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Original Article

Berberine and chemotherapeutic drugs synergistically inhibits cell proliferation and migration of breast cancer cells

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Abstract: Objective: Berberine is widely studied on the antitumor effects of breast cancer cells. However, combination therapy of berberine and chemotherapeutic drugs has not yet been completely clarified. In the present study, we explored the combination therapy of berberine and chemotherapy drugs in proliferation and migration of human breast cancer cells. Material and Method: The cell viability was determined by MTT assay in MCF-7 cells and MDA-MB-231 cells treated with berberine alone, or in combination with cisplatin or 5-Fu. Western blotting was performed to detect the levels of Bax, Bcl-2, caspase-3 and cleaved caspase-3. Transwell assay was performed to determine the migratory ability of MCF-7 cells treated with berberine, cisplatin, or berberine in combination with cisplatin. Results: MTT assay results showed berberine inhibited cell proliferation of human breast cancer cell lines MCF-7 and MDA-MB-231 in a dose-dependent manner. Co-treatment with berberine and cisplatin or 5-Fu significantly inhibited cell viability of MCF-7 cells than that with berberine or chemotherapeutic drugs alone. Western blotting results demonstrated that the level of cleaved caspase-3 obviously increased in MCF-7 cells treated with berberine in combination with cisplatin than the monotherapy alone. Moreover, the ratio of Bax/Bcl-2 was upregulated in the group of combination therapy with berberine and cisplatin than that with monotherapy alone. Transwell assay data showed that berberine in combination with cisplatin significantly decreased cell migratory ability of MCF-7 cells. Conclusion: Berberine showed synergistic effects in combination with chemotherapeutic drugs to remarkably inhibit cell proliferation and suppress cell migration of breast cancer cells.

Keywords: Berberine, chemotherapy drugs, apoptosis, migration

Introduction

Breast cancer is one of the most common female cancers with high incidence among urban than rural [1], which is a heterogeneous tumor and usually classified into various tumor subtype according to many biomarkers, such as estrogen receptor (ER), progesterone receptor (PR), p53, Ki-67 and human epidermal growth factor receptor type 2 (HER2) [2]. The appropriate classification for tumor subtype is needed on early diagnosis, prognosis and therapy for breast cancers. Surgery is one of the main treatment in the therapy of breast cancers, however, for the patients with large tumors or those are not suitable for surgery, preoperative chemotherapy, HER2 targeted therapy or endocrine therapy is helpful to reduce tumors [3]. Presently, chemotherapy is still an important method in breast cancer therapy, but multidrug resistance is a principal obstacle in the treatment of breast cancer [4, 5]. So, it is an urgent problem to minimize the toxicity and improve the treatment efficiency.

Berberine is a common isoquinoline alkaloid with formula of $C_{20}H_{18}NO_4$, which is the active compound extracted from traditional Chinese medicine Barberry and other plants [6, 7]. Pierpaoli, E. has reported that the anticancer effects of berberine and their synthetic analogs NAX012 and NAX014 possessed the greater effectiveness to induce cell apoptosis and senescence in HER-2/neu overexpressing tumor cell lines [8]. Ahmadiankia, N. found that berberine inhibited the distant metastasis of

