过降低肿瘤细胞膜钙泵活性、开放肿瘤细胞膜钙通道及引起肿瘤细胞内钙库释放 3条途径升高细胞内 $[Ca^{2+}]_i$。

参考文献:

[1] 季宇彬,高世勇,孔 琪,等. 海嘧啶抗肿瘤作用的实验研究[J]. 中草药, 2001, 32(6): 524-527.

[2] 季宇彬,张秀娟,孔 琪,等. 海嘧啶对 H_{22}小鼠完整细胞膜脂流动性及人胃腺癌细胞内 DNA含量影响的研究[J]. 中草药, 2001, 32(8): 713-715.

[3] 季宇彬,高世勇,孔 琪,等. 海嘧啶对 SGC-7901人胃癌细胞凋亡的影响[J]. 中草药, 2001, 32(10): 901-904.

[4] 杨易兴. 心力衰竭患者心肌细胞的钙泵活性[J]. 中国危重病急救医学, 1996, 8(1): 44.

[5] Cole K A, Kohn E C. Calcium-mediated signal transdation biology, biochemistry and therapy [J]. Cancer Metastasis Rev, 1994, 13: 33-34.

[6] 曹泽毅. 激素受体及临床应用[M]. 北京: 北京医科大学中国协和医科大学联合出版社, 1993.

[7] 陈 葳,李 旭. 5氟脲嘧啶诱导人卵巢癌细胞凋亡的实验研究[J]. 肿瘤防治研究, 1988, 25(2): 100.

[8] Tauber R. Menmbranes in Tumor Growth [M]. Elsevion Biomedical Press, 1982.

[9] Robinson J M, Smith D F, Davis E M, *et al*. Partial characterization of rat hepotoma cellsurface glucoproteins possession concanavalin A receptor activity [J]. Biochem Biophys Res Commun, 1976, 72: 81-88.

Stellera chamaejasme induced apoptosis of HL-60 cells and regulated expression of *bcl*-2 protein in SGC-7901 cells

JIA Zheng-ping[1], WANG Yan-guang[1], FAN Jun-jie[2], XIE Jing-wen[2], XU Li-ting[2], LIU Sheng[2*]

(1. Department of Chemistry, Zhejiang University, Hangzhou Zhejiang 310027, China; 2. Department of Pharmacy, General Hospital of Lanzhou Command PLA, Lanzhou Gansu 730050, China)

Abstract **Object** To explore the antitumor mechanism of *Stellera chamaejasme* Linn. (SC). **Methods** SC containing-serum (SCCS) was derived from mice pretreated with different doses of SC. Cultured human leukemia HL-60 and human gastric adenocarcinoma SGC-7901 cells were used. Inhibition of proliferation was measured using MTT assay. Morphological assessment of apoptosis was performed with fluorescence microscope. DNA fragmentation was assessed by agarose gel electrophoresis and flow cytometry. Expression of *bcl*-2 protein was measured with immunohistochemistry. **Results** Exposure of exponentially growing HL-60 cells to mice serum containing 10% SC (pretreated with SC 3, 6, and 12 g/kg) for 48 h resulted in growth inhibition in a dose-dependent manner. Typical morphological changes of apoptosis and DNA fragmentation in HL-60 cells were induced. "Apobodies" in the apoptotic cells were observed, "ladder" pattern of agarose gel electrophoresis of DNA from these cells was revealed, and the percentage of apoptotic cells with fractional DNA content increased from 11.7% to 57.4%. Treatment with SC containing serum decreased the percentage of SGC-7901 cells of *bcl*-2 protein positive expression from 78.3% to 32.9%. **Conclusion** SC could induce apoptosis of HL-60 cells and decrease the expression of *bcl*-2 protein of gastric adenocarcinoma SGC-7901 cells.

