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Matrine: A novel inhibitor of growth and proliferation of lung adenocarcinoma cells

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To investigate the inhibitory effects of matrine on lung cancer and explore the potential mechanism, we studied the in vitro anti-proliferation activity of matrine in human lung adenocarcinoma A549 cells. The effects of matrine on the growth and proliferation were evaluated, and the cell cycles and early cell apoptosis were examined after treatment with various concentrations of matrine for 48 and 72 h, respectively. Our data indicated that matrine significantly inhibited the growth of A549 cells through inhibition of cell proliferation, induction of cell apoptosis and blockage of cell cycle in vitro. The inhibitory effects of matrine on growth of A549 cells were in a time- and dose-dependent manner. The fifty percent inhibiting concentration (IC50) of matrine was 0.8 and 0.5 mg/ml at 48 and 72 h, respectively. These results illustrated the anticancer effects of matrine and the potential mechanisms. This study encouraged us to further evaluate the anticancer activity in vivo and clarify the molecular mechanisms responsible for the anticancer effects of matrine.

Key words: Matrine, anticancer, non-small cell lung cancer, cell cycle, apoptosis.

INTRODUCTION

Lung cancer is one of the malignant tumors with higher mortality and morbidity. About eighty percent lung cancer is non-small cell lung cancer (NSCLC) (Liberman et al., 2006). With ageing of population, the process of Industrialization and environmental deterioration, incidence of lung cancer is increasing in singulos annos. Use of complex treatment strategy including surgery, chemotherapy and radiotherapy treatment has led to a decrease of this malignancy in Western countries. However, NSCLC continues to be the leading cause of death from cancer among males in most developing countries. Till now, the 5-year survival rate is less than 15 % for NSCLC (Quadrelli et al., 2009). Therefore, new strategies to improve the therapy of NSCLC have received much attention.

Natural medicinal plants provide rich resources for anticancer drug discovery. Based on the ancient and modern Chinese herbal medicine books, there are many other anticancer plants or herbal formulations which could provide a guide for identification of new anticancer compounds or a source of alternative cancer therapy. It is widely accepted that it has only low toxicity, so it may be a better choice in treatment of cancers (Schwartsmann et al., 2002).

Matrine, one of the major active components found in the traditional Chinese medicine Sophora flavescens roots, has long been regarded as an anticancer herb in China (Niu, 1997). The intensive investigation of the pharmacologic and clinical applications of these alkaloids remains a focus of Chinese medical research. In clinical practice, it is mainly applied in treatment of viral hepatitis, hepatic fibrosis, arrhythmia, atherosclerosis and skin diseases in China (Sun et al., 2004; Yang et al., 2004; Lu et al., 2003; Wu et al., 2003). Recently, interest has been generated in the anti-tumor activity of matrine. Studies have indicated that matrine can inhibit tumor cell proliferation and induce cell differentiation and apoptosis (Zhang et al., 2001; Liu et al., 2006). The proposed major mechanisms involve the following: inhibition of human telomerase reverse transcriptase (hTERT)-mRNA
expression and activation of telomerase (Chen et al., 2008), regulation of the expression of cyclin, cyclin-dependent kinase and cyclin-dependent kinase inhibitors (Si et al., 2001); suppression of C-myc and Bcl-2 expression; and promotion of N-ras and p53 expression (He et al., 2002; Deng et al., 2004); cell apoptosis via down-regulating the Bcl-2/Bax ratio and upregulating the activity of cleaved caspase-3 (Wang BH et al., 2007); and selective inhibition of gene transcription, protein expression and functional activity of cyclooxygenase-2 (COX-2) (Huang et al., 2005). Moreover, matrine exhibits anti-tumor activities by interrupting cell-cell adhesion between the endothelial cells and tumor cells in vitro, which could potentially prevent tumor invasion (Zhang et al., 2007; Fang et al., 2008). Although matrine has been shown to be very effective in treating a variety of malignancies, few studies have reported the effects of matrine on the lung cancer cells and the mechanisms underlying the anticancer effect of matrine remain to be further elucidated.

To gain insight into the role of matrine in lung cancer, we studied the in vitro anti-proliferation activity of matrine in lung cancer cells and explored the possible mechanism. A549 cell line derived from human lung adenocarcinoma was employed in our study. The effects of matrine on the growth and proliferation were evaluated, and the cell cycles and early cell apoptosis were examined after treatment with various concentrations of matrine. Our data indicated that matrine significantly inhibited the growth of A549 cells through inhibition of cell proliferation, induction of cell apoptosis and blockage of cell cycles in vitro. These results prompted us to further evaluate the anticancer activity in vivo and clarify the underlying mechanisms.

