Alkaloids from root tubers of *Stephania kwangsiensis* H.S.Lo and their effects on proliferation and apoptosis of lung NCI-H446 cells.

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**Abstract**

To study the chemical constituents of *Stephania kwangsiensis* Lo. and to explore the effects of corydine on proliferation and apoptosis of lung adenocarcinoma NCI-H446 cells. Chemical structures were elucidated by MS, 1H-NMR and 13C-NMR. Effects of different concentrations of corydine on proliferation of lung adenocarcinoma NCI-H446 cells were determined by MTT assay. Effects of corydine on apoptosis rate of NCI-H446 cells were determined by flow cytometry. Three types of alkaloids were isolated from root tubers of Menispermaceae Stephania plant Stephania kwangsiensis Lo. Three different concentrations of corydine (20, 10, 5 Lg/ml) could all significantly increase the apoptosis rate of NCI-H446 cells after 48 h of treatment compared to the control group. Corydine can inhibit the proliferation of lung cancer NCI-H446 cells and induce their apoptosis.

**Keywords:** *Stephania kwangsiensis* Lo., corydine, small cell lung cancer, apoptosis.

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**Introduction**

*Stephania kwangsiensis* Lo. is a perennial deciduous herbaceous vine in the genus Stephania of the family Menispermaceae, which is mainly distributed in China's Guangdong, growing in mountain shrubs in limestone areas [1]. As a common Chinese herbal medicine, the root tuber extract of *Stephania kwangsiensis* Lo. is the main raw material of rotundine, which has analgesic, sedative, antipyretic effects and is thus widely used in clinical practice [2]. Stephania root tubers contain a variety of constituents such as alkaloids and terpenes [3,4]. Among them, alkaloids are the highest containing active constituent, with content reaching 3%~4% [5]. To further clarify its constituents, this study isolates three compounds from *Stephania kwangsiensis* Lo., namely: palmatine (1), corydine (2) and sinoacutine (3).

Small cell lung cancer is a highly malignant tumor, which is characterized by rapid growth, propensity to early systemic metastasis and higher incidence. Epidemiological [6,7] surveys have shown that small cell lung cancer accounts for 12%~15% of all lung cancers. Currently, small cell lung cancer is treated mostly by combined chemotherapy and radiotherapy in clinical practice. However, such therapy has large toxic side effects, where patients can hardly adhere to the treatment or have ineffective treatment. Therefore, the search for drugs with good efficacy and low toxic side effects is one major direction of cancer therapeutic research at present. According to studies in recent years, alkaloids are the main active constituents of *Stephania kwangsiensis* Lo. are alkaloids, which have antibacterial and insecticidal activities [8,9]. However, their effects on tumor cells have rarely been reported. This project studies the anti-human small cell lung cancer NCI-H446 cell activity of corydine, a main constituent in root tubers of *Stephania kwangsiensis* Lo., and explores its effects on proliferation and apoptosis of NCI-H446 cells, in order to provide experimental data and theoretical basis for clinical application of corydine in treatment of human small cell lung cancer.

**Experimental Section**

**Chemistry**

**Reagents and instruments:** API 4000 triple quadrupole LC-MS system (Applied Biosystems, USA); AVANCE 600 superconducting actively shielded Fourier transform NMR spectrometer (TMS as internal standard, Bruker, Switzerland); N1100 rotary evaporator (EYELA, Japan); Sephadex LH-20 gel (GE, USA); ODS-A reversed phase silica gel (YMC, Japan); silica gel (Qingdao Haiyang Chemical Plant); chemical reagents (AR grade, Tianjin Weilong Chemical Reagents Co., Ltd.). Medicinal material was purchased from Fangzheng Pharmacy, which was identified by Associate Professor Wang Qingjia at School of Chinese Materia Medica, Chengdu University of TCM as the root tubers of Menispermaceae Stephania plant *Stephania kwangsiensis* Lo.

