synergistic anticancer mechanism of DOX and BER.

Additionally, the results of this study showed that IC_{50} of DOX and BER in MCF-7 mammospheres was higher than that in MCF-7 monolayer cells. The higher degrees of chemoresistance in spheroids was attributed to the increased proportions of CSCs.[23] Alternatively, it could also be explained by the drug barrier created by the three-dimensional (3D) structure of the spheroids that conferred a higher degree of resistance to chemotherapeutics drugs as compared to monolayer cultures.[24] Recent studies have also tested whether spheroids have higher drug resistance in two-dimensional (2D) culture condition and are able to rule out the possibility of the physical barrier created by the 3D structure of the spheroids. Interestingly, higher drug

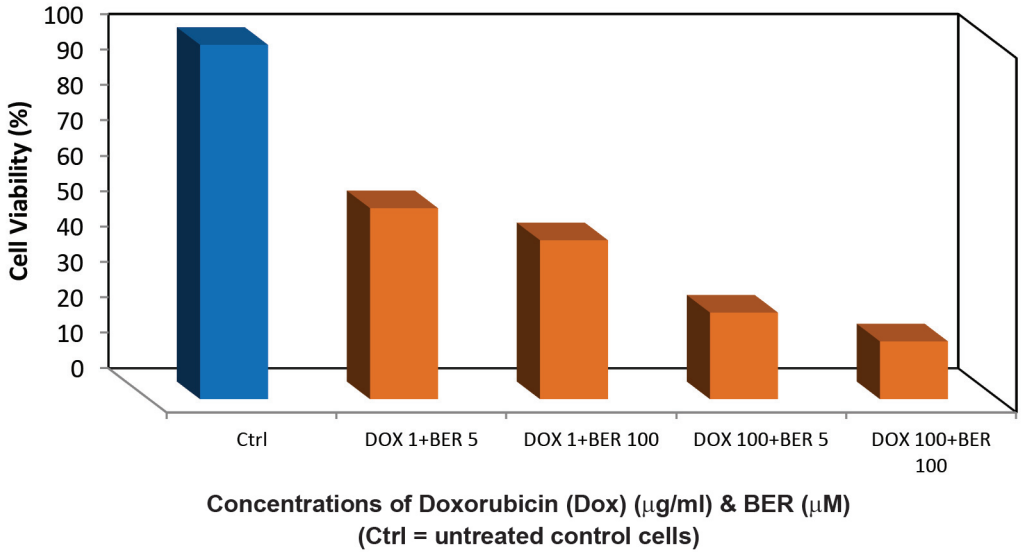

Figure 7. Bar chart representing the viability (%) of MCF-7 mammospheres of untreated control and cells treated with combination of different concentrations of doxorubicin and berberine.

resistance was obtained in spheroids cultured in 3D or 2D as compared to the monolayer cultures. Importantly, these results suggest that the increased proportions of CSCs could be the most probable contributor of the higher drug resistance in the spheroids.[25]

Nanog is a homeodomain-containing transcription factor that maintains the self-renewal and pluripotency of ESCs.[26] Several studies have provided consistent evidence for the role of Nanog as a potential human oncogene.[27] Aberrant expression of Nanog during tumor development was observed in a variety of different tumor types and cell lines, including breast cancer.[28] Many studies have demonstrated that Nanog plays additional roles in determining the malignant potential of tumor cells. For instance, elevated expression of Nanog increased the drug resistance properties of cancer cells and significantly enhanced the invasive potential of many human cancer cells.[29,30] However, down-regulation of Nanog gene expression resulted in the reversal of drug resistance, suppressed tumor growth and reduced metastatic potential.[31,32]

In this study, we evaluated the effect of DOX and/or BER on Nanog gene expression in MCF-7 mammospheres. The results revealed that Nanog gene expression in mammospheres treated with DOX and/or BER at IC_{50} was significantly lower than untreated mammospheres. Moreover, Nanog gene expression in mammospheres treated with BER was insignificantly higher compared to treatment with DOX. Interestingly, we observed that Nanog gene expression in mammospheres treated with DOX combined with BER was significantly lower than that treated with either DOX or BER alone.