MATERIALS AND METHODS

Cell line and reagents

Matrine was obtained from Xian Botany Garden (Shanxi, China), and its purity was > 99% as assessed by high-performance liquid chromatography (Figure 1). Matrine stock solution was prepared in di-distilled water (ddH2O) at 10 mg/ml followed by filtration through 0.22 μm disk filter for sterilization and stored at -20°C. Human lung adenocarcinoma A549 cell line was obtained from China center for type culture collection (CCTCC) and cultured in our laboratory. The cells were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin and 100 μg/ml streptomycin (all from Gibco/BRL, USA).

Morphologic observation

After treatment with matrine at different concentrations for 24 h, the morphological changes of A549 cells were observed under inverted light microscope (Olympus, Japan).

Cell viability analysis

Cells in logarithmic phase were incubated at 2.5 × 10^5 cells/ml in a 12-well plate (Corning, USA) in 1 ml volumes. Different concentrations of matrine (0.1, 0.2, 0.5 and 1.0 mg/ml) were then added into different wells. Cell viability was assessed by trypan blue dye (Sigma, USA) exclusion method. After treated with various concentrations of matrine, cells were collected and trypan blue solution was added to the cells. Cells were counted using hemacytometer after 5 - 10 min of incubation at room temperature for 6 consecutive days and the growth curves were plotted.

Cell growth inhibitory rate

The cell growth inhibitory rate was examined using the 3-(4, 5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Briefly, 3 × 10^4 cells/well was dispensed within 96-well microtiter plates in 200 μl volumes. Different concentrations of matrine (0.1, 0.2, 0.5, 0.8, 1.0 and 1.2 mg/ml) were then added into different wells. Every one of the concentrations above was regarded as one treated group, while the control group contained no matrine. Each matrine-treated or control group contained 6 parallel wells. After culture plates were incubated for 24 and 48 h, 20 μl of MTT working solution (5 mg/ml in ddH2O) was added to each well and incubated continuously for 4 h at 37°C. MTT/medium was then removed and formazan was dissolved in dimethyl sulphoxide (DMSO), of which 150 μl/well was added. Finally, the absorbance (A value) of each well was measured at 570 nm using an enzyme-linked immunosorbent assay (ELISA) plate reader. The growth inhibitory rates (IR) were calculated as [(1-Atest/Acontrol)] × 100%. IC50 was determined from dose-response data. The results were determined by at least three independent experiments.

Cell cycle analysis

Analysis of the cell cycle of control and treated cancer cells was determined. Using standard methods (Yu et al., 2000), the DNA of cells was stained with PI, and the proportion of non-apoptotic cells in different phases of the cell cycle was recorded. Briefly, 5 × 10^4 A549 cells were treated with various concentrations of matrine (0.2, 0.5 and 1.0 mg/ml) for 48 or 72 h. Then the cells were collected and washed twice with phosphate buffer solution (PBS) and fixed in ice cold 70% (v/v) ethanol. After RNase A (10 mg/ml in Tris-EDTA buffer) digestion, the cells were subsequently incubated with propidium iodide (PI, 2 mg/ml in PBS) in dark at room temperature for 1h. The fluorescence-activated cells were sorted in a FACSscan flow cytometer (Becton Dickinson, NJ) using CellQuest 3.0.1 software and the data were analyzed using ModFit V3.0 software. In the nonapoptotic population (as 100% after excluded sub-G1 population), the percentage of cells in each phase of the cell cycle was determined as least triplicate and expressed as mean ± SD. The cell proliferation index (PI) was calculated as: (S+G2/M)/(G1+S+G2/M) × 100%.

Annexin V–fluorescein isothiocyanate/propidium iodide (AnnexinV-FITC/PI) affinity assay

Externalization of PS and membrane integrity were quantified using an Annexin V-FITC Staining Kit (KeyGen Biotech, China). In brief, cells were grown in a 6-well microtiter plates at 3 × 10^5 cells/well in 1 ml volumes. The cells were treated with various concentrations of matrine (0.2, 0.5 and 1.0 mg/ml) for 48 or 72 h. Treated and control cells were harvested and resuspended in 250 μl binding buffer at 1 × 10^5 cells/ml. Then the cells were incubated with Annexin V/FITC (10 μg/ml) and PI (10 μg/ml) in dark for 15 min at room temperature. After staining, all cells were analyzed for the
percentage of Annexin V positive and PI negative cells by flow cytometry (FACScan). The data were determined by three independent experiments.

