Antiproliferative Effect of Matrine on Renal Cell Carcinoma 786–0 cells

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Abstract **Objective** To investigate the antiproliferative effects of matrine on renal cell carcinoma (RCC) 786−0 cells. **Methods** RCC 786−0 cells in culture medium were treated with matrine at concentrations of 0.3, 0.5, 0.8, 1.0 and 1.5g/L, respectively. The inhibitory rate of the cells was measured by MTT assay after 24 h, 48 h and 72 h. The cell apoptotic rate was detected by flow cytometry (FCM). The expression of Bcl−2 and Bax were displayed with immunocytochemical. Quantitative image analysis was performed with computer assisted pathological image analysis system. **Results** Matrine inhibited the growth of RCC 786−0 cells and induced apoptosis significantly at 0.3~1.5g/L concentrations. The suppression were in time and dose dependent manners. After treatment with matrine in different doses for 24 h, the cell apoptosis rate increased significantly compared with the control group ($P < 0.05$). With 1.5g/L of matrine, the protein expression of Bcl−2 was trended to down regulate without significant difference from control group, while the protein expression of Bax was increased significantly. **Conclusion** Matrine exhibits antiproliferative effects to RCC 786−0 cells by inducing apoptosis and inhibiting cell growth in vitro.

Key words Matrine; Renal cell carcinoma; Apoptosis

Renal cell carcinoma (RCC) accounts for 3% of all malignant tumors in man and is the second common urological cancer after bladder cancer. The incidence of RCC is increasing with a percentage of 2% every year. Approximately 75% of kidney cancers are clear cell, 15% are papillary, 5% are chromophobe and 5% are oncocytoma. The prognosis of renal cell carcinoma remains poor as one third of patients already have a metastatic disease at the initial presentation while 30−40% who undergo surgery for primary tumor develop distant metastases [1]. RCC is generally resistant to cytotoxic chemotherapy or radiation therapy, with no regimen consistently producing response rates above 20% [2]. Patients with untreated metastatic RCC have an overall median survival of no more than 12 months and a 5−year survival rate of less than 10%. Because conventional therapy regimens such as single−agent chemotherapy or hormonal therapy have shown unsatisfactory results, new strategies are needed [3].

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Matrine is a traditional Chinese drug, derived from legume, such as Sophora Flavescenes Ait, S. subrostrate, and is one of the main alkaloid compositions in Sophora Flavescene Ait. Matrine has been demonstrated to have cytotoxicity effect against various cancer cell lines with much fewer negative effects [4, 5]. Our previous study showed that matrine could inhibit the proliferation of human renal cell carcinoma cell line GRC−1, which was granular cell carcinoma of kidney cancer, and the effects maybe related to down regulating the ratio of Bcl−2/Bax protein expression and promoting the apoptosis [6].

Clear cell carcinoma is the most common renal cell carcinoma, so the investigation of clear cell carcinoma treatment with matrine is more significance than other else and there was no similar report up to now. In the present study, we tested the feasibility of using matrine to inhibit proliferation of RCC clear cell carcinoma and investigated the mechanism of antiproliferative effects and provided the experimental data to expand its indications in clinical trials.

MATERIALS AND METHODS

Chemical reagents

All chemical reagents were of the most pure analyti-

cal grade possible. Matrine were kindly provided by Honson biotechnology Ltd. Co., and dissolved in RPMI1640 (GIBICO/BRL, USA) to make a 10mg/ml stock solution and stored at 4℃. The 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide(MTT) was purchased from Sigma (St Louis, Missouri, USA) and dissolved in RPMI1640 to make a 5 mg/ml solution. Annexin Ⅴ-FITC/ PIwas obtained from Jingmei BioTech Co.Ltd. Antibodies to Bcl-2, Bax and SP histostain-plus kits obtained from Beijing Zhongshan Golden Bridge Biotechnology Co.Ltd.

Cell culture

Renal clear cell carcinoma cell line 786-0, were obtained from Institute of Biochemistry and Cell Biology (IBCB, Chinese Academy of Sciences Institute). Cells were cultured in RPMI1640 supplemented with 10% fetal bovine serum at 37℃ in humidified atmosphere containing 5% CO_2.

