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Cytotoxic activity of trewiasine in 4 human cancer cell lines and 5 murine tumors[1]

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ABSTRACT Trewiasine (TWS) is a mytansinoid compound. It possessed a significant cytotoxic activity against various human cancer cell lines *in vitro*. U937 cells, which were more sensitive to the TWS, required TWS 1 $\mu g \cdot ml^{-1}$ to inhibit cell growth over 90% ($P<0.01$). TWS also showed activities against murine tumors *in vivo*, such as the ascitic tumors S180, hepatoma, U14, and solid tumor Lewis lung carcinoma.

Depression of leukocytes was not seen when mice were given ip TWS 10 or 50 $\mu g \cdot kg^{-1} \cdot d^{-1} \times 7$ d. TWS 0.1-1 $\mu g \cdot ml^{-1}$ caused no sister chromatid exchange induction in Chinese hamster cell line V79.

KEY WORDS trewiasine; maytansine; cultured tumor cells; experimental neoplasms; phytogenic antineoplastic agents

In previous studies of *Trewia nudiflora* L seeds, Powell *et al* reported structures of TWS and other maytansinoid compounds[1].

In the present study, TWS was isolated from seeds of *Trewia nudiflora* L obtained from the Xishuangbanna area of China. Antitumor activities of TWS against leukemia P388, sarcoma 180 (S180), and Ehrlich ascites tumor were reported previously and its antitumor effect may be associated with inhibiting action on protein synthesis[2]. In this paper, a further evaluation of TWS was performed in human cancer cell lines *in vitro* and other experimental tumor models *in vivo*.

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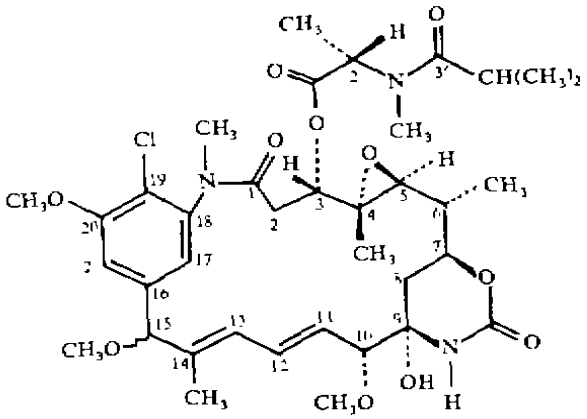

Trewiasine

MATERIALS AND METHODS

TWS was supplied by Dr LI Bing-Jun. Its purity was 99%. TWS was first solubilized in Me_2SO and then diluted with 0.9% NaCl.

***In vitro* assay of growth inhibition** The human cancer cell lines including lung cancer A544, gastric cancer SGC-7901, liver cancer BEL-7402, and acute monocytic leukemia U937 were used for the assay[3-5]. U937 was maintained as suspension culture and other 3 cell lines were grown as monolayers in RPMI 1640 medium (Gibco) supplemented with 10-15% fetal calf serum and antibiotics (penicillin 100 U and streptomycin 100 $\mu g \cdot ml^{-1}$). The cultures were incubated at 37℃ in a humidified atmosphere of 5% CO_2. Logarithmically growing cells were seeded into 40-well plates and incubated for 24 h at 37℃. TWS was cultured with cells for 24 or 48 h. The number of living cells was counted

with the Trypanblau dye exclusion test.

In vivo **treatment** S180, ascitic hepatoma (Hep A), and carcinoma of uterine cervix (U14) were implanted ip into Kunming (KM) mice and melanoma B16, Lewis lung carcinoma were inoculated sc at the axillary region in C57BL mice. Drug was injected ip the next day after tumor inoculation. Therapeutic effect of TWS was evaluated by survival time for ip implanted ascitic tumors, and by tumor weight for sc implanted solid tumors. The *t* test was used for statistical analysis.

RESULTS

Cytotoxicity toward human cancer cells When monolayer cells of A544, SGC−7901, and BEL−7402 were cultured with TWS for 48 h, their growth inhibitions were seen (Tab 1). U937 cells were grown in suspension containing TWS for 24 h, and were the most sensitive to TWS 1.3−130 $\mu mol \cdot ml^{-1}$ with maximal inhibition of 91−95% ($P<0.01$).

