

Original Article

Matrine inhibited proliferation and increased apoptosis in human breast cancer MCF-7 cells via upregulation of Bax and downregulation of Bcl-2

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Abstract: Purpose: The aim of the present study was to investigate the effects of matrine on proliferation and apoptosis in human breast cancer MCF-7 cells and its relevant molecular mechanisms. Methods: Breast carcinoma cell line MCF-7 was cultured with series concentrations of Matrine in vitro. The proliferation and apoptosis of MCF-7 cells were investigated by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, flow cytometry, and Mitochondrial membrane potential (MMP) measurements. The expression levels of Bax and Bcl-2 proteins were detected by Annexin V/propidium iodide coupled staining. The morphological changes of MCF-7 cell were examined. Results: The inhibition rates of MCF-7 cells were 6.01%-37.01%, 7.56%-53.92%, and 10.86%-70.23% for 24, 48, and 72 hours after Matrine treatment, respectively. The proliferation of MCF-7 cells was significantly inhibited by Matrine administration, with a time and dose dependent manner. The rates apoptotic cells was between $4.17\pm 0.25\%$ and $19.63\pm 0.17\%$ in 0.25-2.0 mg/ml Matrine groups, which had significant increased compare with the control groups ($1.10\pm 0.08\%$, $P<0.05$). Meanwhile, increased Bax expression, but decreased Bcl-2 expression was observed in MCF-7 cell line. MMP were significantly decreased by Matrine treatment. Conclusions: Matrine significantly inhibited the growth and induced apoptosis in breast carcinoma MCF-7 cells, which is related to Bax, Bcl-2 signaling and MMP.

Keywords: Matrine, breast cancer, proliferation, apoptosis, Bax, Bcl-2, mitochondrial

Introduction

Breast cancer is the most common female cancer worldwide [1]. Meanwhile, some breast cancers develop multidrug resistance to chemotherapies rapidly, and acquired multidrug resistance severely blocks the effective therapies for breast cancer [2, 3]. Recently, numerous phytochemicals compounds and active alkaloids have been reported to have cancer preventive activity [4-6]. Matrine, a kind of effective components found in the traditional herb medicine *Sophora flavescens*, has many pharmacological activities and is confirmed to have anti-inflammatory, anti-virus, anti-fibrotic and immune regulations [7, 8]. Recently, matrine has been shown to have anti-tumor activity in various cancer cells, including hepatocellular carcinoma, lung cancer, gastric carcinoma and colorectal cancer [9-13]. Many studies have

indicated that matrine inhibits the proliferation and transfer of tumor cells, inducing apoptosis and differentiation. But so far, there are few reports investigated the mechanisms of anti-breast cancer of matrine [14-16]. In order to provide the basis of the development of Matrine, the influence of Matrine was tested in the human breast cancer MCF-7 cell line for cell viability (MTT assay) and flow cytometry detection.

Materials and methods

Materials and reagents

Human breast cancer cell line MCF-7 was obtained from the Life Science Research Center of Hebei North University. Matrine was purchased from Beijing Shuanglu Pharm Ltd. Matrine was dissolved in RPMI 1640 (KeyGEN

