Cytotoxic Activity of Acutiaporberine, a Novel Bisalkaloid of Plant Origin, on Several Human Carcinoma Cell Lines *

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Abstract: The cytotoxic activity of a novel ether-linkaged bisalkaloid isolated from the root of *Thalictrum acutifolium* (Hand.-Mazz.) Boivin., acutiaporberine, on several human carcinoma cell lines was described for the first time. Acutiaporberine was added to the culture in 7 concentrations ranging from 2.5 μg/mL to 100 μg/mL. It showed cytotoxic activity to four cultured human carcinoma cell lines, i.e. Hela (IC_{50} = 37 μg/mL), LCC (IC_{50} = 60 μg/mL), MGC (IC_{50} = 65 μg/mL), NSCLC (IC_{50} = 82 μg/mL), and two normal cell lines, L-02 (IC_{50} = 65 μg/mL) and NIH 3T3 (IC_{50} = 78 μg/mL). But it was less active to HepG2 and Mcf-7 (IC_{50} > 100 μg/mL).

Keywords: acutiaporberine; *Thalictrum acutifolium* (Hand.-Mazz.) Boivin.; cytotoxic activity
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Although chemotherapy treatments to human tumors have been improved a lot, there still lack highly effective and low toxic agents for treating some common malignant tumors, especially solid tumors. One hopeful resource for searching for these agents is the medicinal herbs.

Study of the plants of *Thalictrum* genus is of interests, many species of this genus are often used as Chinese folk medicine. They are used in antitumor, anti-inflammation, anti-virus and antibacterial treatments. They contain many kinds of alkaloids which show antiproliferative activities on a broad range of animal and human tumor cell lines *in vitro*[1,2] and have antitumor activities on several tumor models *in vivo*[3,4]. Among these alkaloids, Thaliblastine (chemical name thalicarpine)[5] is an outstanding example. In clinical trials it was well tolerated without sever toxities[6~8] and positive results have been obtained especially to some drug resistant cancers[7,8].

From the root of the herb *Thalictrum acutifolium* (Hand.-Mazz.) Boivin, which is indigenous to China, a novel bisalkaloids, acutiaporberine, was isolated and identified[9]. Previous studies of the same plant have led to isolation of 8 compounds, i. e. trilobinine, *cis*-9-*cis*-12-methyloctadecadienoate, oxyberberine, methyl palmitate, methyl linoleate, β-sitosterol, nonacosane and n-pentatriacontane[10]. Continuing study of the root constituents of this plant led to the isolation of acutiaporberine, a new ether-linkaged bisalkaloid composed of an aporphine and a protoberbertine. It has a novel chemical structure (Fig.1) confirmed by IR, MS, ^{1}H NMR, ^{13}C NMR and 2D-NMR (^{1}H-^{1}H COSY, ^{13}C-^{1}H COSY, NOESY and HMNC) experiments[9]. By comparing its structure with thaliblastine's (Fig.2), resemblance can be found, hinting that acutiaporberine might have similar activities.

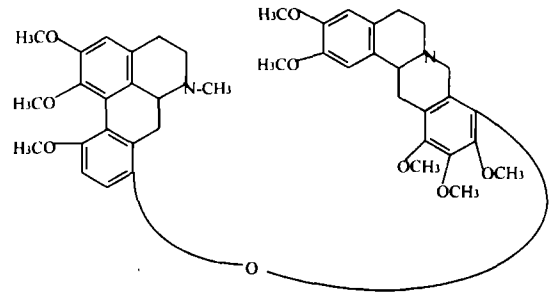

Fig.1 Chemical structure of acutiaporberine

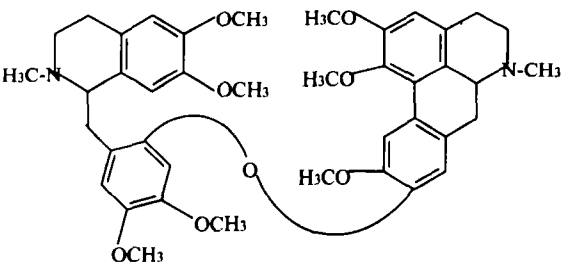

Fig.2 Chemical structure of thalicarpine

In this study, acutiaporberine was subjected to evaluation for cytotoxic activity. It showed cytotoxic