Therapeutic effect on implanted tumors TWS exhibited significant life−prolonging effects on ip−ip models of S180, Hep A, and U14 ($P<0.01$) (Tab 2). TWS 10 $\mu g \cdot kg^{-1} \cdot d^{-1}$

Tab 1. Growth inhibitory activity of trewiasine toward various human cancer cell lines. $n=3-6$, $\bar{x} \pm s$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

Cell line (origin)	Concn / $\mu mol \cdot ml^{-1}$	$10^{-4} \times$ Number of cells $\cdot ml^{-1}$	Growth inhibition / %
A544	NS	17 ± 3.0	
(lung	1.3	8.5 ± 2.0	50**
carcinoma)	13	7.3 ± 2.0	56***,
	130	5.4 ± 1.6	68***
SGC−7901	NS	61 ± 8.0	
(gastric	1.3	32 ± 5.0	48**
carcinoma)	13	21.2 ± 2.3	65***
	130	17.7 ± 2.7	71***
BEL−7402	NS	34 ± 9.0	
(liver	1.3	14.5 ± 0.8	57***
carcinoma)	13	15.8 ± 2.5	54***
	130	9.0 ± 3.0	72***
U937	NS	82 ± 17.0	
(acute	1.3	6.9 ± 1.9	91***
monocytic	13	5.7 ± 1.2	93***
leukemia)	130	4.4 ± 0.9	95***

Tab 2. Antitumor activity of TWS against ascitic tumors and solid tumors in mice. $\bar{x} \pm s$. $^{*}P>0.05$, $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control. Number of mice in parentheses.**

Tumor	Dose, $\mu g \cdot kg^{-1} \cdot d^{-1}$ × 7 d	Ascitic forms (ip−ip) Survival, d Treated / Control	Increase in lifespan, %	Solid forms (sc−ip) Tumor weight, g Treated / Control	Inhibition, %
S180	2.5	40 ± 12 (8) / 16.4 ± 2.1 (16)	144***	1.6 ± 0.4 (10) / 1.5 ± 0.6 (20)	0
	5.0	49 ± 11 (8) / 16.4 ± 2.1 (16)	199***	1.4 ± 0.6 (10) / 1.5 ± 0.6 (20)	6.7*
	10.0	48 ± 9 (8) / 16.4 + 2.1 (16)	193***	1.3 ± 0.6 (10) / 1.5 ± 0.6 (20)	13*
Hep A	2.5	46 ± 15 (8) / 18 ± 5 (16)	155***		
	5.0	37 ± 9 (8) / 18 ± 5 (16)	105***		
	10.0	52 ± 12 (8) / 18 ± 5 (16)	189***		
U14	5.0	34 ± 16 (8) / 14.5 ± 1.2 (16)	134***		
	10.0	38 ± 17 (8) / 14.5 ± 1.2 (16)	162***		
B16	2.5			1.8 ± 0.7 (10) / 1.4 ± 0.7 (20)	0
	5.0			1.8 ± 0.7 (10) / 1.4 ± 0.7 (20)	0
	10.0			1.6 ± 0.7 (10) / 1.4 ± 0.7 (20)	0
Lewis lung	2.5			1.1 ± 0.4 (10) / 1.4 ± 0.4 (15)	21*
carcinoma	5.0			1.2 ± 0.5 (10) / 1.4 ± 0.4 (15)	14*
	10.0			1.1 ± 0.5 (20) / 1.7 ± 0.9 (34)	35**

× 7 d showed a 35% inhibition ($P<0.05$) on the growth of Lewis lung carcinoma. No effect was seen on S180 and melanoma B16 in the sc–ip system.

Toxicity of TWS in mice LD_{50} was 497 $\mu g \cdot kg^{-1}$ after single ip injection and 34.5 $\mu g \cdot kg^{-1}$ by daily ip injection for 7 d. Successive injections attained a lower total dose than a single injection.

The leukocytes counts were found to be 11.8×10^3–13.5×10^3 cells / mm^3 after ip TWS 10 and 50 $\mu g \cdot kg^{-1} \cdot d^{-1} \times 7$ d. The leukocytes counts obtained in treated groups were not significantly elevated *vs* those of control.