Effects of Matrine on MCF-7 cells

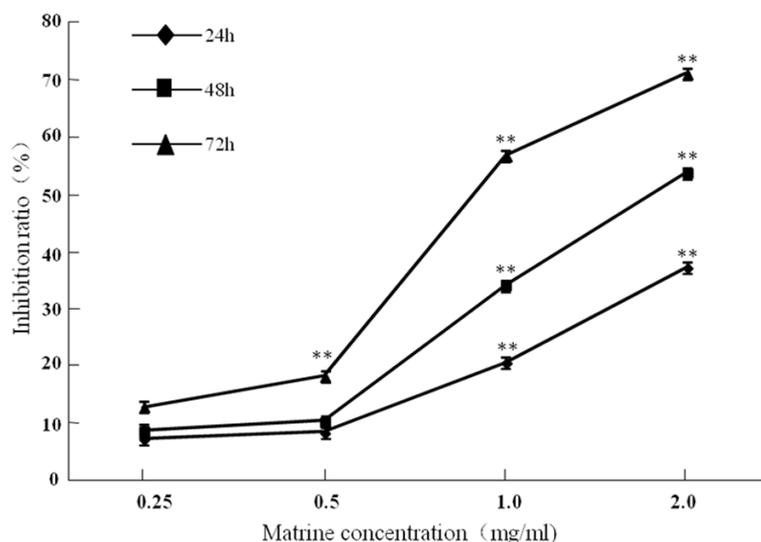


Figure 1. The inhibition ratio of MCF-7 cells treated with Matrine in MTT assay. ** $P < 0.05$ compare to 0.25 mg/ml Matrine groups.

Table 1. Growth inhibition effects of Matrine on MCF-7 cells (%)

Duration	Concentration of Matrine (mg/ml)			
	0.25	0.5	1.0	2.0
24 h	6.61±0.50	7.67±0.64	19.50±2.12 ^{a,b}	35.58±1.38 ^{a,b,c}
48 h	7.97±0.41	9.61±0.67	32.56±1.60 ^{a,b}	51.56±1.72 ^{a,b,c}
72 h	11.98±1.11	17.14±1.21 ^a	54.46±2.93 ^{a,b}	68.31±2.13 ^{a,b,c}

a: Compared with 0.25 mg/ml groups, $P < 0.05$; b: Compared with 0.5 mg/ml groups: $P < 0.05$; c: Compared with 1.0 mg/ml groups: $P < 0.05$.

BioTECH, Beijing), stored at -20°C at a concentration of 20 mg/ml, and then diluted with culture medium before using. 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) and Annexin V-fluorescein isothiocyanate (FITC) Detection kit were purchased from Beijing BIOSS biological technology Ltd. The antibodies FITC-Bax and PE-Bcl-2 were obtained from BD Biosciences (NJ, USA).

Cell viability assay

The MCF-7 cells were grown in RPMI 1640 containing with 10% heat-inactivated fetal bovine serum (FBS) and 100 U/ml gentamicin-streptomycin. The cells were incubated in a stable environment with 5% CO_2 at 37°C in a humidified incubator. The medium was replaced every 24 hours. Cells were grown to about 80% confluence prior to Matrine treatment, and then exposed to Matrine at different concentrations (0.25, 0.5, 1.0, 2.0 mg/ml). Cells grown in a medium containing an equivalent volume of

1640 without Matrine served as control. Each groups of cells were seeded in 96-well microtiter plates and incubated for 24, 48, 72 hours. At different time points, 20 μl of MTT was added to each well followed by 4 hours incubation. The medium was discarded and 150 μl of DMSO was added into each well, and incubated for 20 min. The OD (optical density) 492 nm was measured. The proliferation inhibition rate was calculated as: $(1 - \frac{\text{OD of the experimental group}}{\text{OD of the control group}}) \times 100\%$. Each experiment was repeated three times.

Determination of apoptosis

MCF-7 cells were collected and centrifuged at $1,000 \times g$ for 5 min and re-suspended in fresh RPMI-1640 medium at a density of 2×10^5 cells/ml. Apoptotic and necrotic cells were evaluated by Annexin V (AV) binding and propidium iodide (PI) uptake. Samples

were analyzed by flow cytometry. Each of the concentrations was repeated three times, the final result is expression as the mean of three times.

Detection of mitochondrial membrane potential

The levels of mitochondrial membrane potential (MMP) were determined by flow cytometry after cells staining with Rhodamine 123 (Rh123). Briefly, the MCF-7 cells were seeded in Six-well plates at a density of 1×10^6 cells/well, following Matrine treatment, MCF-7 cells were collected and incubated in PBS containing 10 $\mu\text{g}/\text{ml}$ Rh123 for 20 min at 37°C . Then the cells was centrifuged at $1000 \times g$ for 5 min, cell sample was washed and re-suspended in PBS for flow cytometric assay.