In a few studies, BER reduced cancer stem cells.[33, 34] A recent study on the pancreatic cancer cell lines clearly showed that BER could be employed as a targeting agent for pancreatic cancer treatment. This is because in human pancreatic cancer cell line PANC-1, BER was able to reduce both the population of cancer cells and the proportion of CSCs. In addition, BER treatment down-regulated the expression of Nanog gene in the pancreatic cancer cell lines.[34] The results of the present study suggest that BER may either reduce Nanog gene expression or potentially interfere with Nanog function, ultimately enhancing the sensitivity of breast cancer cell line to DOX. These results show that the growth inhibitory effect of BER treatment on MCF-7 cells may partly be due to its effects on Nanog gene expression. More research is required to explore whether BER is a novel therapeutic drug

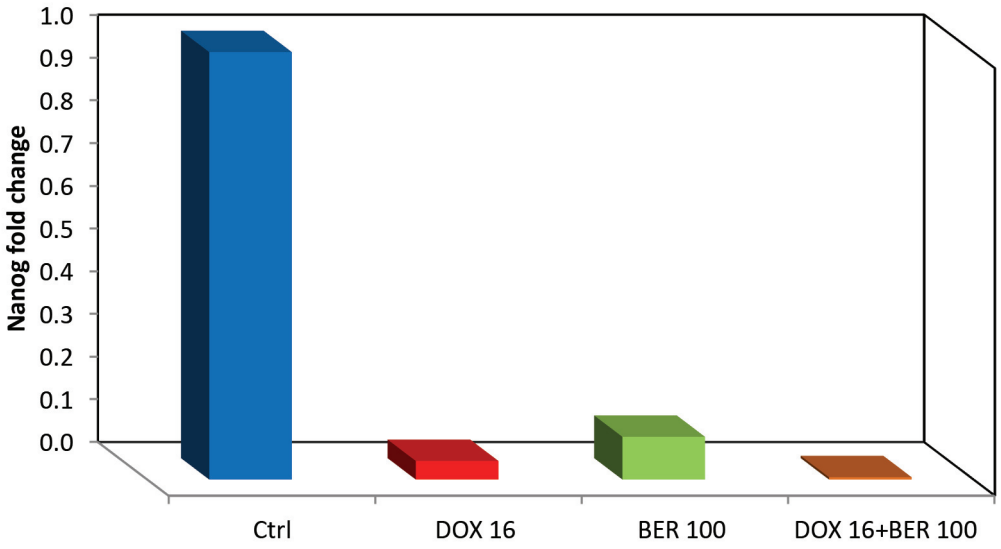

Figure 8. Bar chart representing the effect of DOX (16 µg/mL), BER (100 µM) and DOX (16 µg/mL)+BER (100 µM) treatment on Nanog gene expression in MCF-7 mammospheres (Ctrl=untreated control).

for targeting breast cancer stem cells.

Drug resistance and cancer recurrence remain major causes of death in patients with breast cancer.[35] Among various factors regulating cancer aggressiveness, miRNA-21 has been recently indicated as an important contributing factor. It was not only identified as one of the most up-regulated miRNAs in breast cancer,[36] but also shown to confer resistance to chemotherapy in various cancers. In several studies, the overexpression of miRNA-21 dramatically reduced the therapeutic efficacy of chemotherapy via down-regulating the expression of PTEN/Akt pathway.[37, 38] However, the inhibition of miRNA-21 reversed this effect, hence the reduction in oncogenicity.[37, 39]

The present results showed that miRNA-21 gene expression in MCF-7 mammospheres treated with DOX and/or BER at IC_{50} was significantly lower compared to untreated mammospheres. Moreover, miRNA-21 gene expression in mammospheres treated with either DOX or BER alone was nearly in the same range. Furthermore, miRNA-21 gene expression in mammospheres treated with combined DOX and BER was significantly lower than treatment with either DOX or BER alone.