Cytotoxicity assay

Tumor cells were seeded in 96-well microtiter plates (6000 cells/well) and incubated for 4 h in the medium, and then matrine was added to final concentration of 0.3, 0.5, 0.8, 1.0, 1.5g/L. There were a control group and a blank group. Each group has 5 parallels. The plates were put into incubator and incubated at 37℃ in a 5% CO_2 atmosphere for 24, 48 or 72 hours. Afterwards, 20μL of MTT stock solution was added into each well for another 4 h incubation (37℃, 5% CO_2). After 4 h of incubation, the supernatant was removed and 150 ml of dimethyl sulfoxide was added into each well and the optical density was read at 570 nm with microspectrophotometer (BMG, Germany). The experiments were repeated 3 times. Flow cytometry apoptosis by Annexin Ⅴ-FITC and PI double staining 786-0 cells were seeded at 6-well plates (2.5×105 cells/well). The cells were cultured at 37℃ in a 5% CO2 atmosphere for 12h before they were exposed to various concentrations of matrine for 12h. At the end of treatment, the cells were harvested by trypsin-EDTA soluting, washed twice in cold PBS to produce single cell suspension. Annexin Ⅴ-FITC and PI double staining kit were used to access apoptosis. Flow cytometric analysis of apoptosis was performed on a BD FACSCalibur (Becton Dickinson, San Jose, California, USA). Early apoptotic cells were localized in the lower right quadrant of a dot-plot graph using Annexin Ⅴ-FITC and PI.

Immunocytochemistry

Immunocytochemical staining for Bcl-2 and Bax protein was carried out by the standard streptavidin-peroxidasebiotin technique using SP kit. Cells were treated with 1.0 and 1.5g/L matrine for 24h and plated on slides. Each coverslip was fixed with acetone for 10min and washed with PBS. Then each coverslip was treated for 10min with newly diluted 3% H_2O_2 to destroy internal peroxidase, followed by blocking with normal goat serum at 37℃ for 20min to block non-specific background staining. Cells were immunostained with monoclonal antibody at 4℃ overnight. After three further washes with PBS, a secondary biotinylated rabbit anti-mouse antibody was applied for 1h at room temperature and then streptavidin conjugated to peroxidase was added. Following extensive washes with PBS, DAB was used for color development, and hematoxylin was used for counterstaining. The negative controls were performed by substituting the primary antibody with PBS. Hematoxylin stained cells were examined under light microscope and photographed. Gray value of positive cell was measured by the Motic Med 6.0 image analysis system (Motic, Beijing).

Statistical analysis

The data were presented as mean ± SD and analyzed with SPSS 12.0 statistical software. Student's t test was used and a P value less than 0.05 was considered statistically significant.

RESULTS

Cytotoxicity assay

Using the MTT method, we determined the cytotoxic activity of matrine in human renal cell carcinoma cell line 786-0 and the dose-response curves and time-response curves are shown in Fig 1. With treatment of 1.0, 1.5g/L matrine after 72h, the growth-in-

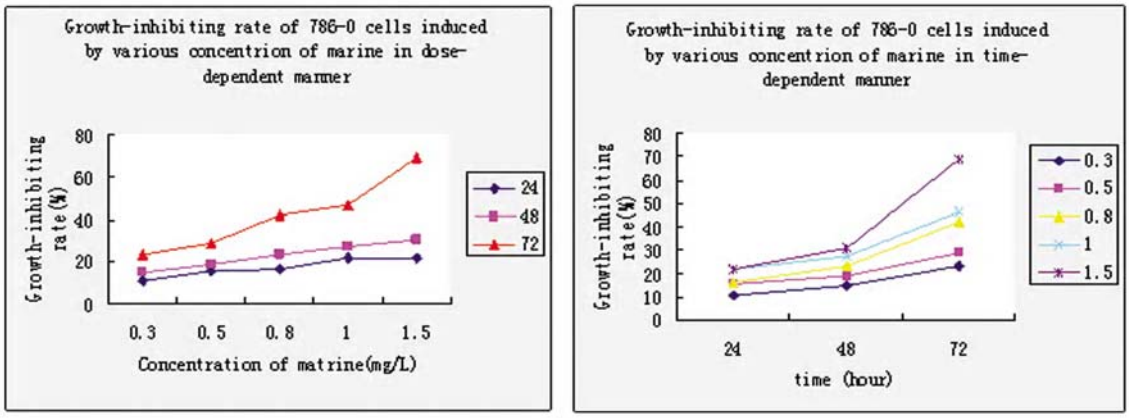


Fig. 1 Growth-inhibiting effects of matrine on human renal cell carcinoma cell line 786-0

hibiting rate of 786-0 cells were 46.44%, 66.83% respectively. RCC cell line 786-0 cells exhibited dose- and time-dependent sensitivity to matrine with 0.3-1.5g/L at 24h, 48h and 72h.