Measurement of Bax and Bcl-2 protein

MCF-7 cells were digested by pancreatic enzyme and seeded into six-well plates with cover

Effects of Matrine on MCF-7 cells

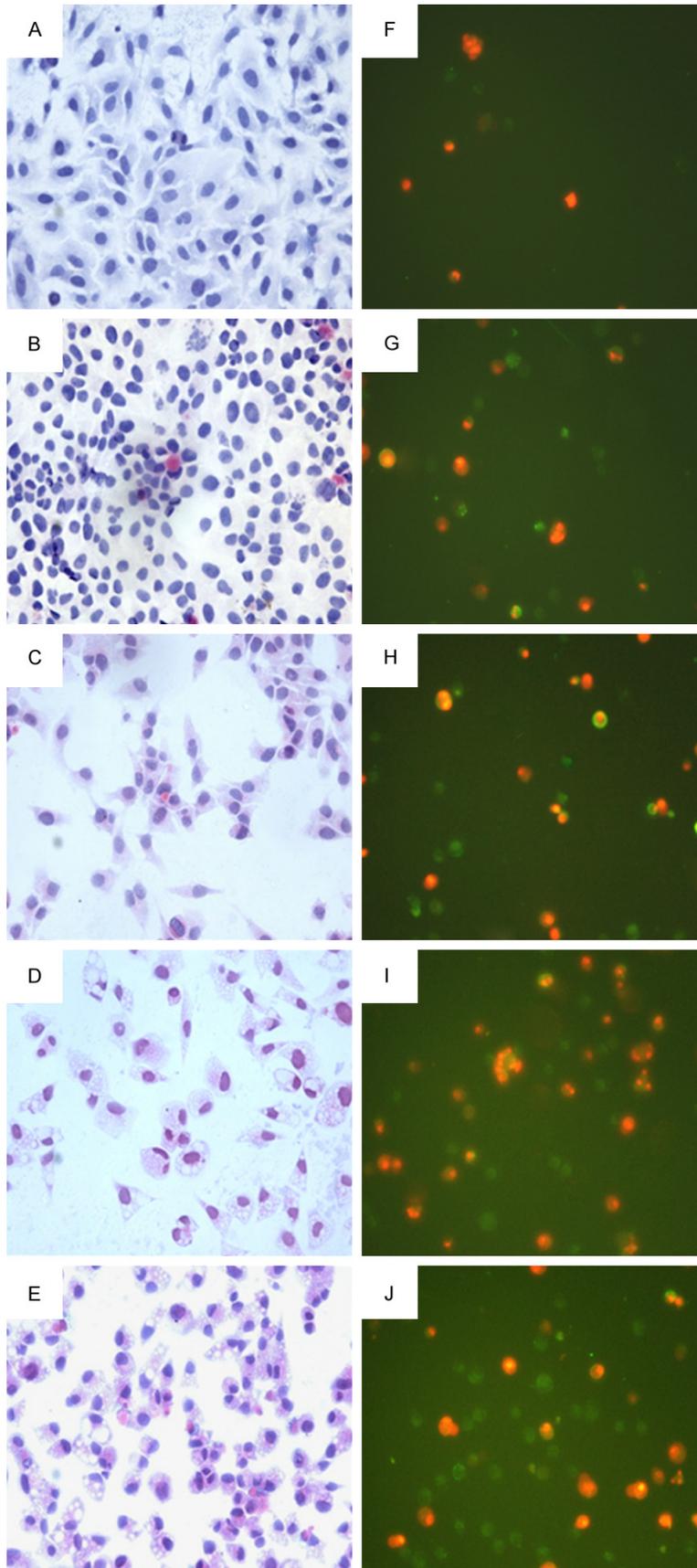


Figure 2. Morphological changes of MCF-7 cells treated with different concentrations of Matrine under H&E staining (A-E) and fluorescence staining (F-J). Note MCF-7 cells have showed shrinkage, rounding, partial detachment, and increased of the suspension dead cells. ($\times 100$). (A, F) negative controls; (B, G) 0.25 mg/ml; (C, H) 0.5 mg/ml; (D, I) 1.0 mg/ml; (E, J) 2.0 mg/ml Matrine.