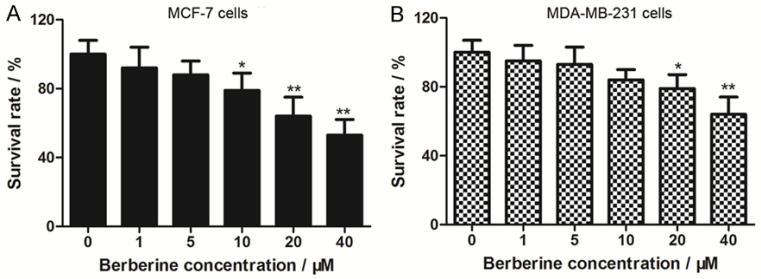

Figure 1. Berberine inhibits cell growth of MCF-7 cells and MDA-MB-231 cells. MCF-7 cells (A) and MDA-MB-231 cells (B) were plated into 96-well plate and cultured for 6 hours. The cells were treated with increasing concentration of berberine at the final concentration of 1 μM, 5 μM, 10 μM, 20 μM and 40 μM of berberine for 24 hours. Cell survival rate was shown in histogram. The data showed that cell survival rate was decreased as the increasing concentration of berberine (*p<0.05, **p<0.01, compared with untreated breast cancer cells). The MCF-7 cells (MDA-MB-231 cells) were used as negative controls being treated with 0 μM of berberine.

breast cancer cells, and they found berberine decreased cell migration by targeting chemokine receptor genes [9]. It has also reported that berberine decreased the metastatic potential of highly metastatic breast cancer cells by suppressing the levels of MMP2 and MMP9 through the Akt/nuclear factor kappa B (NF-kappaB) and activator protein-1 (AP-1) signaling pathways [10]. The effects of the concomitant administration of berberine and radiation was explored by Wang, J., in which they found berberine was a promising radiosensitizer for the treatment of breast cancer by inducing cell cycle arrest and downregulating homologous recombination repair protein, RAD51 [11]. One of the probable molecular mechanism of berberine was widely investigated and the growth inhibitory effects of berberine treatment on MCF-7 cells might be partly via effects on side population (SP) cells and ABCG2 expression [12].

However, the combination therapy of berberine with other antitumor drugs was little to be explored till now. A group of researcher found that a combination therapy of berberine with lapatinib, a novel tyrosine kinase inhibitor of HER2/EGFR, induced cell apoptosis of lapatinib-resistant cells suggesting treatment with berberine could overcome lapatinib resistance in HER2-positive breast cancers [13]. Barzegar, E. thought berberine alone or in combination with doxorubicin remarkably induced cell apoptosis, inhibited cell growth and altered cell cycle distribution of breast cancer cells [14]. In the present study, we explored the combination therapy of berberine and chemotherapeutic drugs, such as cisplatin and 5-Fu and further investigated the molecular mechanism of combination therapy of berberine with cisplatin in breast cancer cells.

Material and methods

Cell lines and agents

MCF7 (ATCC® HTB-22™) and MDA-MB-231 (ATCC® HTB-26™) were purchased from ATCC and kept in our laboratory. MCF-7 cells and MDA-MB-231 cells were cultured in DMEM medium containing 10% fetal bovine serum. The berberine was obtained from Sigma Inc. (Sigma, Saint Louis, MO) with purity of more than 98%.

MTT assay

Cell viability was determined by MTT assay. Briefly, breast cancer cells were plated into 96-well plate and cultured for 6 hours. The MCF-7 cells or MDA-MB-231 cells were treated with 1 μM, 5 μM, 10 μM, 20 μM and 40 μM of berberine for 24 hours. In other experiment, MCF-7 cells were plated into 96-well plate and treated with berberine in combination with cisplatin or 5-Fu for 24 hours. The breast cancer cells treated with 0.2% DMSO for 24 hours were used as negative controls. Four hours before test, 10 μL of 3-(4,5-cimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (5 mg/mL, MTT agent, Sigma) was added into the medium. Finally, the absorbance of each well in 96-well plate was read at 490 nm.

Western blotting

MCF-7 cells were plated into 48-well plate and treated with 5 μM of berberine, 10 μM of cisplatin or 5 μM of berberine in combination of 10 μM of cisplatin for 48 hours. The levels of caspase-3, cleaved caspase-3, Bax and Bcl-2 was determined by western blotting analysis as described [15-17]. The primary antibodies used in the experiment were shown as follows: anti-caspase-3 antibody (Cat. No. ab44976) or anti-active caspase-3 antibody (Cat No ab2302) was a rabbit polyclonal to caspase-3 and obtained from Abcam Co. BAX Antibody (N-term) (Cat. #AP13211a).