Statistical analysis

The data are presented as mean ± standard deviation (SD). The statistical significance of the results was tested using Student’s t-test (two-tailed) for normally distributed values using SPSS 13.0 software. Statistical significance was accepted at the level of p < 0.05.

RESULTS

Morphological changes of A549 cells after matrine treatment

A549 cells treated with different concentrations of matrine for 24 h were observed under inverted light microscope. As shown in Figure 2, in general, untreated A549 cells were found to be long spindle shape or cobblestones-like. The cell nucleus was irregular and the karyopyknosis phenomenon was seldom seen. There were less cytoplasmic granules and vacuoles in cytoplasm. Cells treated with 0.1 mg/ml matrine grew well, and only scattered granules were noted around nuclei. Cells of 0.2 mg/ml matrine treatment grew slowly, irregular cells and vacuoles with different sizes were observed, resulting in cell body enlargement. After 0.5 mg/ml matrine treatment, cytoplasmic vacuoles and swollen cells were observed, but the cell membrane was integrated. When the concentration of matrine was up to 1.0 mg/ml, the cells became more atrophic and the cell debris and suspended cells apparently accumulated.

Cell viability analysis

As shown in Figure 3a, the number of viable A549 cells were gradually decreased by trypan blue exclusion method after treatment with different concentrations of matrine (0.1, 0.2, 0.5 and 1.0 mg/ml). The inhibitory effect of matrine on A549 cells viability was significantly increased when the concentration of matrine was higher than 0.2 mg/ml. After been counted for 5 days, the ratio of viable cells to the total cells was significantly reduced especially in cells with 0.5 and 1.0 mg/ml matrine treatment.

Inhibitory effects of matrine on A549 cell growth

The antiproliferative effect of matrine on lung cancer A549 cell lines was determined by MTT assay (Table 1 and Figure 3b). A slight decrease of cell vitality was observed after treatment with 0.1 mg/ml matrine for 48 and 72 h (5.4 and 8.3%, respectively). After exposure of more than 0.2 mg/ml matrine, the cell proliferation was significantly suppressed after 48 or 72 h treatment compared to the untreated cells (p < 0.05, student’s-test). The IC50 values of matrine in A549 cells were 0.8 mg/ml for 48 and 0.5 mg/ml for 72 h, respectively. The results indicated that over 0.2 mg/ml matrine had marked inhibitory effect on cell proliferation and presented with a concentration and time dependent manner in A549 cells.

Effects of matrine on cell cycle of A549 cells

The cell cycle was analyzed in A549 cells exposed to different concentrations of matrine for 48 and 72 h. The relative frequencies of cells in each cell cycle phase were estimated by PI mediated cellular DNA content measurement. As summarized in Table 2 and Figures 4, 5 and 6, as compared to the control cells, there was a significant reduction in population of cells in S phase after cells were treated with matrine, while a accumulation of cells in G0/G1 phase. In cells treated with matrine for 48 h,
Figure 2. Matrine causes morphologic changes in A549 cells. A549 cells were treated with different concentrations of matrine for 24 h and observed under inverted light microscope (200×). The cellular growth rate decreased and the number of apoptotic and karyopyknotic cells increased as the concentration of matrine increased. Cytoplasmic vacuoles and swollen cells were observed in 0.5 mg/ml matrine treated cells. After treated with over 1.0 mg/ml matrine, more and more atrophic and dead cells were founded. (a) - (f) referred to the control A549 cells and the cells treated with 0.1, 0.2, 0.5, 1.0 and 1.2 mg/ml matrine, respectively.

The populations of cells in G0/G1 phase increased to 43.78 ± 3.81% (0.5 mg/ml) and 76.65 ± 4.67% (1.0 mg/ml) from 35.23 ± 2.19% (0.2 mg/ml) with the increasing of matrine concentration compared to the control cells, respectively. Meantime, the populations of cells in S phase decreased to 55.12 ± 4.32% (0.5 mg/ml) and 23.45 ± 2.85 (1.0 mg/ml) from 60.70 ± 3.95 (0.2 mg/ml), respectively. After cells were treated with matrine for longer time (72 h), the trends of cell cycles change was also observed. These results suggested that matrine could induce cell cycle arrest in G1 phase and behaved with a time-dependent manner in A549 cells.
Table 1. Inhibitory effects of matrine on proliferation of A549 cells (MTT assay) (x ± s, n=6).