glasses, cultured for 2-3 days, treated with different concentrations of matrine for 24 h, collected, fixed with 4% paraformaldehyde, stained HE, dehydrated, sealed pieces. MCF-7 cells were treated with various concentrations of Matrine for 24 and 48 hours, digested, washed with PBS, centrifuged three times. The FITC-Bax (100 μ l) and PE-Bcl-2 (10 μ l) were added to each sample. The experimental and control cells were incubated in dark at 4°C for 30 min, then, determined by flow cytometry. The experiments were performed in triplicate. The relative content of protein is IF: (the protein fluorescence intensity of sample- the average fluorescence intensity of normal sample)/the average fluorescence intensity of normal sample. FI >1.0 was judged to be positive expression, FI ≤ 1.0 was determined to be negative expression.

Statistical analysis

All data were expressed as the Mean values \pm standard deviation (SD). Comparisons were made with an one-way analysis of variance (ANOVA) using SPSS software (version 11.0). $P < 0.05$ was considered statistically significant.

Effects of Matrine on MCF-7 cells

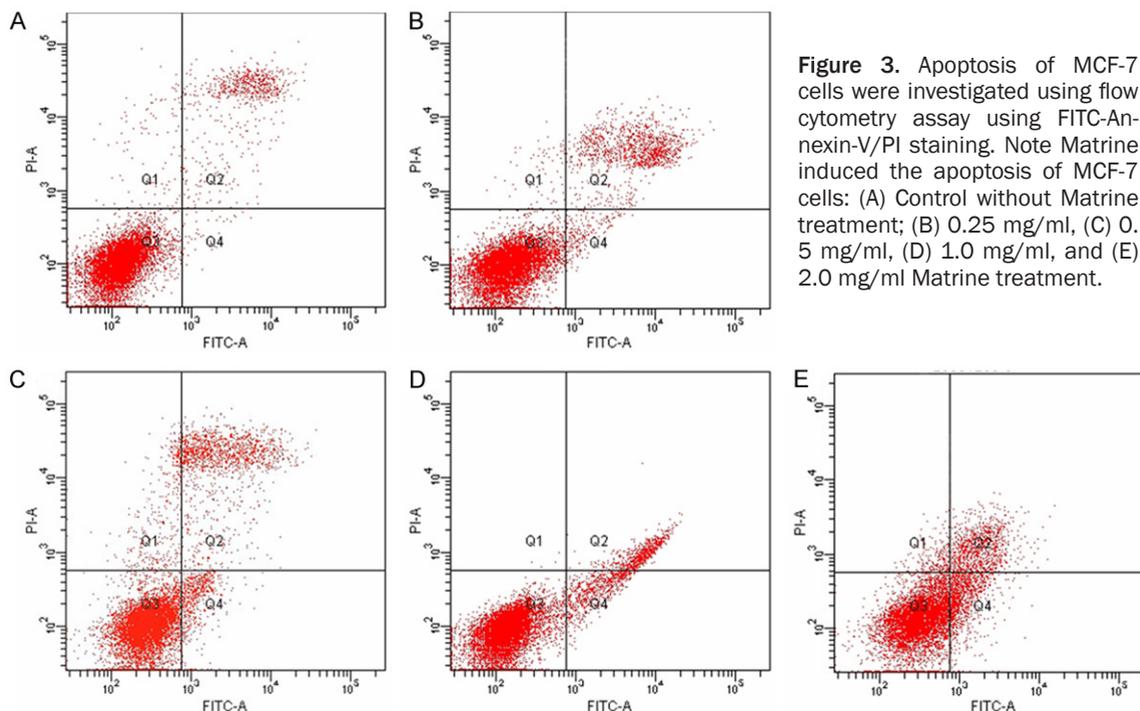


Figure 3. Apoptosis of MCF-7 cells were investigated using flow cytometry assay using FITC-Annexin-V/PI staining. Note Matrine induced the apoptosis of MCF-7 cells: (A) Control without Matrine treatment; (B) 0.25 mg/ml, (C) 0.5 mg/ml, (D) 1.0 mg/ml, and (E) 2.0 mg/ml Matrine treatment.