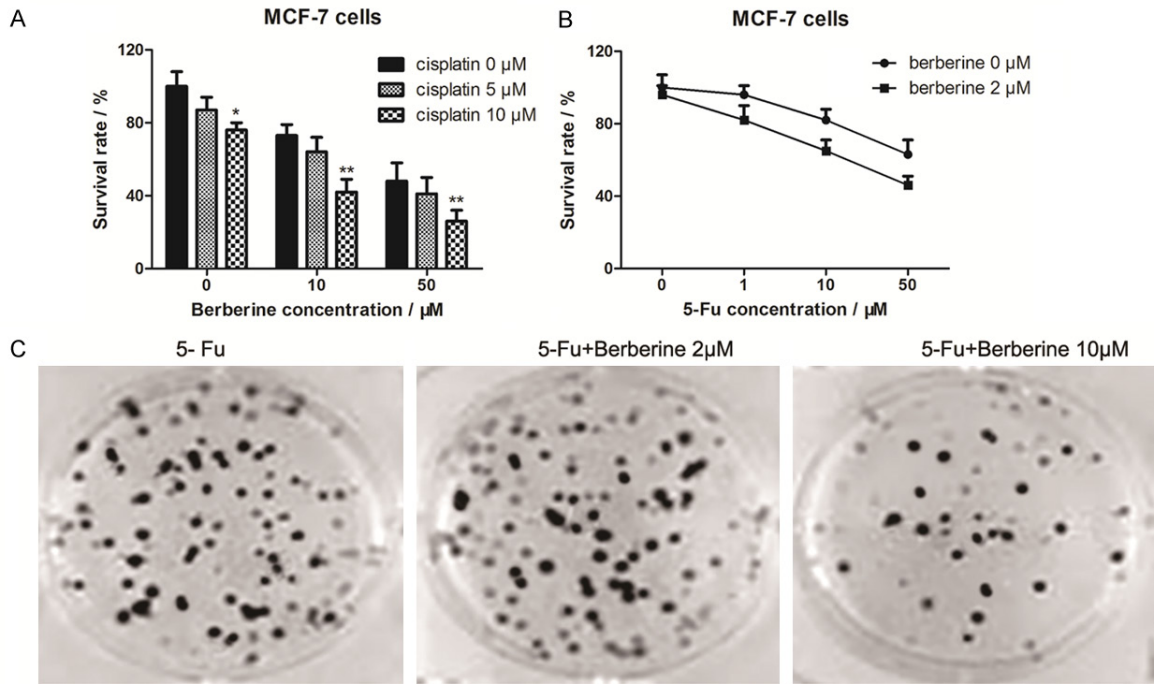

Figure 2. Berberine sensitizes the effects of chemotherapeutic drugs cisplatin and 5-Fu. A. MCF-7 cells were treated with berberine at the final concentration of 10 μM and 50 μM with or without 5 μM and 10 μM of cisplatin for 24 hours. B. MCF-7 cells were treated with 1 μM, 10 μM and 50 μM of 5-Fu with or without 5 μM of berberine for 24 hours. The cell viability was determined by MTT assay. $^{*}p<0.05$, $^{**}p<0.01$, compared with untreated MCF-7 cells. C. Colony formation assay. MCF-7 cells were plated into 6-well plate at the density of 100 cells per well. The cells were treated with 1 μM of 5-Fu, 1 μM of 5-Fu plus 2 μM of berberine or 10 μM of berberine, then cultured for 12 days. The colonies with diameter of more than 0.5 mm and containing more than 50 cells were recorded.

BAX Antibody (N-term) (Cat. #AP13211a) Bax antibody (N-term) (Cat. #AP13211a) and Bcl2 antibody (Center) (Cat. #AP19560c) was a purified rabbit polyclonal antibody and purchased from Abgent (Wuxi, China).