Sister chromatid exchange (SCE) analysis The number of SCE in Chinese hamster cell line V79[16] was counted under microscope (× 1000). TWS did not produce any SCE induction at the doses of 0.1–1 $\mu g \cdot ml^{-1}$.

DISCUSSION

TWS showed marked activity only against cultured cells and murine tumor models in ip–ip system. For S180, it inhibited markedly the ascitic form of mouse tumor, but not the solid form. These results suggested that TWS showed a narrow spectrum of activities against murine tumor models.

The structure of TWS was similar to that of maytansine which also isolated from *Trewia nudiflora* L seeds. The *in vivo* data showed that maytansine was active against melanoma B16 and Lewis lung carcinoma[7]. Our tests demonstrated that TWS was moderately active against Lewis lung carcinoma but had no effect on the other two solid tumor models. Clinical studies indicated that maytansine was an agent with some activity in lung cancer and breast cancer[8,9]. In China, it was also shown to have some therapeutic effects against small cell lung cancer.

On the basis of the antitumor activity in animal models and the clinical data, it is suggested that Lewis lung carcinoma model and A544 lung carcinoma may be useful for screening the new maytansinoids against human lung cancer.

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特利回生对 4 株人癌细胞株及 5 种小鼠肿瘤的细胞毒作用

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提要 特利回生(trewiasine、TWS)是美登素类化合物. 体外研究表明对4种人体肿瘤细胞株有明显抗癌活性，其中对白血病U937最为敏感，1 $\mu g \cdot ml^{-1}$ 抑制率大于90% ($P<0.01$). 特利回生对多种实验动物肿瘤如腹水型的S180，肝癌，U14及实体型的Lewis肺癌等均有明显疗效. 剂量为10 $\mu g \cdot kg^{-1}$ 及50 $\mu g \cdot kg^{-1}$ 时，对小鼠白细胞无明显影响. 特利回生也不诱导SCE形成.

关键词 特利回生；美登素；培养的肿瘤细胞；实验性肿瘤；植物性抗肿瘤药

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Effect of schisandrin B on lipoperoxidative damage to plasma membrane of rat liver *in vitro*

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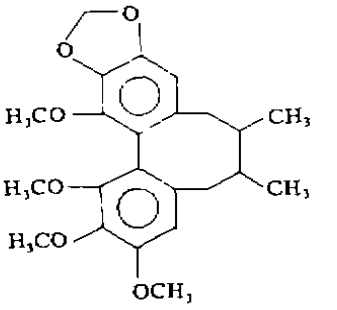
ABSTRACT The effect of schisandrin B (Sin B) on oxygen free radicals-induced lipoperoxidative damage to plasma membrane of rat hepatocytes was investigated. When the plasma membrane of rat hepatocytes was incubated with iron / cysteine or Vit C / NADPH, the production of malondialdehyde (MDA) and consumption of NADPH increased, while the membrane fluidity reduced. Addition of Sin B (3-25 $\mu g \cdot ml^{-1}$) to the incubation mixture inhibited all these alterations of the plasma membrane induced by iron / cysteine and Vit C / NADPH. The results indicated that Sin B could maintain membrane stability of rat hepatocytes under oxidative stress.

KEY WORDS schisandrin B; membrane fluidity; lipid peroxidation; liver; cell membrane; malondialdehyde; NADP

Lipid peroxidation induced by oxygen free radicals is a deteriorative reaction, which results in cell injury[1]. Plasma membrane of cells is rich in polyunsaturated fatty acid and sensitive to lipid peroxidation. Our previous studies demonstrated that schisandrin B (SinB) with the following structure, one of the active components isolated from *Fructus Schisandrae Chinensis* (Turcz) Baill, has significant protective effect against lipoperoxidative damage to cultured rat hepatocytes[2]. The release of transaminase and lactic dehydrogenase (LDH) and malondialdehyde (MDA) production as well as damages of cell surface of hepatocytes were all counteracted[2]. In this paper, the effects of Sin B on MDA production, NADPH consumption and decrease of membrane fluidity of plasma membrane of rat hepatocytes



Schisandrin B

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