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activity to four cultured human carcinoma cell lines, i.e. Hela (cervix adenocarcinoma), LCC (hepatocellular carcinoma), MGC (gastric carcinoma), NSCLC (non-small cell lung carcer), and two normal cell lines, L-02 (human liver cell) and NIH 3T3 (mouse fibroblast). But it was less active to another human hepatocellular carcinoma cell line HepG2 and human mammary carcinoma cell line Mcf-7.

1 Materials and methods

1.1 Cell cultures

Six human carcinoma cell lines, i.e. Hela (cervix adenocarcinoma), LCC (hepatocellular carcinoma), MGC (gastric carcinoma), NSCLC (non-small cell lung carcer), HepG2 (hepauocellular carcinoma), Mcf-7 (human mammary carcinoma) and two normal cell lines, i.e. L-02 (human liver cell) and NIH 3T3 (mouse fibroblast) were used. Hela, HepG2, MCF-7 and NIH3T3 were bought from Shanghai Cell Institute, Chinese Academy of Science. L-02 was obtained from Bio-physics Institute, Chinese Academy of Science. LCC, MGC and NSCLC were obtained from the First Military Medical University of China. These cells were cultured in RMPI 1640 medium containing 10% fetal bovine serum, at 37 ℃ in a humidified atmosphere with $\varphi = 5\%$ CO_2 in the air.

1.2 Drug

Acutiaporberine is a light yellow crystalline flake with a relative molecular mass of 724.85, $\theta_{mp} = 183.5 \sim 184$ ℃, optical rotation $[\alpha] = -14.2°$ (3% CH_3OH). It was readily soluble in water containing 10% DMSO and kindly provided by Dr. LIM Cui-wu and co-workers. The drug was diluted with RPMI 1640 medium before use. RPMI 1640 medium was supplied by Gibico Co. (USA) and fetal bovine serum by Hyclone Co. (USA).

1.3 Evaluation of cytotoxic activity

Cytotoxic activities of the components isolated were detected by a modified MTT assay. Cells were seeded into 96-well tissue culture plate (Falcon) at the density of 1×10^5 cells/mL using fresh medium supplemented by 10% fetal bovine serum. After 24 hours, the liquid in each well was removed and medium containing drug were added to establish cultures at 7 drug concentrations ranging from 2.5 to 100 μg/mL in triplicate. After 48 hours of incubation, cell viability was measured by MTT assay. 20 μL of 5 mg/mL MTT were added to each well, and the plates were incubated at 37 ℃ for a further 4 hours. Liquid in each well was carefully removed. Formazan crystals were redissolved in DMSO for 10 min with shaking. The plate was read immediately on a microplate reader (ELX800, BIO-TEK Instruments, Inc.) at a wavelength of 490nm. IC_{50} value was calculated from linear regression of the percent of absorbance reduction versus the log of the drug concentration.

2 Results and discussion

As shown in Table 1, acutiaporberine displayed significant cytotoxic activity to four of the carcinoma cell lines, i.e. Hela ($IC_{50} = 37$ μg/mL), LCC ($IC_{50} = 60$ μg/mL), MGC ($IC_{50} = 65$ μg/mL) and NSCLC ($IC_{50} = 80$ μg/mL), while to HepG2 and MCF-7 it was less cytotoxic ($IC_{50} > 100$ μg/mL). But to the two tested normal cell lines, it also showed cytotoxic activity.