Down-regulation of miRNA-21 expression by DOX is in agreement with a recent study; which reported that DOX down-regulated miRNA-21 expression, thereby up-regulating the protein expression of miRNA-21 target gene caspase-9 in MCF-7 human breast cancer cells.[22] Another recent study showed that the direct inhibitory effect of BER on oral squamous cell carcinoma cancer stem cells via targeting miRNA-21, attenuated tumor growth in vivo. Most importantly, BER potentiated chemotherapy through the down-regulation of miRNA-21 expression.[40] Reduced expressions of miRNA-21 may account for the significantly decreased percentage of viable cells following BER treatment. Consistent with these findings, we proposed that BER may affect the viability of cancer cells through the down-regulation of miRNA-21 expression.

We recommend that in vivo experiments be performed to corroborate the obtained results. Further work is needed to explore whether BER is a novel therapeutic drug for targeting breast cancer stem cells.

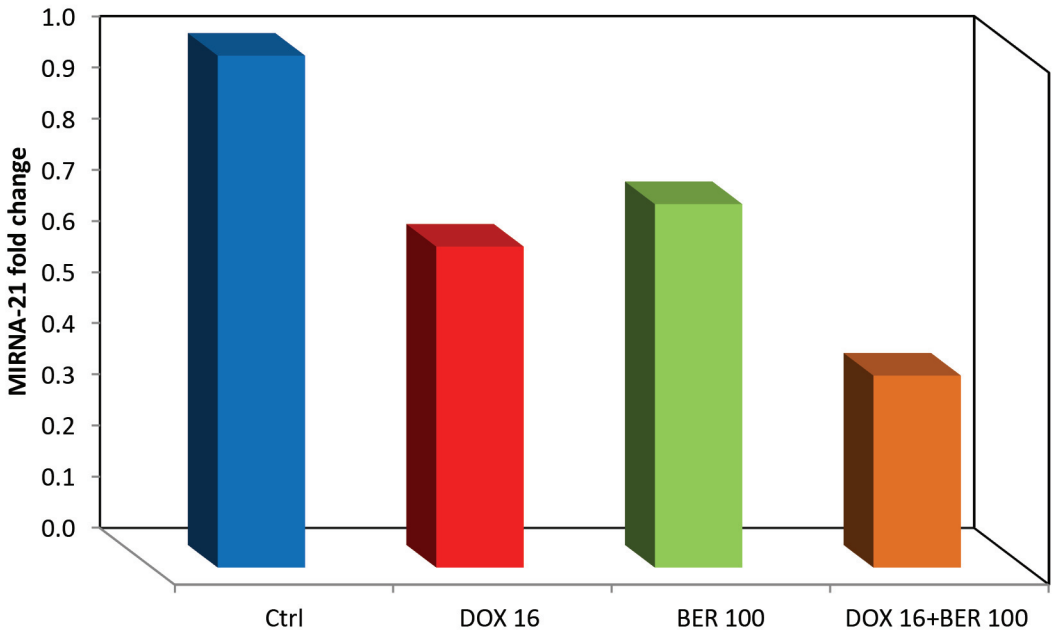

Figure 9. Bar chart representing the effect of DOX (16 µg/mL), BER (100 µM) and DOX (16 µg/mL)+BER (100 µM) treatment on the miRNA-21 gene expression in MCF-7 mammospheres.

Conclusion

From this study we may conclude the following:

a) Combination of DOX and BER generated synergistic anticancer effects on MCF-7 monolayer cells and MCF-7 mammospheres and BER may be an effective chemotherapeutic agent against breast cancer.

b) BER may affect the viability of breast cancer cells through the down-regulation of Nanog and miRNA-21 gene expression, ultimately enhancing the sensitivity of breast cancer cell line to DOX.

Conflicts of Interest

None declared.

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