Transwell assay

Transwell assay was performed to detect the migration ability of breast cancer cells. MCF-7 cells (1×10^5 cells) were plated into millicells in 100 μl of serum-free medium and treated with 10 μM of cisplatin or 10 μM of berberine in combination of 10 μM of cisplatin. Then, 600 μl of medium containing 20% FBS was added into the bottom chambers as the chemotactic factor. After 24 hours, the migrated cells were fixed using methyl alcohol and stained using 0.1% crystal violet. The migratory MCF-7 cells were captured (original magnification × 200) using a light microscope.

Colony formation assay

MCF-7 cells were digested and plated into 6-well plate at the density of 100 cells per well. DMEM containing 1% FBS was added for 12 days of culture. The cells were treated with 1 μM of 5-Fu, 1 μM of 5-Fu plus 2 μM of berberine, and 1 μM of 5-Fu plus 10 μM of berberine. The colonies with diameter of more than 0.5 mm and containing more than 50 cells were recorded. The assay was repeated for three times.

Statistical analysis

The data was analyzed by SPSS statistical package (11.5, Chicago, IL, USA). The data was analyzed by used by independent samples t-test analysis and shown as mean ± standard error of the mean. The experiment was done twice and each sample was given three replicates. *P* values <0.01 were defined as significantly statistical difference.

Results

Berberine inhibits cell growth of MCF-7 cells and MDA-MB-231 cells

In order to test whether berberine inhibited cell viability of human breast cancer cells, we used

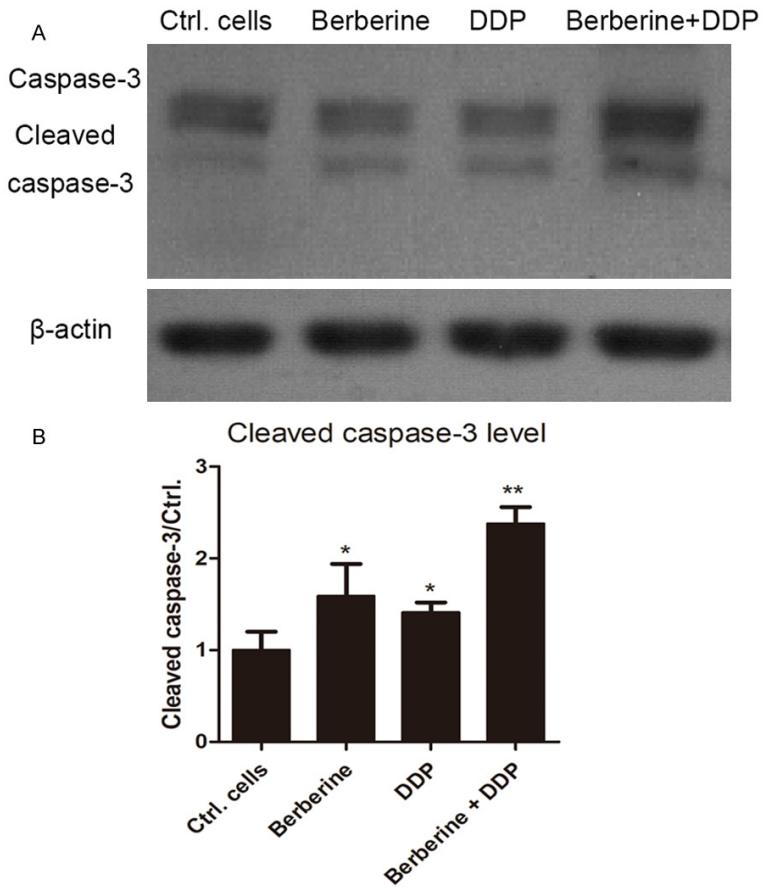

Figure 3. Berberine combinated with cisplatin promotes the activation of caspase-3. A. MCF-7 cells were treated with 5 μM of berberine, 10 μM of cisplatin or 5 μM of berberine in combination of 10 μM of cisplatin for 48 hours. The level of caspase-3 and cleaved caspase-3 were detected by western blotting analysis. Beta-actin was used as internal reference gene in MCF-7 cells. B. Histogram of cleaved caspase-3 expression was shown. *p<0.05, **p<0.01, compared with control cells.

