

Original Article

Effects of matrine on the proliferation and apoptosis of myeloma RPMI 8226 cells

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Received January 10, 2016; Accepted March 22, 2016; Epub June 1, 2016; Published June 15, 2016

Abstract: Objective: To investigate the effects of matrine on the proliferation and apoptosis of RPMI 8226 cells *in vitro*. Methods: RPMI 8226 cells were cultured *in vitro*, in the presence of different concentrations of matrine. The effect of matrine at different concentrations on the proliferation and apoptotic rate of RPMI 8226 cells was detected by MTT assay and flow cytometry. The mRNA expression of cyclooxygenase-2 (COX-2) was determined by reverse transcription polymerase chain reaction (RT-PCR) while the expression level of COX-2 protein was evaluated by Western blotting. Results: Matrine inhibits the proliferation and induces apoptosis of RPMI 8226 cells in a time- and concentration-dependent manner. In addition, it induced COX-2 mRNA expression and decreased COX-2 protein expression in RPMI 8226 cells. Conclusion: Matrine can inhibit the proliferation and induce apoptosis of RPMI 8226 cells possibly via the downregulation of COX-2 gene as one of the mechanisms.

Keywords: Matrine, myeloma, cell proliferation, cell apoptosis, COX-2

Introduction

Multiple myeloma (MM) is a type of malignant plasmacytic disease affecting people mostly older than 40 years, and its tumor cells are derived from bone marrow plasmacytes. MM is characterized by abnormal proliferation of bone marrow plasmacytes accompanied by the overproduction of monoclonal immunoglobulins or light chains (M protein); a small portion of MM patients might have non-secretory MM without M protein production. MM is often accompanied by multiple osteolytic lesions, hypercalcemia, anemia, and kidney damage. Patients are prone to a variety of bacterial infections due to the suppressed production of normal immunoglobulins. Because MM predominantly affects adults older than 40 years, most MM patients are not able to tolerate the standard dose of chemotherapy and complete bone marrow transplantation, and thus, their five-year survival rate is extremely low. The incidence rate of MM is rising gradually and has attracted an increasing amount of attention in recent years. Currently, MM patients outside China are main-

ly treated with bortezomib, lenalidomide, and other new drugs as well as bone marrow transplantation [1]. However, most patients are unable to afford new MM drugs owing to their high cost. Meanwhile, bone marrow transplantation is not widely applied because of the generally poor physical condition of MM patients who mostly have a late disease onset age and the difficulties in finding suitable donors. Therefore, it is important to develop new drugs and treatment approaches to prevent and treat MM, thus improving the survival rate of patients. Taking advantage of Chinese traditional medicine, the medical field has turned its attention to screening natural agents for antitumor activities, which could be further developed into chemopreventive drugs for MM [2].

Application of natural agents in myeloma treatment has been explored extensively in recent years. A variety of natural agents including *Sophora* were found to have antitumor activities against MM. Using RPMI 8226 MM cells as a cellular model in this study, we investigated the role of matrine in inhibiting cell proliferation

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and inducing apoptosis, with an aim of providing new theoretical and experimental basis for the treatment of MM.

Materials and methods

Main materials and reagents

Human MM cell line RPMI 8226 was purchased from the Institute of Hematology, Tongji Medical College, Huazhong University of Science and Technology. Matrine (purity 85.63%) was extracted from *Sophora* at the Department of Medicinal Chemistry, and verified by the Department of Chinese Medicine Authentication at the School of Chinese Pharmacy, Chengdu University of Traditional Chinese Medicine. Dimethylsulfoxide (DMSO) was purchased from Sigma. RPMI 1640 medium and trypsin were purchased from Gibco. Methyl thiazolyl tetrazolium (MTT) was purchased from Zhuhai Libao Biotech Co. Ltd. and 10% fetal bovine serum (FBS) was purchased from Hangzhou Sijiqing Biotech Co. Ltd. Annexin V-FITC kits were obtained from COULTER (US).

Methods

Cell culture: Adherent RPMI 8226 cells were cultured in 10% FBS containing RPMI 1640 medium in a 5% CO₂ incubator at 37°C.