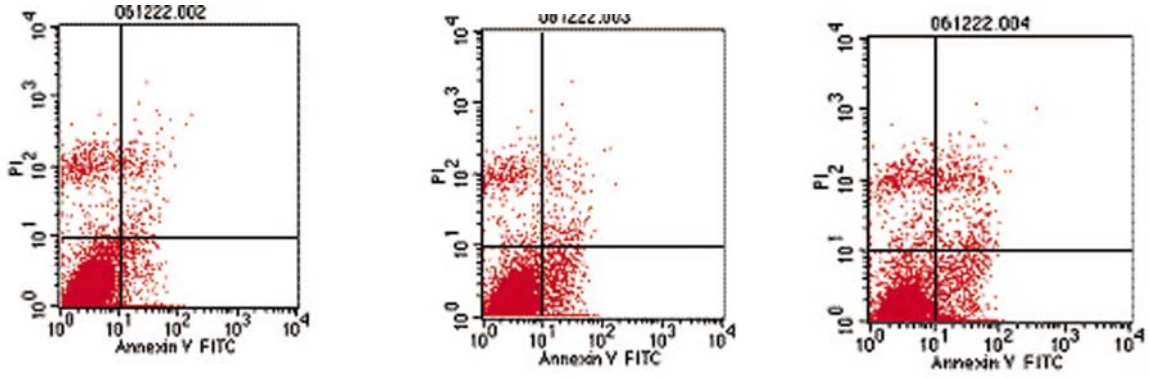
Apoptosis assay for the anti-RCC activity of matrine against 786-0

After treated with different concentrations of matrine 24h, 786-0 cells were harvested. Apoptotic rate was measured by flow cytometry on a FACScan. The apoptotic rate of 786-0 cells treated with 0.5, 1.0, 1.5g/L concentrations of matrine were 8.68%, 12.84%, 23.62% respectively (Fig 2). Our experiments showed that matrine could increase the apoptotic rate of RCC 786-0.

Immunocytochemical stain of Bcl-2 and Bax

The immunocytochemical stain of Bcl-2 and Bax in 786-0 cells was shown in Fig. 3, 4. Immunocytochemical analysis was used to detect Bcl-2 and Bax expression in the RCC 786-0 cells. Immunostaining of Bcl-2 and Bax were primarily in the cytoplasm of 786-0 cells, and the positive cells were stained brown. The gray value is negative correlation to intensity of immunocytochemical stain. The gray value of Bcl-2 and Bax was listed in table1.The expressions of Bax had significant differences between control group and 1.5g/L matrine group ($P < 0.05$). Although expression of Bcl-2 protein was no significant change in 1.5g/L matrine group compared with control group, it was trended to regulate downwards.

DISCUSSION



0.5g/L 1.0g/L 1.5g/L

Fig. 2 Apoptosis are shown: cells were treated with 0.5,1.0,1.5g/L of matrine for 24 h.Apoptotic rate was detected by FCM.

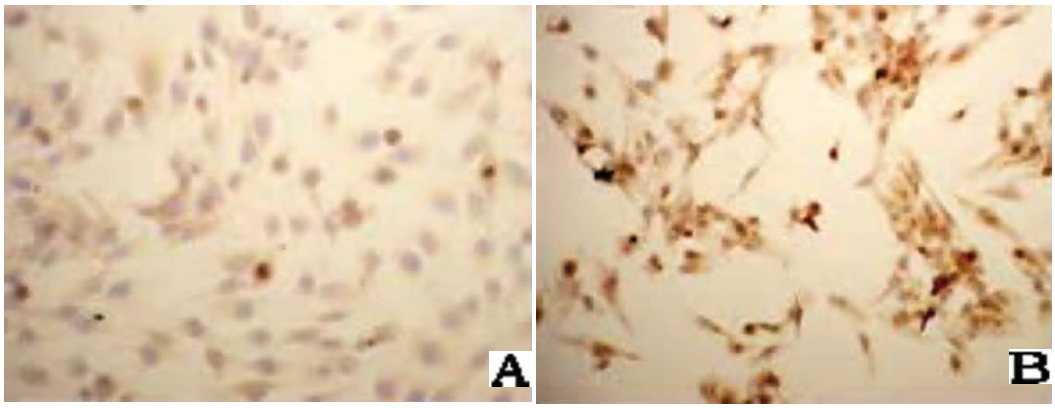


Fig. 3 Bcl−2 immunocytochemical staining of 786−0 cells in vitro A: control group; B: 1.5g/L matrine group