Key words *Stellera chamaejasme* Linn.; apoptosis; *bcl*-2 protein; cultured tumor cells; flow cytometry; agarose gel electrophoresis

瑞香狼毒诱导 HL-60细胞凋亡和调节 SGC-7901细胞 *bcl*-2蛋白表达

贾正平[1],王彦广[1],樊俊杰[2],谢景文[2],徐丽婷[2],刘 盛[2]

(1. 浙江大学 化学系,浙江 杭州 310027; 2. 兰州军区兰州总医院 药剂科,甘肃 兰州 730050)

* 收稿日期: 2000-10-12

基金项目: 国家自然科学基金资助项目 No29972054

作者简介: 贾正平,男,40岁,理学博士,兰州军区兰州总医院主任药师,解放军第四军医大学硕士生导师,教授。现在浙江大学化学系做博士后研究。主要研究方向:中药及肿瘤药理学。完成的鬼臼毒素衍生物抗肿瘤药理学研究获军队科技进步二等奖。Tel (0931) 8975478

摘 要:目的 探索瑞香狼素(SC)抗肿瘤机制。方法 以 HL-60和 SGC-7901为靶细胞,用 MTT比色法测定细胞增殖抑制,荧光显微镜观察凋亡细胞的形态学改变,DNA电泳和流式细胞仪检测 DNA断裂,免疫组化检测 *bcl*-2蛋白表达。结果 含 SC药物血清处理细胞 48 h后,HL-60细胞增殖呈剂量依赖性抑制,并表现出典型的凋亡细胞形态学改变及 DNA断裂:即染色体聚集、核固缩、断裂及阶梯状 DNA电泳条带,G_1期前细胞从 11.7% 增至 57.4%;而 SGC-7901细胞 *bcl*-2蛋白表达率从 78.3% 下降到 32.9%。结论 SC可诱导肿瘤细胞凋亡,降低 *bcl*-2蛋白表达。

关键词:瑞香狼毒;凋亡;*bcl*-2蛋白;培养的肿瘤细胞;流式细胞计数;琼脂凝胶电泳
中图分类号:R286.91 文献标识码:A 文章编号:0253-2670(2001)12-1097-05

Stellera chamaejasme Linn. (SC) used as a Chinese herb medicine is the radix of the plant *S. chamaejasme*[1]. In our previous work[2-4], it was found that SC inhibited growth of transplantable mouse tumors, sarcoma 180, hepatic carcinoma HePS and lung carcinoma Lewis. *In vitro*, the *Stellera chamaejasme* containing-serum (SCCS) of mouse exhibited remarkable inhibitory effects on proliferation, clone formation and incorporation of [^{3}H] deoxythymidine into DNA of mouse leukemia L_{1210} cells. In order to obtain further insight of its mechanism of antitumor activity, this study is focused on whether SC could induce apoptosis in human leukemia HL-60 cells and regulate expression of *bcl*-2 protein in human gastric adenocarinoma SGC-7901 cells.

1 Materials and methods

1.1 Reagents: SC was purchased from the Department of Chinese Herb Medicine, Lanzhou Center of Drug Supply and its authenticity identified by professor Xie Jingwen. 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) and RPMI-1640 medium were the products of Sigma.

1.2 Preparation of SCCS: 200 g SC coarse powder was extracted with 500 mL water at 100℃ for 30 h, cooled and filtered. The filtrate was concentrated to 200 mL to give the SC extract. 40 mice were divided into four groups, and treated orally with the SC extracts at doses of 0, 3, 6, 12 g/kg respectively. After 2 h, blood of mice was collected under aseptic condition, and the SCCS was obtained by centrifuging (1 000× g for 10 min.).

1.3 Cell culture and drug treatment: Human leukemic HL-60 cells and human gastric adenocarcinoma SGC-7901 cells were purchased from Shanghai Institute of Cell Biology, Chinese Academy of Sciences, and maintained in RPMI-1640 medium supplemented with 10% heat-inactivated calf serum, penicillin 100 kU/L, and streptomycin 100 mg/L in a humidified atmosphere containing 5% CO_2 at 37℃. Exponentially growing cells (5×10^7 cells/L) were exposed to 10% SCCS derived from mice pretreated orally with different doses of SCCS for 48 h. The cells were harvested by centrifuging at 200× g and washed with BPS.