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentrations (mg/ml)</th>
<th>A 570</th>
<th>Proliferation rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.517 ± 0.11</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.312 ± 0.05</td>
<td>60.4^b</td>
</tr>
<tr>
<td>48 h</td>
<td>0.1</td>
<td>0.466 ± 0.08</td>
<td>90.1^a</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.272 ± 0.01</td>
<td>52.7^b</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.125 ± 0.04</td>
<td>24.3^b</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>0.089 ± 0.07</td>
<td>17.2^b</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.814 ± 0.09</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.726 ± 0.06</td>
<td>89.2^a</td>
</tr>
<tr>
<td>72 h</td>
<td>0.8</td>
<td>0.449 ± 0.04</td>
<td>55.2^b</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.304 ± 0.02</td>
<td>37.4^b</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>0.110 ± 0.03</td>
<td>13.6^b</td>
</tr>
<tr>
<td></td>
<td>1.2</td>
<td>0.134 ± 0.02</td>
<td>8.2^b</td>
</tr>
</tbody>
</table>

^aCell proliferation rate in control group was 100%. The number of trial times was three. All values are expressed as mean ± sd.
Different upper letters a or b, p < 0.01 or p < 0.05, compared with the cell proliferation rate in control group.

Effects of matrine on apoptosis of A549 cells

Previous studies revealed matrine induced chromatin change in A549 cells. Shrinkage of cells and disorganization of chromatin might be related to apoptosis. Simultaneous staining with Annexin V-FITC and PI can distinguish the intact cells, early apoptosis and late apoptosis or cell death (Engeland et al., 1998). In A549 control culture, 1.06% of the cells were in early apoptosis and 1.03% were in late apoptosis or cell death (Figure 7a). After A549 cells exposed to different dosages of matrine for 48 h, the apoptotic cells were significantly increased with a dose-dependent manner. According to previous data, treated with 0.2 mg/ml matrine for 48 h, the cells were characterized by morphological changes and proliferation inhibition, but this did not affect the cell cycle (P > 0.05). When the concentration of matrine was up to 0.5 and 1.0 mg/ml, the percentages of early apoptosis cells increased to 6.85 and 17.88%, respectively, meantime, the cells in late apoptosis or cell death increased to 13.49 and 22.22%, respectively. The difference between these groups and control group or low-concentration groups was significant (P < 0.01). Moreover, apoptotic cells gradually increase in a time-dependent manner after exposure to matrine for a longer time (72 h). These results (Figures 8 and 9) suggested that matrine at a final concentration of 0.5 - 1.0 mg/ml induced more Annexin-V-stained cells in a time- and dose-dependent manner.

DISCUSSION

In recent years, many researchers have studied the anti-cancer effects of active constituents of traditional Chinese medicines. It has been suggested that there are multiple active ingredients in Chinese medicinal herbs which may inhibit the malignancies effectively through one or more of the following mechanisms of tumormetastasis: (i) induction of cells differentiation, (ii) interruption of DNA synthesis or blockage of cell division, (iii) induction of cell apoptosis (Xiao, 2002).

Matrine, an alkaloid compound isolated from Sophora roots, not only inhibits tumor cell proliferation, but also induces cellular differentiation and apoptosis. Catassi found that matrine significantly induced cell cycle arrest at the G2/M phase in A549 cell growth, and the c-Myc down-regulation was observed in matrine-treated cells, so they inferred that mechanisms of action of matrine was associated with the down-regulation of c-Myc (Catassi et al., 2006).