MTT colorimetric assay: The cells in the logarithmic growth phase (4-5 d) were digested with V-T solution (containing 2.0 g/L trypsin and 0.5 mmol/L EDTA). The density of the cell suspension was adjusted to 4×10⁸ cells/L, and then the cells were seeded into 96-well culture plates with 50 µL of suspension for each well. After 24 h of incubation, the culture medium was replaced with fresh medium containing matrine at a final concentration of 250, 500, 1000, or 1500 mg/L, with five replicate wells for each concentration and three independent repeats for each experiment. Cells without matrine treatment served as the negative control group, while wells without cells served as the blank control. MTT solution (10 µL; 5 g/mL) was added to each well after 24 h and 48 h, respectively. The reaction in each well was terminated by adding 100 µL DMSO after 4 h of incubation. The plates were shaken at 37°C for 30 min to dissolve the crystals, and the absorbance of each well at wavelength 570 nm (A) was recorded using a microplate reader. Inhi-

tion rate of tumor cell growth (%) was calculated as $[A_{\text{control}} - A_{\text{matrine}}]/A_{\text{control}} \times 100\%$.

Flow cytometry: RPMI 8226 cells (5×10⁸ cells/L) were cultured in flasks for 3 days before its culture medium was discarded. Matrine was added at final concentrations of 250, 500, 1000, and 1500 mg/L, and negative controls were set up simultaneously. Cells were collected separately after 24 h and 48 h, and washed with cold PBS (centrifuged at 4°C, 500 g, 5 min). After removing the supernatant, cell staining was performed with Annexin V-FITC kit according to the manufacturer's instructions. After the cell density was adjusted to 10⁵-10⁶ cells/mL with ice-cold binding buffer, the cell suspension was added with Annexin V (a type of phospholipid-binding protein) and propidium iodide (PI) and then subjected to flow cytometry (COULTER, USA) for cell apoptosis detection within one hour.

Determination of cyclooxygenase-2 (COX-2) mRNA using RT-PCR: RPMI 8226 cells treated with 1500 mg/L matrine for 48 h as well as the negative control cells were harvested. The sequences of the primers were as follows: COX-2 forward primer: 5'-ATCCTTGCTGTTCCCA-3'; COX-2 reverse primer: 5'-CTTTGACACCCAAGGGAGTC-3'; the length of the amplified target fragment was 386 bp. The length of the internal reference GAPDH fragment was 321 bp. Total RNA was extracted from each group of cells using the Trizol method, followed by cDNA production by reverse transcription using RT-PCR kit. An appropriate amount of cDNA was added with target gene primers for PCR reaction to generate DNA amplification products, which allowed semi-quantitative determination of the target gene mRNA expression. The remaining cDNA was stored at -20°C. In this study, the total volume of the PCR reaction system was 25 µL, and the reaction conditions were as follows: pre-denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 30 s, annealing for 30 s (the annealing temperature for the internal reference GAPDH was 53°C, while that for COX-2 was 55°C), and extension at 72°C for 1 min, followed by a final extension of 72°C for 7 min. The experimental data of optical density (OD) ratio of COX-2/GAPDH DNA was calculated. Each experiment was conducted in triplicates and the average value was used as the final outcome.

Effects of matrine on RPMI 8226 cells

Table 1. Effect of matrine on proliferation of RPMI 8226 cells

Matrine concentration (mg/L)	Inhibition of cell proliferation (%)			
	24 h	P value	48 h	P value
0	3.88±0.86		5.57±1.01	
250	5.79±0.90	0.055	7.78±1.42	0.052
500	11.31±2.07	0.002	17.90±1.90	<0.001
1000	23.44±2.52	<0.001	30.36±2.86	<0.001
1500	28.90±2.31	<0.001	39.42±2.94	<0.001

Note: The *P* value of each group was derived from comparison with the negative control.

Table 2. Effects of matrine on apoptosis of RPMI 8226 cells

Matrine concentration (mg/L)	Apoptotic rate (%)			
	24 h	P value	48 h	P value
0	1.79±0.66		3.02±1.19	
250	2.96±1.02	0.054	4.98±1.37	0.054
500	5.33±0.98	0.001	9.75±1.13	<0.001
1000	13.60±2.82	0.001	18.48±2.02	<0.001
1500	23.38±3.11	<0.001	31.10±2.98	<0.001

Note: The *P* value of each group was derived from comparison with the negative control.