different concentrations of berberine to treat breast cancer cell lines, such as MCF-7 and MDA-MB-231 for 24 hours. MCF-7 cells were treated with 1 μM, 5 μM, 10 μM, 20 μM and 40 μM of berberine for 24 hours. As shown in **Figures 1**, **2C**, the data showed that berberine suppressed MCF-7 cell survival rate and cell proliferation by colony formationand there was a statistical difference between untreated MCF-7 cells and 10 μM of berberine treated MCF-7 cells ($^{*}p<0.05$). Beberine treated MCF-7 cells for 24 hours at the concentration of 20 μM and 40 μM and the data showed beberine significantly inhibited survival rate of breast cancer cells compared with untreated MCF-7 cells ($^{**}p<0.01$). This effect was further confirmed in MDA-MB-231 cells, in which the survival rate of MDA-MB-231 cells was decreased as the increasing concentration of berberine, and there was a statistical difference between untreated cells and 20 μM and 40 μM of berberine treated MDA-MB-231 cells ($^{*}p<0.05$, $^{**}p<0.01$).

Berberine sensitizes the effects of chemotherapeutic drugs cisplatin and 5-Fu

Chemotherapeutic drugs are normally used in clinical therapy of breast cancer patients. In the present study, we used two chemotherapeutic drugs, cisplatin and 5-Fu to treat human breast cancer cells. We also further detected whether the combination of berberine and chemotherapeutic drugs effectively suppressed the cell proliferation of breast cancer cells. As shown in **Figure 2A**, MCF-7 cells were treated with berberine in combination with 5 μM and 10 μM of cisplatin for 24 hours. Cell viability was determined by MTT assay and the results showed 10 μM of cisplatin in combination with berberine significantly reduced cell survival rate of MCF-7 cells than that treated in berberine alone ($^{*}p<0.05$, $^{**}p<0.01$). Moreover, the increasing concentration of 5-Fu plus 5 μM of berberine obviously decreased the survival rate of MCF-7 cells compared with the cells treated with 5-Fu alone ($^{*}p<0.05$, $^{**}p<0.01$). This was confirmed by colony formation assay (**Figure 2C**). All the data revealed that berberine in combination with chemotherapeutic drugs remarkably suppressed cell proliferation of breast cancer cells and berberine could sensitize the effects of chemotherapeutic drugs.

Berberine combined with cisplatin increases the level of cleaved caspase-3

One method to kill cancer cells is to induce cell apoptosis and the obvious characteristic of cell apoptosis is caspase-3 activation. In order to determine whether berberine combined with