**Extraction and isolation:** *Stephania kwangsiensis* Lo. root tubers were ground with plant mill, and passed through a 60 mesh sieve. 10 Kg dry powder was then weighed, placed in a
Alkaloids from root tubers of Stephania kwangsiensis H.S.Lo

Biology

Reagents and instruments: Corydine compound was prepared by the laboratory, while human lung cancer NCI-H446 cell lines (batch No.: 20150113) were provided by Shanghai Institute of Cell Biology, CAS. RPMI 1640 medium (GIBCO, USA, each L containing 0.33 mg of L-glutamine, 10% FBS, 100 U of streptomycin, 100 U of penicillin, pH 7.4); trypsin (MERCK, USA); AR grade H2O2 (Tianjin Weilong Chemical Reagents and instruments); DMSO and FBS (provided by Nanjing Senbeijia Biotechnology Co., Ltd.); Annexin-V-FITC (PI) double staining reagent (provided by Nadika Biotechnology Co., Ltd.). Corydine was dissolved in DMSO, and then filtered and sterilized through a 0.22 μm nylon membrane to give a 100 mg/ml stock solution, which was stored in a -20 refrigerator.

Cell cultivation: Human small cell lung cancer NCI-H446 cells were cultured in a pH 7.4, 10% FBS containing RPMI 1640 medium under 37, 5% CO2 incubator conditions. Culture medium was replaced once every 48 h and the cells were passaged at a 1:5 ratio.

NCI-H446 cell proliferation inhibition rate: Logarithmic phase NCI-H446 cells were prepared into a 1×105/ml cell suspension with RPMI 1640 medium, seeded in 96-well plates at 200 μl per well, and cultured for 24 h. After cells were adherent, culture medium was aspirated off. 1) Treatment groups were added with different concentrations of corydine (final mass concentrations of 20, 10, 5 μg/ml), respectively, at 200 μl per well. 2) Control group was added with an equivalent volume of blank RPMI 1640 medium with binding buffer. Afterwards, 5 ml of the density-adjusted cells were placed into culture tubes, added with 5 μl of Annexin-V-FITC and 5 μl of PI, and reacted under dark conditions in the refrigerator at 4 for 30 min, followed by determination of apoptosis rate with flow cytometer.

Statistical processing: All the experimental data were expressed as mean ± standard deviation ( ± s), with 95% reference range. F-test, as well as chi-square test of apoptosis rate between different treatment groups were performed using SPSS 16.0 for Windows at a significance level α=0.05.

Results and Discussion

Chemistry

Structure elucidation

Compound 1: Bright yellow acicular crystals (methanol); soluble in water, methanol, ethanol, acetone, etc. 1H-NMR (600 MHz, DMSO-d6) δ: 7.05 (1H, s, H-1), 7.56 (1H, s, H-4), 3.29 (2H, t, J=6.4 Hz, H-5), 4.92 (2H, t, J=6.4 Hz, H-6), 9.74 (1H, s, H-8), 8.10 (1H, d, J=8.8, H-11), 8.02 (1H, d, J=8.8, H-12), 8.77 (1H, s, H-13), 4.18 (3H, s, COCH3), 4.12 (3H, s, COCH3), 3.88 (3H, s, COCH3), 3.94 (3H, s, COCH3); 13C-NMR (150 MHz, DMSO-d6) δ: 110.2 (C-1), 151.1 (C-2), 153.7 (C-3), 112.4 (C-4), 27.9 (C-5), 56.7 (C-6), 146.6 (C-8), 152.7 (C-9), 145.3 (C-10), 121.9 (C-11), 124.2 (C-12), 128.2 (C-13), 139.7 (C-14), 23.7 (C-15), 130.2 (C-16), 121.5 (C-17), 134.3 (C-18), 56.6 (C2-OCH3), 57.2 (C3-OCH3), 62.7 (C9-OCH3), 56.2 (C10-OCH3). The above data were basically consistent with the literature [10], so the compound was identified as palmatine.