Detection of cellular COX-2 protein expression using Western blotting (WB): RPMI 8226 cells treated with 1500 mg/L matrine for 48 h and the negative controls were harvested for protein extraction. Protein concentrations were measured using a bicinchoninic acid (BCA) kit (Beyotime Biotechnology Co., Ltd, China). Equal amounts of proteins were loaded for protein separation using sodium dodecyl sulfate-polyacrylamide electrophoresis (SDS-PAGE). After the proteins were transferred onto a PVDF membrane, the membrane was then agitated at room temperature for 4 h for non-specific signal block. After addition of mouse anti-human COX-2 antibody, the membrane was incubated at 4°C overnight. After washing the membrane at room temperature, alkaline phosphatase-labeled goat anti-mouse IgG antibody was added. The membrane was incubated at room temperature for 1 h, followed by washes to remove unbound secondary antibodies. Freshly prepared chemiluminescence reagent was added to the membrane, and then the membrane was incubated in dark for 20 min before terminating the reaction. The band intensity of COX-2 protein was normalized against the internal reference β -actin. Each experiment was conducted in triplicates and the average value was used as the final result.

Statistical analyses

SPSS 19.0 was used for data processing. Experimental data were expressed as $\bar{x} \pm S$. Value differences between groups were determined using t-test, and $P < 0.05$ was considered statistically significant.

Results

Inhibitory effect of matrine on proliferation of RPMI 8226 cells

After 24 h, 250 mg/L matrine did not significantly inhibit the proliferation of RPMI 8226 cells ($P > 0.05$). Matrine at concentrations of 500 mg/L, 1000 mg/L, and 1500 mg/L showed inhibitory effect on cell proliferation, and the effect

was statistically significant ($P < 0.05$). In particular, the inhibitory effect of 1500 mg/L matrine on the proliferation of RPMI 8226 cells was the most pronounced. After 48 h, the inhibitory effect of 500 mg/L, 1000 mg/L, and 1500 mg/L matrine on the proliferation of RPMI 8226 cells showed statistically significant differences from that of the negative control ($P < 0.05$). In addition, this inhibitory effect increased with increasing concentrations of matrine, indicating that the effect of matrine was dose-dependent. Further, the inhibitory effects of 500 mg/L, 1000 mg/L, and 1500 mg/L matrine on RPMI 8226 cells showed statistically significant differences at different time points ($P < 0.05$). This suggests that the inhibitory effect of matrine on RPMI 8226 proliferation might be time-dependent. Detailed results are tabulated in **Table 1**.

Apoptosis-inducing effect of matrine on RPMI 8226 cells

After 24 h, 250 mg/L of matrine did not induce detectable apoptosis of RPMI 8226 cells ($P > 0.05$). Compared to the negative control, the cells treated with 500 mg/L matrine showed statistically significant apoptosis ($P < 0.05$); in particular, 1500 mg/L matrine

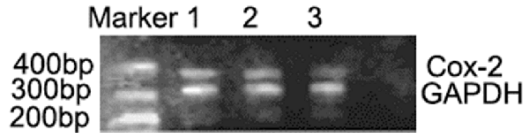


Figure 1. Effect of matrine on COX-2 mRNA expression in RPMI 8226 cells. Note: 1, positive controls of GAPDH and COX-2; 2, mRNA expressions of GAPDH and COX-2 in RPMI 8226 cells prior to matrine treatment. 3, mRNA expression of GAPDH and COX-2 in RPMI 8226 cells after matrine treatment.

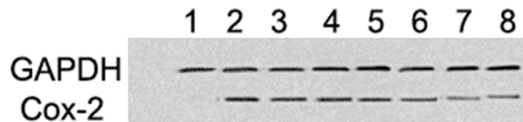


Figure 2. Effect of matrine on the expression of COX-2 protein in RPMI 8226 cells. Note: 1, positive control of GAPDH and the negative control of COX-2; 2, positive controls of GAPDH and COX-2; 3, 4, 5 expressions of GAPDH and COX-2 before matrine treatment; 6, 7, 8, expressions of GAPDH and COX-2 after matrine treatment.

showed the most pronounced effect on RPMI 8226 cell apoptosis. After 48 h, compared to the negative control, the cells treated with 500 mg/L matrine showed statistically significant apoptosis ($P < 0.05$). The apoptosis-inducing effect of matrine on RPMI 8226 cells increased with increasing concentrations, indicating that the effect of matrine was dose-dependent. The apoptosis-inducing effect of matrine at 500 mg/L, 1000 mg/L, and 1500 mg/L concentrations showed statistically significant differences ($P < 0.05$) at different time points, suggesting that the apoptosis induction of RPMI 8226 cells by matrine might be time-dependent. Detailed results are presented in **Table 2**.