1.4 Cell growth and morphological assessment: Cell viability was determined by MTT assay. For observation of chromatin condensation, cells were stained by 4′, 6-diamido-2-phenylindole hydrochloride[6]. The condensed chromatin parts containing some cytoplasm that appeared as "dots" under fluorescence microscope were called as "Apobodies".

1.5 Determination of DNA content by flow cytometry: The DNA degradation of apoptotic cells was determined directly by flow cytometry[5]. Briefly, the cells cultured with SCCS for 48 h were washed twice with PBS and fixed in cold 70% ethanol for 24 h at 0℃~ 4℃. After removal of ethanol, the cells were incubated in PBS containing RNase A 50 mg/L at 37℃ for 1 h and stained with 10 mg/L propidium iodide (PI, Sigma) at 37℃ for 1 h. Distribution of cells with different DNA contents was determined on flow cytometry (Becton Dickinson). Apoptotic cells were calculated by determining the percentage of cells with a DNA content less than that of G_1 phase.

1.6 DNA gel electrophoresis: After 48 h incubation with SCCS, fragmented DNA was analyzed by electrophoresis. The cellular DNA was extracted, dialyzed and electrophoresed in 1.8% agarose gel.

DNA was visualized with ethidium bromide.

1.7 *Bcl*-2 immunohistochemical analysis: The SGC-7901 cells grown on coverglass were treated with SCCS for 48 h. Washed with 0.01 M PBS buffer twice, the cells were fixed with acetone containing 1% H_2O_2 at 25 ℃ ~ 30 ℃ for 20 ~ 30 min, then washed with 0.01 M PBS 3 times, and incubated with anti-*bal*-2 protein mouse monoclonal antibody (1∶40, Boehringer Mannheim) at 4 ℃ for 24 h. After washed with 0.01 M PBS 3 times, biotinylated goat antimouse Ig G (1∶200 for 30 min, Vector), avidin-biotin complex (1∶100 for 45 min, Vector), and DBA were used. Finally, the cells were dehydrated with absolute alcohol, cleared in xylene, and then cover-slipped. The percentage of positive cells was independently assessed in blind fashion by two pathologists (2 000 consecutive cells in each sample were counted).

1.8 Statistical analysis: Data were expressed as $\bar{x} \pm s$ and analyzed by *t*-test.

2 Results

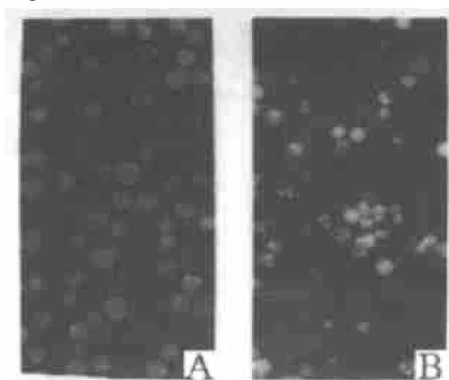
2.1 Cell growth-inhibitory effect: A dose-dependent inhibition of HL-60 cell proliferation was found after treatment with 10% SCCS for 48 h (Tab 1). IC_{50} was 6.5 g/kg (the doses of SC were preadministrated orally to the mice for 2 h).

Tab 1. Effects of *S. chamaejasme* (SC) on proliferation and apoptosis in HL-60 cells, *n* = 6 wells (5×10^7 cells/L, 200 μL/well). The cells exposed to 10% SCCS derived from mice administrated orally with SC for 48 h. $\bar{x} \pm s$. $^{**}P < 0.01$ *vs* control

SC (g/kg)	MTT (A_{540})	Inhibition (%)	Apoptotic cells (%)
0	1.38 ± 0.05		11.7
3	0.97 ± 0.08**	29.7	27.3
6	0.65 ± 0.05**	52.9	39.6
12	0.36 ± 0.04**	73.9	57.4

2.2 Cell morphological assessment: After treatment with SCCS for 48 h, the "dotted" chromatin in HL-60 cells, which was condensed chromatin and divided into "Apobodies" appeared in a large cell subpopulation under fluorescence microscope (Fig 1).