Infinite division and out-of-control proliferation are the two salient attribute of malignant cells distinguished from the normal cells. Therefore, inhibition of cell proliferation and induction of apoptosis are the principal therapeutic strategies for malignant tumors. In the present study, we investigated the effects of matrine on the proliferation and apoptosis of A549 cells and tried to determine the possible mechanisms of its anti-tumor effects. We found that matrine had antiproliferative effect to human lung cancer A549 cells and a time- and dose-dependent manner was found. The result of cell cycle analysis evaluated by flow cytometry analysis showed that matrine could induce G0/G1 arrest in A549 cells. Disturbance of the cancer cell cycle is one of the therapeutic targets for development of new anticancer drugs (Carnero, 2002). So we supposed that exploration of targeting regulatory molecules involving G1→S transition (e.g. cyclinE1/2, cyclinD1/2/3, cyclinA, CDK2/4/6, CDC25) (Tong et al., 2005) might be contributed to elucidate
Figure 3. Matrine inhibits the growth of A375 cells in a dose-dependent manner. A549 cells were treated with various concentrations of matrine for 1 – 5 days, and then the viable cells was counted for the grow curve. The curves of the 0 and 0.1 mg/ml matrine groups were similar, but the proliferation of A549 cells treated with 0.2, 0.5 and 1.0 mg/ml matrine was suppressed compared with the control group and (A). The cell growth inhibitory rate in A549 cells were assayed using MTT method after treatment with various concentrations of matrine for 48 h and 72 h, respectively. The inhibitory effect was dose and time dependent (B). Six parallel wells were included in each group and data were presented as mean ± SD.

contributed to elucidate the mechanism underlying this effect of matrine in A549 cells.

Apoptosis is an important homeostatic mechanism that balances cell division and cell death, thus contributing to stable the cell quantity and preventing formation of malignant tumors (Hale et al., 1996). Induction of apoptosis in cancer cells has been viewed as one of the strategies for anticancer drug development (Martin et al., 1995; Hu et al., 2003). There are several crucial cellular and molecular biological features involving apoptosis, they include cell shrinkage, disorganization of chromatin, externalization of PS and activation of caspase (Martin et al., 1995; Salvesen et al., 1997). PS externalization is an early feature if apoptosis can be detected by the binding of annexin V to PS on the cell surface (Engeland et al., 1998). In our results, matrine can cause externalization of PS and induce early apoptosis.

In regulation of apoptosis, the bcl-2 family members are key regulators and the balance between these proteins is essential to cell fate. Bcl-2 and Bcl-xL promote cell
Table 2. Effects of matrine on cell cycle of A549 cells.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Concentrations (mg/ml)</th>
<th>Percentage of cells in different phases (%)</th>
<th>Proliferation index (PI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>G0/G1</td>
<td>S</td>
</tr>
<tr>
<td>48 h</td>
<td>Control</td>
<td>35.49 ± 4.36</td>
<td>63.82 ± 5.21</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>35.23 ± 2.19</td>
<td>60.70 ± 3.95</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>43.78 ± 3.81</td>
<td>55.12 ± 4.32</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>76.65 ± 4.67</td>
<td>23.45 ± 2.85</td>
</tr>
<tr>
<td>72 h</td>
<td>Control</td>
<td>46.23 ± 2.34</td>
<td>52.07 ± 3.45</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>52.55 ± 1.89</td>
<td>45.35 ± 2.61</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>71.58 ± 2.22</td>
<td>26.52 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>77.20 ± 3.11</td>
<td>21.70 ± 1.98</td>
</tr>
</tbody>
</table>

a: p < 0.05 vs. control; b: p < 0.01 vs. control.

After matrine treatment with different concentrations (0.2, 0.5 and 1.0 mg/ml) for 48 and 72 h, the cells were incubated with Propidium Iodide for 30 min and flow cytometry assay was performed for cell cycle analysis. Measurement was repeated three times and data were presented as mean ± SD. Different upper letters a or b, p < 0.01 or p < 0.05, compared with the cell proliferation rate in control group.

Figure 4. Effects of matrine on cell cycle of A549 cells. The percentages of cells in different phases after matrine treatment with different concentrations for 48 and 72 h (A, B). After matrine treatment for 48 and 72 h, the proportion of cells in the G0/G1 phase was significantly increased and that in the S phase was markedly reduced. Detection was repeated thrice and data were presented as mean ± sd. (a) 0 mg/ml (negative control; spontaneous apoptosis rate); (b) 0.2 mg/ml; (c) 0.5 mg/ml and (d) 1.0 mg/ml matrine.
Figure 5. Effects of matrine on cell cycle of A549 cells; Cell cycle analysis with flow cytometry. After matrine treatment for 48 h, the proportion of cells in the G0/G1 phase was significantly increased and that in the S phase was markedly reduced. Detection was repeated thrice and data were presented as mean ± sd. (a) 0 mg/ml (negative control; spontaneous apoptosis rate); (b) 0.2 mg/ml; (c) 0.5 mg/ml and (d) 1.0 mg/ml matrine.