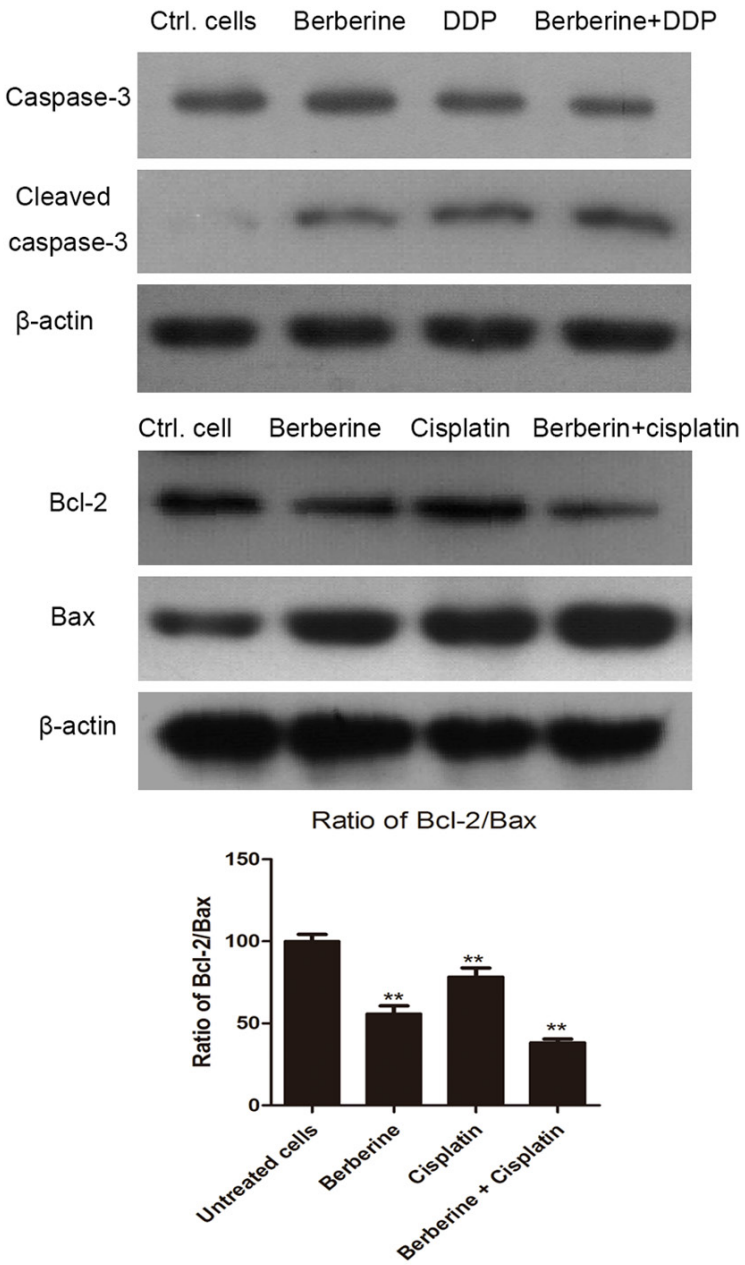

Figure 4. Berberine in combination with cisplatin increases Bax and decreases Bcl-2 expression. MCF-7 cells were treated with 5 μM of berberine in combination with 10 μM of cisplatin for 48 hours. The levels of Bcl-2 and Bax were detected by western blotting analysis. β-actin was used as internal reference gene here and untreated MCF-7 cells were used as negative control cells. $^{**}p<0.01$, compared with untreated cells.

cisplatin increased cell apoptosis than monotherapy, we use western blotting method to analyze the level of total caspase-3 and cleaved caspase-3 in MCF-7 cells. As shown in **Figure 3**, in untreated MCF-7 cells, there was no obvious cleaved caspase-3 to be detected by western blotting analysis. While, berberine in combination with cisplatin remarkably increased the level of cleaved caspase-3, compared with berberine or cisplatin treated MCF-7 cells.

Berberine in combination with cisplatin increases Bax and decreases Bcl-2 expression

In the Bcl-2 family, Bcl-2 and bax are the most important proteins to inhibit apoptosis and promote apoptosis, respectively. The ratio of bax to bcl-2 regulates the apoptosis of cancer cells. In the present study, 5 μM of berberine in combination with 10 μM of cisplatin obviously increased the levels of Bax and remarkably decreased the level of Bcl-2 in breast cancer cells (**Figure 4**), suggesting that berberine combined with cisplatin upregulated the ratio of Bax to Bcl-2 and promoted cell apoptosis of MCF-7 cells.