2.3 DNA fragmentation: Agarose gel electrophoreses of DNA extracted from HL-60 cells treated with SCCS for 48 h revealed a "ladder" pattern (Fig 2).



A—control; B—cells exposed to 10% SCCS of mice administrated with SC 12 g/kg for 48 h

Fig 1. Fluorescence of HL-60 cells stained with AO, ×200

A—DNA marker; B—control; C—cells exposed to 10% SCCS of mice pretreated with SC 3 g/kg; D—with SC 12 g/kg

Fig 2. Agarose gel electrophoresis of DNA extracted from HL-60 cells exposed to SCCS for 48 h

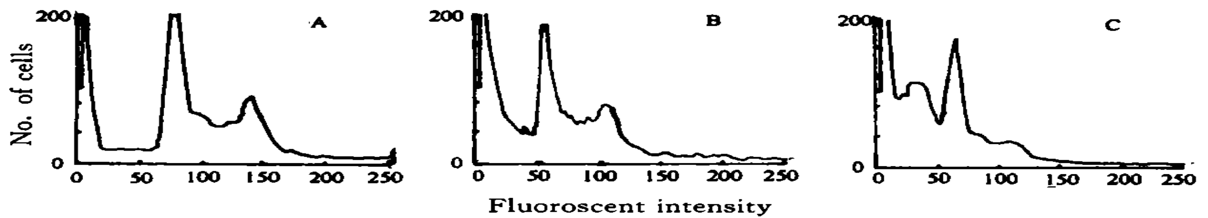
2.4 DNA degradation: Apoptotic cells were distinguished by their fractional DNA content less than that of G_1 phase, while the nonapoptotic cells were classified as G_1, S, and G_2/M phases of the cell cycle. After exposure of HL-60 cells to SCCS for 48 h, the apoptotic cells increased from 11.7% to 59.4% (Fig 3, Tab 1).

The changes in cell cycle distribution of the cells treated with SCCS appeared that cells in G_2/M phases increased in a dose-dependent manner, and cells in G_1 and S phases decreased gradually.

2.5 Expression of *bcl*-2 protein: SCCS decreased the expression of *bcl*-2 protein in SGC-7901 cells which had a higher expression of oncogene protein (Fig 4). Along with the increase of SC dose, the percentage of positive cells changed from 78.3% to 32.9% (Tab 2).

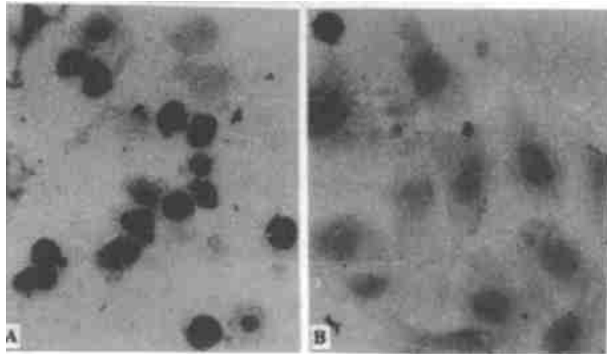
3 Discussion

It was known for a long time that *S. chamaejasme* (SC) was used as an antitumor herb



A-control; B-cells treated with 10% SCCS of mice preadministrated with 3 g/kg SC; C-with SC 12 g/kg

Fig 3. DNA contents, determined by flow cytometry, of HL-60 cells treated with SCCS of mouse for 48 h



A-control; B- cells treated with 10% SCCS of mice pretreated with SC 12 g/kg for 48 h

Fig 4. *Bcl*-2 protein expression of HL-60 cells treated with SCCS of mouse× 400. The protein was detected by immunohistochemistry

Tab 2. Expression of *bcl*-2 protein in SGC-7901 cells treated with SCCS of mouse for 48 h. $n=4$ samples (2 000 cells were measured in each sample). $\bar{x}\pm s$. * $P<0.05$, ** $P<0.01$ *vs* control.