survival, whereas Bax promotes cell death (Oltvai et al., 1993; Adams et al., 1998). Lines of evidence indicate that these proteins regulate apoptosis through controlling cytochrome C release from mitochondria and activation of caspase cascades (Kluck, 1997; Li et al., 1997). It was reported that the molecular mechanism underlying the effect of matrine on apoptosis induction was correlated with cytochrome C-mediated caspase-3 activation and elevated level of Bax expression in human leukemia K562 cells. The treatment with matrine upregulated the expression of proapoptotic Bax protein and caused the release of cytochrome C, which initiated the caspases
Figure 6. Effects of matrine on cell cycle of A549 cells; Cell cycle analysis with flow cytometry. After matrine treatment for 72 h, the proportion of cells in the G0/G1 phase was significantly increased and that in the S phase was markedly reduced. Detection was repeated thrice and data were presented as mean ± sd. (a) 0 mg/ml (negative control; spontaneous apoptosis rate); (b) 0.2 mg/ml; (c) 0.5 mg/ml and (d) 1.0 mg/ml matrine.

cascade. Moreover, Catassi founded that matrine induced a cascade of events including Bad dissociation from Bcl-XL, cytochrome C release, leading to mitochondria apoptotic pathway in matrine-treated A549 cells (Catassi et al., 2006). Taken together, the results suggest the regulation of Bcl-2 family members and the mitochondrial apoptotic pathway probably is partly responsible for the apoptosis-inducing activity of matrine in A549 cells.

In addition, the observation that A549 cells become swollen and intracellular vacuoles and karyopyknosis was seen after matrine treatment (24 h) indicates that the inhibitory effect of matrine on the proliferation of A549 cells may also be related to changes in cellular morphology. Therefore, the inhibitory effect of matrine on
Matrine induces the apoptosis of A549 cells. The percentages of early apoptosis (Annexin V<sup>+</sup>/PI<sup>-</sup>) cells and the late apoptosis (Annexin V<sup>+</sup>/PI<sup>+</sup>) cells for 48 h and 72 h (A,B) after treatment with different concentrations of matrine. The numbers of apoptotic cells in the 0.5 and 1.0 mg/ml matrine groups were significantly different from those in the control and low-concentration groups. Apoptosis induced by matrine was observed in a dose and time dependent manner. Furthermore, the percentage of apoptotic cells increased as the matrine concentration increased. Detection was repeated thrice and data were presented as mean ± SD. (a) 0 mg/ml (negative control; spontaneous apoptosis rate); (b) 0.2 mg/ml; (c) 0.5 mg/ml and (d) 1.0 mg/ml matrine.

In summary, our study showed that matrine could inhibit the proliferation of A549 cells in vitro via causing cell cycle arrest in G1/S phase and inducing apoptosis. Matrine is widely used in clinical practice for other diseases rather than proliferation of lung cancer cells may involve several different mechanisms, and further studies are required to understand the exact mechanisms of its anti-cancer action.
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Figure 8. Matrine induces the apoptosis of A549 cells. The percentages of cell apoptosis analysis with flow cytometry for 48 h and after treatment with different concentrations of matrine. The numbers of apoptotic cells in the 0.5 and 1.0 mg/ml matrine groups were significantly different from those in the control and low-concentration groups. Apoptosis induced by matrine was observed in a dose and time dependent manner. Furthermore, the percentage of apoptotic cells increased as the matrine concentration increased. Detection was repeated thrice and data were presented as mean ± SD. (a) 0 mg/ml (negative control; spontaneous apoptosis rate); (b) 0.2 mg/ml; (c) 0.5 mg/ml and (d) 1.0 mg/ml matrine.

Figure 9. Matrine induces the apoptosis of A549 cells. The percentages of cell apoptosis analysis with flow cytometry for 72 h and after treatment with different concentrations of matrine. The numbers of apoptotic cells in the 0.5 and 1.0 mg/ml matrine groups were significantly different from those in the control and low-concentration groups. Apoptosis induced by matrine was observed in a dose and time dependent manner. Furthermore, the percentage of apoptotic cells increased as the matrine concentration increased. Detection was repeated thrice and data were presented as mean ± SD. (a) 0 mg/ml (negative control; spontaneous apoptosis rate); (b) 0.2 mg/ml; (c) 0.5 mg/ml and (d) 1.0 mg/ml matrine.
than antitumor application, so it is necessary to further demonstrate the in vivo antitumor effects of matrine. Understanding of the molecular mechanisms responsible for the anticancer effects of matrine may encourage us for further evaluation and application of matrine and elucidating its anticancer profile.

REFERENCES


