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
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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: (batch No.: 20150316); MTI (Sigma, USA); 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 for later use.

The extract was taken out, dissolved in water, and extracted sequentially with petroleum ether, ethyl acetate and n-butanol to give petroleum ether phase, ethyl acetate phase and n-butanol phase. The ethyl acetate phase was isolated and recrystallized by repeated normal phase silica gel, reversed phase silica gel and sephadex LH-20 column chromatographies to obtain three compounds.

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), 4.38 (1H, 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), 27.3 (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), 235.3 (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), 62.9 (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. EI-MS(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 (C1), 150.6 (C2), 113.2 (C3), 29.6 (C4), 62.5 (C5), 63.7 (C6), 35.9 (C7), 125.2 (C8), 112.4 (C9), 154.1 (C10), 145.2 (C11), 126.7 (C12), 119.2 (C13), 121.5 (C14), 132.1 (C15), 129.5 (C16), 44.4 (C17), 53.5 (C2-OCH3), 56.4 (C10-OCH3), 56.6 (C11-OCH3). The

Alkaloids from root tubers of Stephania kwangsiensis H.S.Lo sequentially with petroleum ether, ethyl acetate and n-butanol extract weighing 3.5 Kg, which was then stored in a refrigerator for later use.

The extract was taken out, dissolved in water, and extracted sequentially with petroleum ether, ethyl acetate and n-butanol to give petroleum ether phase, ethyl acetate phase and n-butanol phase. The ethyl acetate phase was isolated and recrystallized by repeated normal phase silica gel, reversed phase silica gel and sephadex LH-20 column chromatographies to obtain three compounds.
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.

<p>| Table 1: Effects of corydine on NCI-H446 cell proliferation (rate of cell apoptosis, ± s). Note: Comparison with the control group, *P&lt;0.05, **P&lt;0.01. |</p>
<table>
<thead>
<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>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>-</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Treatment group</td>
<td>20</td>
<td>48.39 ± 2.23**</td>
<td>59.01 ± 4.71**</td>
<td>75.12 ± 5.91</td>
<td>86.81 ± 7.99**</td>
</tr>
<tr>
<td>Treatment group</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>
</tr>
<tr>
<td>Treatment group</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>
</tr>
</tbody>
</table>

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

<p>| Table 2: Effects of different mass concentrations of corydine on apoptosis rate of NCI-H446 cells (n=6). Note: Comparison with the control group, *P&lt;0.05. |</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Mass concentration (μg/ml)</th>
<th>Apoptosis rate (%)</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0</td>
<td>1.02</td>
<td>0.41</td>
</tr>
<tr>
<td>Treatment group</td>
<td>20</td>
<td>8.77*</td>
<td>1.29</td>
</tr>
<tr>
<td>Treatment group</td>
<td>10</td>
<td>9.12*</td>
<td>2.01</td>
</tr>
<tr>
<td>Treatment group</td>
<td>5</td>
<td>12.38*</td>
<td>2.12</td>
</tr>
</tbody>
</table>

Changes in cell morphology are shown in Figure 1

![Figure 1: Morphology change of lung NCI-H446 cells treated with corydine. A: Control group. B: 20 μg/ml corydine group](image)

We observed the morphology of lung adenocarcinoma N446
cells with an inverted optical microscope. Experimental results
showed that corydine had some cytotoxic effect on A549 cells
(Figure 1). In the non-treatment group, cells presented intact
morphology, uniform size, clear outline and vigorous growth,
all of which grew adherently. No contact inhibition was
observed between cells, indicating that cells were in a very
healthy state. After treatment with corydine solution, N446
cells exhibited obvious morphological changes, such as
decrease in cellular volume and rounding and shrinkage of
cells. Cells dramatically decreased in number, with almost no
presence of viable cells. Light microscopic results
demonstrated that corydine can effectively inhibit the growth
of lung adenocarcinoma N446 cells and change their
morphology markedly.

**Stephania kwangsiensis** Lo. is a Stephania plant in the family
Menispermaceae, from whose root tubers bioactive substances
having a variety of pharmacological actions [2] can be
extracted, especially anti-tumor actions, since they have multi-
target, multi-channel anti-tumor effects such as induction of
tumor cell apoptosis, promotion of tumor cell differentiation,
inhibition of intratumoral angiogenesis and suppression of
tumor cell metastasis. Many studies have shown [13-15] that
alkaloids like corydine, alone or in combination, can inhibit the
proliferation of various tumor cells, and induce their apoptosis.
These alkaloids have prominent cytostatic and proapoptotic
effects on tumor cells. Moreover, their cytostatic and
proapoptotic mechanisms are associated with the arrest of
tumor cells in G1 phase, which causes cell cycle imbalance,
thereby leading to stoppage of cell proliferation and occurrence
of apoptosis.

**Conclusions**

The results of this study demonstrate that corydine has marked
cytostatic and proapoptotic effects on human small cell lung
cancer NCI-H446 cells, suggesting its potential value for
clinical treatment of small cell lung cancer. This study only
makes a preliminary exploration on the effects of corydine on
proliferation and apoptosis of NCI-H446 cells, while the
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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