At the highest concentration (100 μg/mL), acutiaporberine destroyed all cells in the culture of Hela, LCC, MGC, NSCLC, L-02 and NIH3T3 at the end of culture period. The results were shown in Fig.3: 1 ~ 12. However, in the culture of HepG2 and MCF-7, it only destroyed part of the cells as shown in Fig.3: 13 ~ 16.

Acutiaporberine is a newly identified novel bisalkaloid isolated from a medical herb often used in China. Its chemical structure has some resemblance to the antitumor agent thaliblastine, which is of the same origin and which showed no myelotoxity, immunosuppression, hepatotoxicity, renal toxity or long-lasting side-effects in clinical trials. This *in vitro* experiment shows that acutiaporberine has cytotoxic activity to a range of human solid tumor cell lines. Furthermore, acutiaporberine has a novel structure and is the first one of its kind reported possessing cytotoxic activity. Thus, the biological activities of acutiaporberine are worth investigating.

Overall, results of this study suggest that further *in vitro* and *in vivo* investigations on the antitumor activity of acutiaporberine should be carried out and the study of the mechanism of its action done to find out whether acutiaporberine might be a candidate for antitumor treatment.

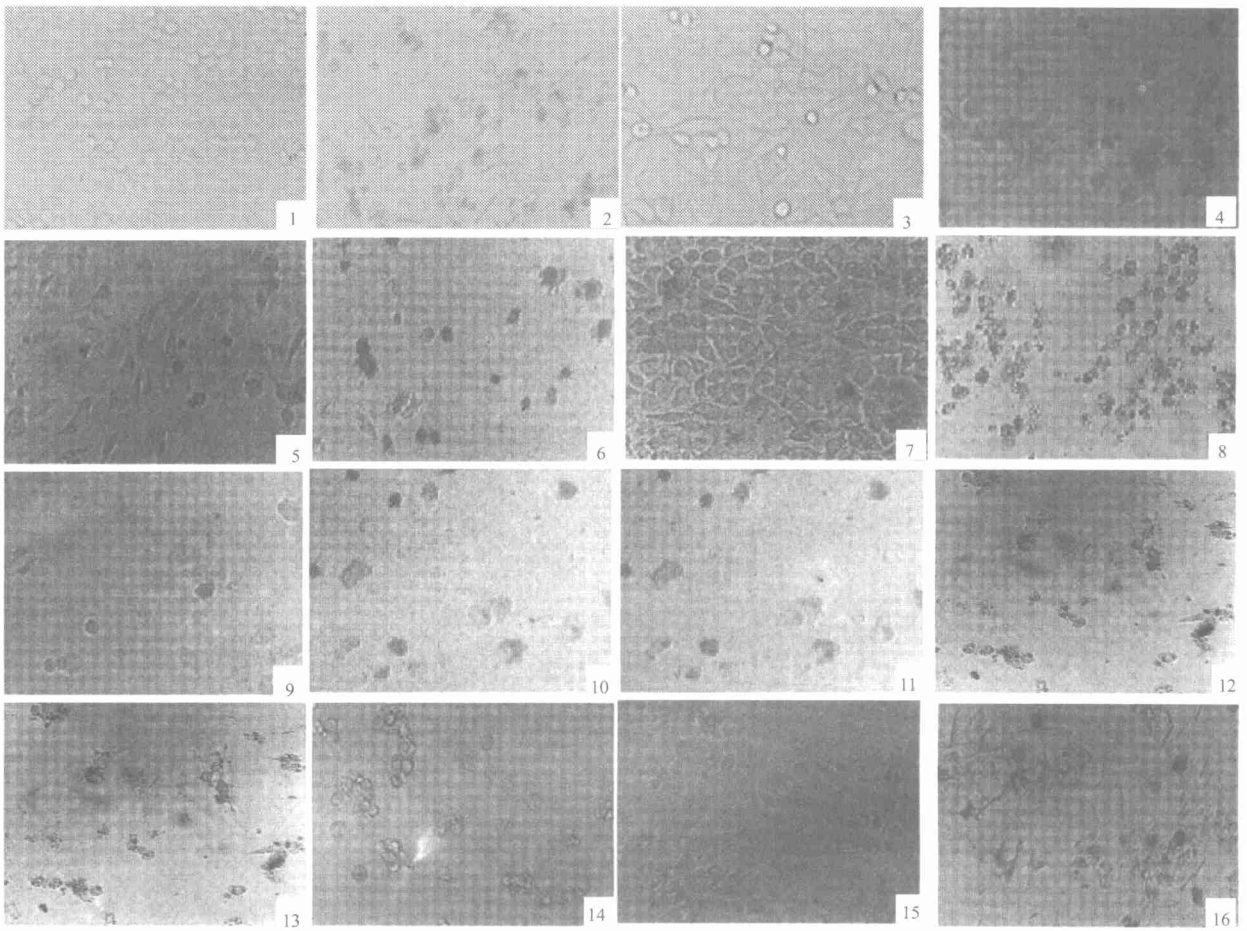

Fig.3　Photo 1, 3, 5, 7, 9, 11, 13 and 15 show control cells of Hela, LCC, MGC, NSCLC, L-02, NIH3T3, HepG2; Photo 2, 4, 6, 8, 10, 12, 14 and 16 show the corresponding cells incubated with 100 μg/mL acutiaporberine for 48 h

Tab.1　IC_{50} values of acutiaporberine (Ⅰ) to several cultured cell lines　μg/mL

	Hela	LCC	MGC-803	NSCLC	HepG2	MCF-7	L-02	NIH3T3
Acutiaporberine (Ⅰ)	37	60	65	80	> 100	> 100	65	78

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新型植物来源生物碱

——唐松草阿原碱对人体肿瘤细胞株的细胞毒性研究