Table 2. The results of mitochondrial transmembrane potential

Rho123 dyeing	Control group	Concentration of Matrine (mg/ml)			
		0.25	0.5	1.0	2.0
Positive staining cells (%)	83.00±1.42	66.03±2.08 ^a	61.28±1.29 ^{a,b}	60.55±0.87 ^{a,b}	56.30±1.87 ^{a,b,c}

^aCompare to control groups, $P < 0.05$; ^bCompare to 0.25 mg/ml groups, $P < 0.05$; ^cCompare to 0.5 and 1.0 mg/ml groups, $P < 0.05$.

Table 3. FI value of Bax and Bcl-2 expressions (n=3)

Groups	Matrine concentration (mg/ml)				
	0 (Control)	0.25	0.5	1.0	2.0
24 h Bax	1.03±0.05	1.80±0.12 ^a	2.13±0.18 ^{a,b}	4.30±0.08 ^{a,b,c}	8.93±0.24 ^{a,b,c,d}
48 h Bax	1.04±0.05	2.15±0.13 ^a	4.73±0.17 ^{a,b}	9.46±0.64 ^{a,b,c}	16.03±0.62 ^{a,b,c,d}
24 h Bcl-2	1.05±0.04	0.99±0.06	0.66±0.12 ^{a,b}	0.44±0.13 ^{a,b,c}	0.17±0.05 ^{a,b,c,d}
48 h Bcl-2	1.02±0.03	0.76±0.18 ^a	0.59±0.23 ^{a,b}	0.20±0.31 ^{a,b,c}	0.04±0.15 ^{a,b,c,d}

a: Compared with the control groups: $P < 0.05$; b: Compared with 0.25 mg/ml matrine groups: $P < 0.05$; c: Compared with; 0.5 mg/ml matrine groups: $P < 0.05$; d: Compared with 1 mg/ml matrine groups: $P < 0.05$.

Results

Inhibition effects of Matrine on MCF-7 cells

The maximum cell inhibition rates were 6.01%-37.01%, 7.56%-53.92%, and 10.86%-70.23% for 24, 48, and 72 hours after Matrine treatment, respectively. The results have showed that cell inhibition rate was significantly increased ($P < 0.05$), except 0.25 and 0.5 mg/

ml matrine concentration for 24 and 48 h. The results of MTT assay also showed that inhibitory effects on the proliferation of Matrine in MCF-7 cells were dose-dependent (**Figure 1; Table 1**). After 24 hours of Matrine treatment, MCF-7 cell showed significantly changes in morphology, including shrinkage, rounding, partial detachment, and proliferation inhibition and increase of the suspension dead cells (**Figure 2**).

Effects of Matrine on MCF-7 cells

Effects of Matrine on apoptosis

In the experimental group, the numbers of apoptotic cells significantly increased with drug concentrations. Nuclear fragmentation and petal-like arrangement were observed at various concentrations, which were considered as one of apoptotic body formation process (**Figure 3**). After 24 hours Matrine treatment, the incidences of early apoptotic cells were $4.17 \pm 0.25\%$, $6.60 \pm 0.18\%$, $15.32 \pm 0.21\%$, $19.63 \pm 0.17\%$, at the concentrations of 0.25, 0.5, 1.0, and 2 mg/ml, respectively. Compare to apoptotic rate of control groups ($1.10 \pm 0.08\%$), Matrine treatment significantly promoted apoptosis of MCF-7 cells ($P < 0.05$). Furthermore, the incidence of apoptosis was also increased with concentration of Matrine (**Figure 3**).

Mitochondrial transmembrane potential

The changes in the number of cells with positive staining of rhodamine reflect the alterations of mitochondrial membrane potential. The rates of rhodamine-positive cells were significantly decreased in the Matrine treated groups compare to the control groups ($P < 0.05$). It means that mitochondrial membrane potential (MMP) was significantly lower in the Matrine groups than those in the control group ($P < 0.05$). In addition, changes of MMP were Matrine concentration dependent, and in consist with rates of apoptosis in cells (**Table 2**).