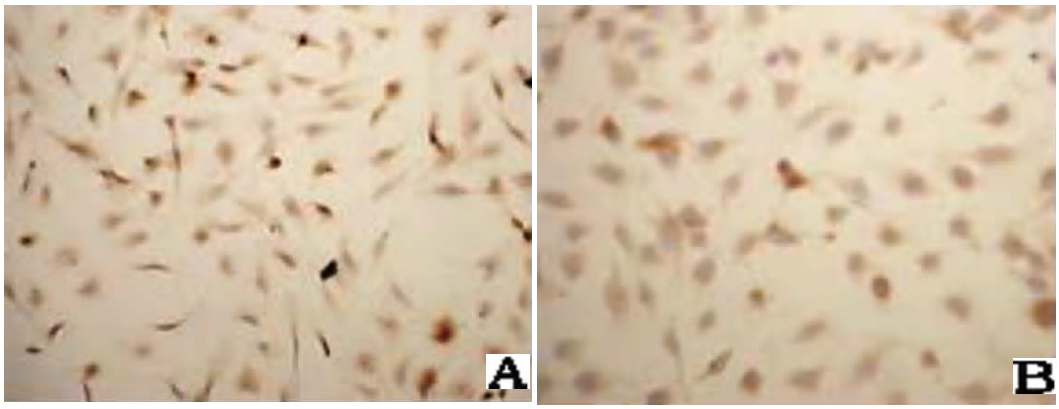


Fig. 4 Bax immunocytochemical staining of 786−0 cells in vitro A: control group; B: 1.5g/L matrine group

Matrine is an alkaloid of Flavescent sophora root, it has been used in viral hepatitis B and C as an antiviral, antifibrotic, immuno −modulating agent as well as an antiarrhythmic agent. Recently, it has been found to be effective in inducing tumor cell apoptosis in vitro, which can inhibit tumor cells proliferation, thus producing an antitumor effect. It is reported that matrine can play a significant effect on the inhibition of proliferation cells and inducing differentiation in K−562 cells [7]. Furthermore, it can inhibit the proliferation of vascular endothelial cells induced by lung cancer and gastric cancer cells, and might play an important role in preventing and treating tumor infiltration and metastasis [8, 9].

At present study, MTT assay showed that the growth of 786 −0 cells was inhibited after treatment with matrine for 24~72h (P<0.05). The effect enhanced with increasing concentration and prolonged treatment time of matrine, which suggested that the inhibition effects are dose−and time−dependent. These results were similar to those reports elsewhere. Apoptosis is known to be a very important mechanism in the anticancer effects induced by chemopreventive and chemotherapeutic agents. To assess whether matrine induced apoptosis, we checked the appearance of apoptotic rate by flow cytometry analysis. The apoptotic rate of 786 −0 cells treated with 0.5, 1.0, 1.5g/L concentrations of matrine were 8.68%, 12.84%, 23.62% respectively. The apoptotic rate was increased with added concentration of matrine. Our date showed that matrine inhibited RCC 786−0 cells proliferation and the effect was correlated to induce cell apoptosis.

As a gene −directed process, apoptosis is modulated by the expression of a number of regulatory genes, such as p53, c−myc and Bcl−2 family. The Bcl−2 family is an important regulator of apoptosis, which consists of anti −apoptotic (such as Bcl −2) and pro −apoptotic members (such as Bax) [10]. Bcl −2 has been shown to have the ability to prevent cytochrome C release from the mitochondria, which is an important event during

Table 1 Expressions of Bcl−2 and Bax protein in 786−0 cells in different group(x ± s)

Group	n	Bcl−2 protein gray value	Bax protein gray value
Control	6	172.55±7.19	161.44±10.97
Matrin(1.5g/L)	6	177.96±12.67	131.02±14.51
t		0.911	4.097
P		>0.05	<0.05

apoptosis mediated by mitochondrial pathways, but overexpression of pro−apoptotic Bax induces the release of cytochrome C from the mitochondria. Whereas, when Bcl−2 heterodimerizes with Bax, it abrogates the ability of Bax and blocks apoptosis in cells [11]. The date in our study showed that expression of Bax significantly increased and the Bcl−2/Bax ratio decreased with treatment of 1.5g/L matrine, which indicated that the decrease of Bcl−2/Bax ratio was involved in matrine induced apoptosis.

In conclusion, matrine significantly inhibited growth of RCC 786−0 cells in vitro and induced cell apoptosis. Much work remains to be done; we should evaluate the effects and explore the antitumor mechanism of matrine to RCC in vivo.

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