RT-PCR detection of COX-2 gene

The ratio of the COX-2 gene and GAPDH in RPMI 8226 cells treated with 1500 mg/L matrine before and after 48 h were $52.65 \pm 4.04\%$ and $28.33 \pm 2.52\%$, respectively ($P = 0.018$; **Figure 1**).

WB detection of COX-2 protein expression

The ratio of the COX-2 protein and the internal reference in RPMI 8226 cells treated with 1500 mg/L matrine before and after 48 h were $85.57 \pm 1.86\%$ and $52.30 \pm 2.05\%$, respectively ($P = 0.003$; **Figure 2**).

Discussion

MM treatment has achieved relatively significant progress in the recent years. Application of thalidomide, lenalidomide, bortezomib, and other new drugs, as well as high-dose chemotherapy combined with hematopoietic stem cell transplantation may improve the remission rate and prolong survival in MM patients. However, MM is still an incurable disease. Most patients are unable to afford the newly developed expensive drugs. In addition, it is very difficult to further promote bone marrow transplantation because MM patients generally have a late onset age and a relatively poor physical condition. It is not easy to find suitable donors for the patients as well. Therefore, it is important to develop new drugs and treatment methods to prevent and treat MM, thus improving the survival rate of patients. Taking advantage of Chinese traditional medicine, the medical field has turned its attention to screening natural agents for prevention and treatment of MM.

Sophora is a traditional Chinese herb widely used in various clinical fields. In addition, it is widely used in antitumor prescription for hematological malignancies. The main pharmacological component of *Sophora* is matrine. Modern pharmacological studies have shown that matrine has a wide range of pharmacological effects such as antiviral, anti-inflammatory, immunomodulatory, renoprotective, and antitumor properties; it has central nervous, cardiovascular, and respiratory effects as well [3]. The antitumor activity of matrine has garnered great attention in recent years, and studies have shown that matrine exhibits antitumor activity both *in vivo* and *in vitro*.

In this study, we investigated the efficacy of matrine and its possible mechanisms in MM treatment by manipulating the concentrations and treatment durations of matrine.

Our *in vitro* experiment demonstrated that matrine could effectively inhibit the proliferation of myeloma cell line RPMI 8226, and this inhibitory effect was both time- and dose-dependent. After 48 h of matrine treatment (1500 mg/L), the proliferation of RPMI 8226 cells was maximally suppressed and the apoptosis rate reached the highest. Thus, the optimal concentration of 1500 mg/L matrine was

adopted in the subsequent experiments to explore the possible mechanisms of matrine.

Recent studies have found that COX-2 is over-expressed in many precancerous lesions and malignant tumors. Because COX-2 has epoxygenase and peroxidase activities, it shows certain correlations to cell cycle, cell death, tumor angiogenesis, tumor invasion, and tumor metastasis during the progression of malignant tumors. Studies by Marco *et al.* (2005), Cetin *et al.* (2005), and Bao *et al.* (2009) indicated that COX-2 was expressed in MM cells, and its expression level could be used as an important prognostic factor [4-6]. Fujita *et al.* (2001) demonstrated that the inhibition of COX-2 expression is one of the mechanisms underlying the efficacy of thalidomide for MM treatment [7].

In this study, we preliminarily explored the inhibitory mechanism of matrine by investigating the changes in COX-2 mRNA and protein levels in RPMI 8226 cells treated with matrine for 48 h. Experimental results showed that matrine inhibited COX-2 expression at both the mRNA and protein levels, thus inhibiting the proliferation of myeloma cells and promoting cell apoptosis.

Traditional Chinese medicine can be used to kill tumor cells owing to its anticancer activities or through regulation of the immune function when combined with chemotherapeutic drugs. Moreover, because Chinese medicinal herbs possess multiple advantages, such as abundant sources, low cost, and few side effects or low toxicity, we can opt for a combination of Chinese and Western medicine in future studies to minimize the dosage of a single drug for better therapeutic effects with reduced toxicity.

Results from this study demonstrated that matrine could suppress myeloma cell line RPMI 8226 *in vitro* by inhibiting cell proliferation and inducing apoptosis. Downregulated COX-2 RNA and protein expressions are one of the possible mechanisms of matrine.

Disclosure of conflict of interest

None.

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