Compound 2: Silver white acicular crystals (acetone); soluble in acetone, ethyl ether, ethyl acetate and chloroform. EIMS(m/z):341(M+), 326, 310, 155. 1H-NMR (600 MHz, DMSO-d6) δ: 6.71 (1H, s, H-3), 7.02 (1H, d, J=8.4, H-8), 7.15 (1H, d, J=8.4, H-9), 2.54 (3H, s, H-17), 3.71 (3H, s, COCH3), 3.83 (1H, s, COCH3), 3.92 (1H, s, COCH3); 13C-NMR(150 MHz, DMSO-d6) δ: 144.2 (C-1), 150.6 (C-2), 113.2 (C-3), 29.6 (C-4), 62.5 (C-5), 63.7 (C-6), 35.9 (C-7), 125.2 (C-8), 112.4 (C-9), 154.1 (C-10), 145.2 (C-11), 126.7 (C-12), 119.2 (C-13), 121.5 (C-14), 132.1 (C-15), 129.5 (C-16), 44.4 (C-17), 53.5 (C2-OCH3), 56.4 (C10-OCH3), 56.6 (C11-OCH3).
above data were basically consistent with the literature [11], so the compound was identified as corydine.

**Compound 3:** White cubic crystals (ethanol); soluble in methanol and ethanol. EI-MS (m/z): 327 (M+), 312, 299, 284. 1H-NMR (600 MHz, DMSO-d6) δ: 6.82 (1H, d, J=8.4 Hz, H-1), 6.67 (1H, d, J=8.4 Hz, H-2), 6.30 (1H, s, H-5), 7.87 (1H, s, H-8), 3.72 (3H, s, COCH3), 3.90 (3H, s, COCH3), 2.43 (3H, s, NCH3); 13C-NMR (150 MHz, DMSO-d6) δ: 123.5 (C-1), 111.7 (C-2), 148.2 (C-3), 146.5 (C-4), 120.1 (C-5), 165.3 (C-6), 183.8 (C-7), 123.3 (C-8), 62.5 (C-9), 34.1 (C-10), 131.2 (C-11), 124.8 (C-12), 45.4 (C-13), 151.9 (C-14), 38.5 (C-15), 48.1 (C-16), 56.5 (C3-OCH3), 55.2 (C6-OCH3), 41.4 (NCH3). The above data were basically consistent with the literature [12], so the compound was identified as sinoacutine.

**Biology**

Effects of corydine on NCI-H446 cell proliferation are shown in Table 1.

<table>
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<tr>
<th>Group</th>
<th>Mass concentration (μg/ml)</th>
<th>24 h</th>
<th>48 h</th>
<th>72 h</th>
<th>96 h</th>
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<tr>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>Treatment group 20</td>
<td>20</td>
<td>48.39 ± 2.23</td>
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<td>75.12 ± 5.91</td>
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<td>Treatment group 10</td>
<td>10</td>
<td>31.34 ± 7.27</td>
<td>44.56 ± 4.36</td>
<td>59.87 ± 0.31</td>
<td>74.51 ± 3.22</td>
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<tr>
<td>Treatment group 5</td>
<td>5</td>
<td>24.85 ± 1.39</td>
<td>31.23 ± 2.12</td>
<td>40.11 ± 3.99</td>
<td>60.12 ± 3.19</td>
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</table>

Effects of different mass concentrations of corydine on apoptosis rate of NCI-H446 cells are shown in Table 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mass concentration (μg/ml)</th>
<th>Apoptosis rate (%)</th>
<th>Standard error</th>
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<td>1.02</td>
<td>0.41</td>
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<tr>
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<td>20</td>
<td>8.77</td>
<td>1.29</td>
</tr>
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<td>10</td>
<td>9.12</td>
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<tr>
<td>Treatment group 5</td>
<td>5</td>
<td>12.36</td>
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Changes in cell morphology are shown in Figure 1.
specific molecular mechanisms of its actions on human small cell lung cancer cells remain to be further studied.

Conflict of Interests
The authors declare that they have no conflict of interests.

References
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