Berberine in combination with cisplatin decreases the invasive ability of MCF-7 cells by invasion assay

Furthermore, we also detected the migration and invasive ability of MCF-7 cells in cisplatin, berberine, cisplatin combinated with berberine treated group, the migration ability was determined by transwell assay. As shown in **Figure 5**, MCF-7 cells were treated with 10 μM of berberine, 10 μM of cisplatin or 10 μM of berberine in combination of 10 μM of cisplatin for 24 hours, and the resultsdemonstrated that the migration and invasive ability of MCF-7 cells was significantly inhibited in berberine combinated with cisplatin treated cells, compared with cisplatin or berberine treated MCF-7 cells, alone.

Discussion

Berberine is an active compound isolated from herbal plants such as *Berberis*, *Hydrastis*

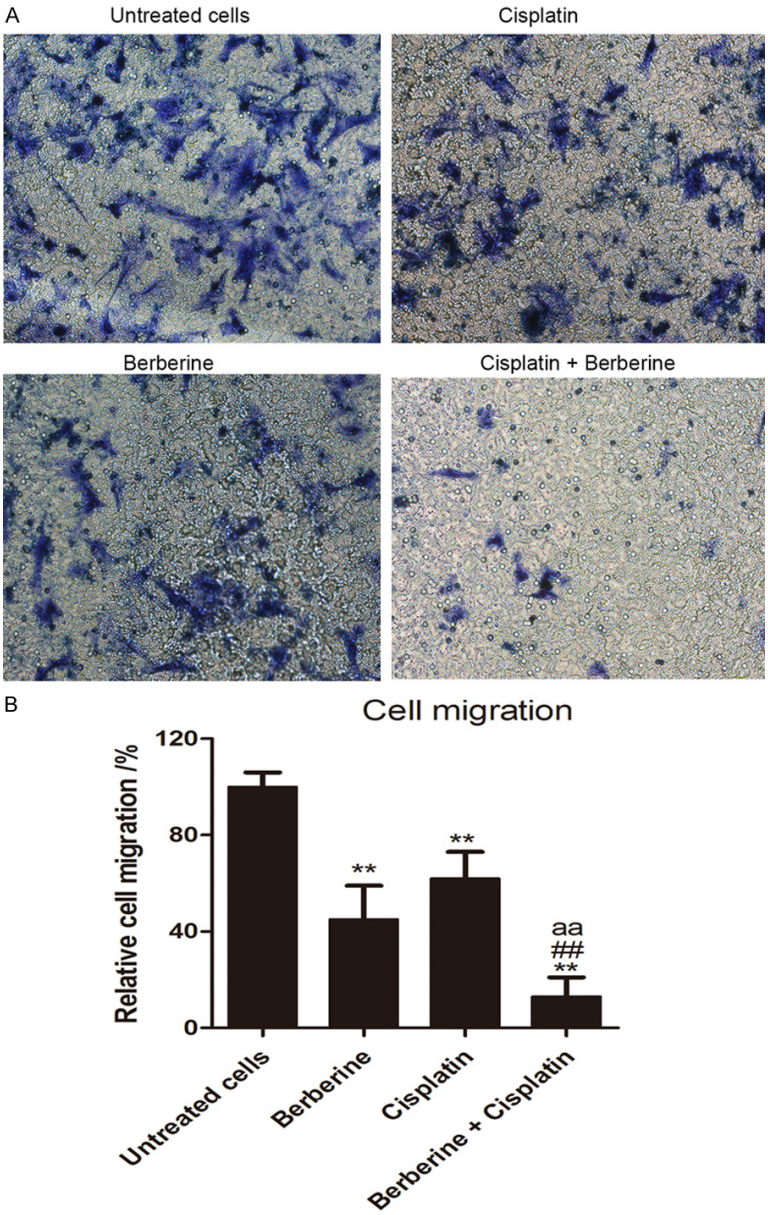

Figure 5. Berberine in combination with cisplatin decreases the invasive ability of MCF-7 cells. MCF-7 cells were cultured in DMEM medium containing 10% FBS and treated with 10 μM of berberine, 10 μM of cisplatin or 10 μM of berberine in combination of 10 μM of cisplatin for 24 hours. A. Transwell assay was performed to detect the invasive ability of MCF-7 cells in three groups. The data suggested berberine combination with cisplatin significantly inhibited cell invasive number of MCF-7 cells. B. The relative invasive ability (invasive cell number) was shown in histogram. Here, untreated MCF-7 cells were used as control cells. $^{**}p<0.01$, compared with untreated cells. $^{\#\#}p<0.01$, compared with berberine treated cells. $^{aa}p<0.01$, compared with cisplatin treated MCF-7 cells.

canadensis and *Coptis chinensis*. It could inhibit anoikis-resistant cells to a greater extent than doxorubicine by inducing cells cycle arrest at G0/G1 [6]. In the present study, we tested the antitumor effects of co-treatment of berberine and chemotherapeutic drugs in human breast cancer cells. The results revealed that lower level of berberine and cisplatin or 5-Fu significantly inhibited the cell proliferation of breast cancer cells, such as MCF-7 and MDA-MB-231. This was consistent with Wang, K.'s results and they found curcumin and berberine had synergistic chemopreventive effects on human breast cancer cells through induction of cell apoptosis and autophagy [18].