SC (g/kg)	Positive cells (%)
0	78.30± 5.5
3	60.24± 7.3*
6	44.30± 3.1**
12	32.94± 3.6**

medicine in China. In clinic, the water extract of SC exhibited a marked antitumor activity against many leukemia and solid carcinoma[1]. However, its antitumor mechanism has not been fully elucidated. In recent years, we have studied the antitumor effects of SC on the growth of transplantable mouse tumors *in vivo* and the proliferation, clone formation and the incorporation of [^{3}H] deoxythymidine into DNA of tumor cells *in vitro*, especially by the methods of serum-pharmacology. It was found that the extract of SC inhibited the growth of transplantable mouse tumors, sarcoma 180, hepatic carcinoma HePS and lung sarcoma Lewis[2,3], and the SCCS of mouse exhibited inhibitory effects on proliferation, clone formation and incorporation of [^{3}H] deoxythymidine into DNA of mouse leukemia L_{1210} cells[4], which suggested that the inhibitory activity was directly against the growth of tumor cells as one of its main antitumor mechanisms. Our another experiment showed that the polysaccharide extracted from SC had a remarkable improving effect on immune function inhibited by CTX in mice[5]. After treatment with this polysaccharide, the thymus of mice, delayed hypresensitivity and the proliferation of mouse splenic lymphocytes activated with Con A of mice treated with CTX was increased markedly in a dose-dependent manner, and the peritoneal macrophage phagocytosis in mice was also improved, the weights of mice spleens, specific antibody formation of splenocytes, serum agglutinin titer, hemolysin HC_{50} in mice immunized with SRBC were all increased significantly. SC polysaccharide also inhibited the lung metastasis of Lewis lung carcinoma in mice remarkably[6]. The present study revealed that *S. chamaejasme* could induce apoptosis of HL-60 cells and decrease the expression of *bcl*-2 protein in SGC-7901 cells.

Lotem and Sachs[7] demonstrated that apoptosis is an active cell death process regulated by endogenous specific enzymes and genes. Inhibited apoptosis is one of the important characteristics of tumor cells. It is also the molecular basis of the occurrence, development of cancer, and may be related to the drug-resistance of tumor cells[8]. It has been reported that *bcl*-2 over-expression could remarkably suppress cell apoptosis induced by *L*-glutamate, free radicals and glutathione depletion[9,10]. So it can be concluded that inducing apoptosis and decreasing apoptosis over-expression in tumor cells may be another important antitumor

mechanisms of SC.

References

[1] Jiangsu New Medical Colloge. Chinese Herb Medicine Dictionary [M]. Shanghai: Shanghai People Publishers. 1977.

[2] Fan J J, Jia Z P, Xie J W, *et al*. Effects of Chinese Stellera (*Stellera chamaejasme* L.) on growth of transplanted mouse tumors [J]. J Lanzhou Med Coll, 1994, 20(4): 228-230.

[3] Fan J J, Jia Z P, Xie J W, *et al*. Antitumor activity of Chinese Stellera (*Stellera chamaejasme* L.) [J]. J Pharmaceutical Practice, 1996, 14(1): 9-12.

[4] Jia Z P, Wang Y G, Fan J J, *et al*. Effect of serum Ruixiang Langdu (*Stellera chamaejasme*) extract on proliferation, clonal formation and DNA synthesis of mouse L_{1210} leukemic cells [J]. Chinese Traditional Herbal Drugs, 2001, 32(9): 807-809.

[5] Fan J J, Jia Z P, Xie J W, *et al*. Effects of polysaccharide in Ruixiang Langdu (*Stellera chamaejasme* L.) on immune function in mice treated with cyclophosphamide [J]. Med J National Defending Forces Northwest China, 2000, 21(4): 263-265.