Effects of Matrine on Bax and Bcl-2 protein

In order to examine the characteristics of Bax and Bcl-2 expressions in Matrine treated MCF-7 cells, FCM analysis was employed. After Matrine treatment, The FI values were 1.80 ± 0.12 , 2.13 ± 0.18 , 4.30 ± 0.08 , and 8.93 ± 0.24 for 24 hours, 1.04 ± 0.05 , 2.15 ± 0.13 , 4.73 ± 0.17 , 9.46 ± 0.64 , and 16.03 ± 0.62 for 48 hours, respectively. They were significantly increased than those in control groups (1.03 ± 0.05 for 24 h and 1.04 ± 0.05 for 48 hs, $P < 0.05$). Inversely, expressions of Bcl-2 were decreased after treatment by different concentrations of Matrine (**Table 3**).

Discussion

Recently, many anti-cancer drugs are derived from natural resources [6, 17]. Many traditional

plant medicines, such as active alkaloids, have showed the some potential effects of anti-tumors. Matrine is kinds of alkaloid components found in the roots of Sophora species. Matrine was considered have anti-inflammatory and anti-virus effects. The current experiment explored the possible mechanism of Matrine against breast cancer MCF-7 cells.

In this study, cell viability was measured using the MTT assay. The growth of MCF-7 cells was significantly inhibited in a dose and time dependent manner when treated with Matrine. The result is consistent with previous study [14]. The morphological changes also demonstrated the cytotoxic effects of Matrine on MCF-7 cells. These changes indicated eosinophilic cytoplasm was increased with treatment of Matrine, thus, we speculate that Matrine may inhibited the proliferation of MCF-7 cells via cytoplasm and nucleic acid metabolism of breast cancer cells in vitro.

Then, we focus on the effects of Matrine on apoptosis whether via Bax/Bcl2 signaling in current studies. Present results have indicated Matrine could up-regulate expression of Bax protein and down-regulate Bcl-2 protein in MCF-7 cells. Apoptosis is one of the most important topics in the field of biology and medicine. Many chemotherapy drugs and natural active products are proved to have anti-cancer potential by induce apoptosis of cancer cell [18, 19]. It is one of the important reference index assessed the clinical application and development of Matrine [20-22]. Bcl-2 and Bax are the important members of the Bcl-2 gene families. They take an important part in the functional regulation of cell apoptosis [23]. Bcl-2 is one of the apoptosis control genes. Studies have shown that Bcl-2 can maintain normal cells and prevent the occurrence of apoptosis through the following channels [24]. Bax is a kind of protein that can promote tumor cell apoptosis. Bax in the form of monomer can shift to the mitochondrial membrane, form polymer or highly oriented oligomer, cause cell death through the mitochondrial permeability transition pore (MPTP). However, some researchers believe p53 can induce the expression of apoptosis proteins and have the ability to start MPTP. As p53 transcriptional target, Bax may be one of the important mediators in p53 dependent apoptosis signaling pathways [25].

Mitochondrial membrane potential (MMP) is an indicator of mitochondrial function which is related with mitochondrial membrane permeability. After treatment with Matrine in different concentrations, MMP decreased significantly compared with the control group in MCF-7 cells ($P < 0.05$). Matrine may influence the structure of MMP channel, leading MMP channel opening, triggering apoptosis. Together, our findings suggested that Matrine activated apoptosis in breast cancer cells was modulated by the mitochondrial and Bax/Bcl-2 pathway.

In conclusion, Matrine promoted the apoptosis of breast cancer MCF-7 cells through up-regulation of Bax and down-regulation of Bcl-2 expression. Furthermore, Matrine also inhibited the proliferation of MCF-7 cells. These findings suggest that Matrine has potential to be developed as a new natural anti-gastric carcinoma agent. It is considered multifactor involved in this is complex process, and need to investigate in future study [26, 27].

Disclosure of conflict of interest

None.

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Effects of Matrine on MCF-7 cells

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