In the present study, we used two kinds of breast cancer cell lines MCF-7 and MDA-MB-231. Firstly, MCF-7 cells and MDA-MB-231 cells were treated with increasing concentrations of berberine for 24 hours and we found that cell viability was significantly decreased in breast cancer cells compared with untreated cells. This was consistent with the other studies in breast cancer cells [19, 20], suggesting berberine had the antitumor effects in breast cancer cells. Additionally, berberine had more anti-prolifeative activity in MCF-7 cells compared with that of MDA-MB-231 cells. Next, the antitumor effects of berberine and chemotherapeutic drugs, such as cisplatin and 5-Fu, alone and in combination were investigated in breast cancer cells MCF7 using MTT cytotoxicity assay. The results revealed that co-administration with berberine and cisplatin or 5-Fu increased the anti-tumor activity in MCF-7 cells more significantly than in MCF-7 cells. The data suggested that berberine and chemotherapeutic drugs, such as cisplatin, had synergistic antitumor effects in human breast cancer cells.

The molecular mechanism of berberine and cisplatin was investigated in human breast cancer cells. We selected an appropriate concentration for berberine and cisplatin to treat human breast cancer cells. The western blotting analysis results showed that cisplatin in combination with berberine increased the level of cleaved caspase-3 and Bax, but the expression of Bcl-2 was obviously decreased. All the results revealed that cisplatin in combination with berberine promoted cell apoptosis of breast cancer cells than cisplatin alone.

Furthermore, transwell assay was performed to detect the metastatic potential of breast cancer cells treated with cisplatin, berberine, or cisplatin in combination with berberine. Our findings showed that both 10 μM of cisplatin and 10 μM of berberine suppressed cell migration of MCF-7 cells. While, berberine in combination of cisplatin would more effectively inhibit cell invasion and migration of human breast cancer cells. Kim, S. found berberine inhibited and decreased TNF-alpha-induced matrix metalloproteinase-9 (MMP-9) expression and cell invasion through suppressing AP-1 DNA binding activity in MDA-MB-231 human breast cancer cells [21]. We speculated that synergistic effects of berberine and cisplatin on the inhibition of breast cancer cells might contribute to the decreased level of MMP-2 and MMP-9. This would be further confirmed in near future.

In conclusion, berberine in combination with chemotherapeutic drugs remarkably inhibited cell proliferation and suppressed the cell migration of breast cancer cells, which would be helpful to minimize the dosage of chemotherapeutic drugs and decrease the toxicity of chemotherapeutic drugs. The research provides some new clues for the treatment of human breast cancers.

Disclosure of conflict of interest

None.

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