[6] Fan J J, Jia Z P, Xie J W, *et al*. Effect of polysaccharide in Ruixiang Langdu (*Stellera chamaejasme* L.) on pulmonary metastases of Lewis lung carcinoma and proliferation of splenocytes in mice [J]. J Chinese Med Materials, 2000, 23 (Suppl): 106-107.

[7] Lotem J, Sachs L. Regulation of *bcl*-X_L and *bax* in the control of apoptosis by hematopoietic cytokines and dexamethasone [J]. Cell Growth Differ, 1995, 6: 647-653.

[8] Nagata S. Apoptosis by death factor [J]. Cell, 1997, 88: 355-365.

[9] Gajewski T F, Thompson C B. Apoptosis meets signal transduction: elimination of a bad influence [J]. Cell, 1996, 87: 589-592.

[10] Hockenberg D M. *Bcl*-2 in cancer development and apoptosis [J]. J Cell Sci, 1994, 18 (Suppl): 51-55.

应用 M-H琼脂进行五倍子等 5种中药对 28株肠球菌的体外抗菌活性观察

李仲兴,王秀华,张立志*,岳云升,赵宝珍,陈晶波,李继红

(河北医科大学第二医院,河北 石家庄 050000)

摘 要: 目的 对五倍子、儿茶、乌梅、黄连和黄芩的体外抗菌效果进行比较。方法 用新的中药抗菌实验方法,进行五倍子等 5种中药对 28株肠球菌的体外抗菌活性检测。结果与结论 5种中药对肠球菌的抑菌作用均较好,其中以五倍子和儿茶的抑菌效果最好,其 MIC_{90}均为 1∶640,而黄连、乌梅和黄芩的抑菌效果也较好,其 MIC_{90}分别为 1∶320, 1∶320和 1∶160

关键词: 五倍子;儿茶;黄连;乌梅;黄芩;肠球菌;抗菌活性

中图分类号: R285.51; R286.8 文献标识码: A 文章编号: 0253-2670(2001)12-1101-03

Evaluation of *in vitro* antibacterial activities of *Melaphis chinensis etc*. 5 TCMs against 28 strains of enterococci with M-H agar medium

LI Zhong-xing, WANG Xiu-hua, ZHANG Li-zhi; YUE Yun-sheng, ZHAO Bao-zhen, CHEN Jing-bo, LI Ji-hong

(Second Hospital Affiliated to Hebei University of Medical Sciences, Shijiazhuang Hebei 050000, China)

Abstract Object To compare the *in vitro* antibacterial activities of 5 TCMs including *Melaphis chinensis* (Bell) Bake, *Acacia catachu* (L.) Milld, *Armeniaca mume* Sieb, *Coptis chinensis* Franch and *Scutellaria baicalensis* Georgi.. **Methods** Activities of the 5 drugs against 28 strains of *Enterococci* were determined by the new antibacterial test for TCM. **Results and Conclusion** All 5 TCMs showed antibacterial activities against *Enterococci*, with *M. chinensis* and *A. catachu* being the most potent. Both had the MIC_{90} of 1∶640. *A. mume*, *C. chinensis* and *S. baicalensis* followed next with their MIC_{90} of 1∶320, 1∶320 and 1∶160 respectively.

Key words *Melaphis chinensis* (Bell) Bake; *Acacia catachu* (L) Milld; *Armeniaca mume* Sieb; *Coptis chinensis* Franch; *Scutellaria baicalensis* Georgi; *Enterococci*; antibacterial activity

---

收稿日期: 2001-02-10

基金项目: 国家中医药管理局重点课题资助项目(编号: 97Z009)

作者简介: 李仲兴(1938-),男,河北玉田人,教授,1958年毕业于张家口医专检验专业,现工作在河北医科大学第二医院,一直从事临床微生物检验工作。研究方向为“机会致病菌生理、生化特性研究;机会致病菌对抗生素、中草药敏感及耐药谱型研究”。在国内外发表论文 80余篇,著有《临床细菌学》及《诊断细菌学》等著作,获省部级科技进步奖 8项。

* 河北省人民医院