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摘 要：研究从毛茛科唐松草属植物尖叶唐松草根部提取的新型醚键双生物碱——唐松草阿原碱对几种人体肿瘤细胞株的毒性作用. 唐松草阿原碱以质量浓度从 2.5 μg/mL 到 100 μg/mL 作用于 6 种体外培养的肿瘤细胞株及 2 种正常细胞株，对其中 4 个肿瘤细胞株种表现了强烈的杀伤作用，IC_{50}分别为：宫颈癌 Hela 37 μg/mL，肝癌 LCC 60 μg/mL，胃癌 MGC 65 μg/mL，非小细胞肺癌 PLA-801 82 μg/mL. 对 2 个正常细胞株的毒性分别为：肝细胞 L-02 65 μg/mL，小鼠成纤维细胞 NIH3T378 μg/mL. 而对另一株肝癌 HepG2 及乳腺癌细胞 MCF-7 无明显作用（$IC_{50} > 100$ μg/mL）.

关键词：唐松草阿原碱；尖叶唐松草；细胞毒性
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The Biological Studies of Growth and Development as well as Larval Rearing in Marine Fishes

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Abstract: The fish growth and developmental biology is the theoretical bases of larval rearing in marine fishes. Recent advances in fish growth and developmental biology provided fundaments for the improvement of larval rearing in marine fishes. The major advances are: elucidated the secretion and action of the hormones from the brain-pituitary-liver axis which control the growth of fish, promoted growth hormone (GH) release and enhanced growth rate in marine fishes; the morphological and physiological characteristics of larval growth and development in marine fish as well as digestion and absorption of diet; the essential nutrients of marine fish larvae during growth and development; the application of dietary supplements to facilitated ingest and digest of diets in marine fish larvae; the effect of environmental factors on the utilization of nutrients and growth in marine fish larvae, etc. The advances up till the present moment are confined to few cultured marine fish, a great deal of fundamental theories are remained to be further investigation. In future, in order to coordinate the development of proliferation and culture of marine fish, the deeply and systematic research of growth and developmental biology in the major cultured marine fishes are necessary, and then establish new techniques of larval rearing based on these research.

Keywords: marine fishes; growth; development; larval rearing