[3] Maria M, Simona M, Daniela D R, *et al*. Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample [J]. *Btol Proced Online*, 2001, 3(1): 19-25.
[4] Wang L, Zhang G M, Feng Z H. Down-regulation of survivin expression reversed multidrug resistance in adriamycin-resistant HL-60/ADR cell line [J]. *Acta Pharm Sin* (药学学报), 2003, 24(12): 1235-1240.
[5] Adel B, Akira Y, Yuji W, *et al*. Expression of the anti-apoptotic gene survivin in myelodysplastic syndrome [J]. *Int J Oncol*, 2003, 22: 59-64.
[6] Lock R B, Rose W E. *Inhibition of p34cdcL kinase activity* by etoposide or irradiation as a mechanism of G2 arrest in Chinese hamster ovary cells [J]. *Cancer Res*, 1990, 50: 3761-3766.
[7] Tsujimoto Y, Cossman J, Jaffe E, *et al*. Involvement of the bcl-2 gene in human follicular lymphoma [J]. *Science*, 1985, 228(4706): 1440-1443.
[8] Lam M, Dubyad G, Chen L, *et al*. Evidence that bcl-2 repress apoptosis by regulating endoplasmic reticulum-associated Ca^{2+} fluxes [J]. *Proc Natl Sci USA*, 1994, 91: 6569.
[9] Susin S A, Zamzami N, Castedo M, *et al*. Bcl-2 inhibits the mitochondrial release of an apoptogenic protease [J]. *J Exp Med*, 1996, 184(4): 1331-1341.
[10] Oltvai Z N, Milliman C L, Korsmeyer S J. Bcl-2 heterodimerizes *in vivo* with a conserved homolog, bax, that accelerates programmed cell death [J]. *Cell*, 1993, 74(4): 609-619.
[11] Rosse T, Olivier R, Monney L, *et al*. Bcl-2 prolongs cell survival after bax-induced release of cytochrome c [J]. *Nature*, 1998, 391(6666): 496-499.
[12] Jurgensmeier J M, Xie Z, Deveraux Q, *et al*. Bax directly induces release of cytochrome c from isolated mitochondria [J]. *Proc Natl Acad Sci USA*, 2001, 251: 45-52.
[13] Antonsson B, Conti F, Ciavatta A, *et al*. Inhibition of bax channel-forming activity by bcl-2 [J]. *Science*, 1997, 277 (5324): 370-372.
[14] Fengzhil A G, Chu E Y. Control of apoptosis and mitotic spindle checkpoint by survivin [J]. *Nature*, 1998, 396: 580-588.
[15] Mahotka C, Wenzel M, Springer E, *et al*. Surviving-delta EX3 and survivin-2B: two novel splice variants of the apoptosis inhibitor surviving with different anti-apoptotic properties [J]. *Cancer Res*, 1999, 59: 6097-6103.
[16] Amdrosini G, Adida C, Altieri D. A novel anti-apoptosis gene, surviving, expressed in cancer and lymphoma [J]. *Nat Med*, 1997, 3: 917-921.
[17] Kawasaki H, Alteieri D C, Lu C D, *et al*. Inhibition of apoptosis by survivin predicts shorter survival rates in colorectal cancer [J]. *Cancer Res*, 1998, 58: 5071-5074.

Apoptotic effect of oridonin on NB4 cells and its mechanism

LIU Jia-jun[1], LI Qiao[2], PAN Xiang-lin[3], PENG Jun[3], WU Xiang-yuan[1], LI Ming-quan[1], LIN Dong-jun[1], LIN Qu[1], HUANG Ren-wei[1]

(1. Department of Hematology, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou 510630, China;
2. Department of Biomedical Engineering, Medical College of Shandong University, Jinan 250012, China;
3. Department of Hematology and Oncology, Qilu Hospital of Shandong University, Jinan 250012, China)

Abstract: Objective To investigate the mechanisms of oridonin inducing apoptosis on acute leukeamia NB4 cells and its mechanism. **Methods** NB4 cells in culture medium *in vitro* were given with different concentrations (8, 16, 24, and 32 μmol/L) of oridonin. The inhibitory rate of the cells was measured by MTT assay, cell apoptotic rate was detected by flow cytometry (FCM), morphology of apoptosis was observed by Hoechst 33258 fluorescence staining, DNA fragmentation was assayed by agarose gel electrophoresis, caspase-3 expression was detected by Western blotting, and caspase-3 activity was assayed with colorimetric assay kit before and after apoptosis occurred. **Results** Oridonin (over 16 μmol/L) could inhibit the growth of NB4 cells and cause apoptosis significantly, the suppression was both in a time- and dose-dependent manner. Marked changes of apoptosis including condensation of chromatin and nuclear fragmentation were observed very clearly by Hoechst 33258 fluorescence staining and a characteristic "ladder" of DNA fragments was elicited by agarose gel electrophoresis; Western blot analysis revealed that caspase-3 was activated by the loss of caspase-3 proenzyme (32 kDa) and the appearance of its 20 kDa subunit, and that along with the apoptotic process caspase-3 activity was increased concurrently. **Conclusion** Oridonin can induce apoptosis in NB4 cells via activation of caspase-3. These results will provide laborato-

收稿日期: 2005-01-07
基金项目: 国家自然科学基金资助项目 (30300312)
作者简介: 刘加军(1966—),山东省日照市人,主治医师,讲师,硕士生导师,山东大学博士,中山大学博士后,主要从事血液肿瘤的分子生物学及细胞凋亡机制等研究。Tel: (020) 85516867-2227

ry evidence for the clinical treatment of acute leukemia with oridonin.

Key words: oridonin; caspase-3; apoptosis; NB4 cell

冬凌草甲素对白血病 NB4 细胞的诱导凋亡作用及其机制

刘加军[1]，李 桥[2]，潘祥林[3]，彭 军[3]，吴祥元[1]，李铭权[1]，林东军[1]，林 曲[1]，黄仁魏[1]

(1. 中山大学附属第三医院 血液科，广东 广州 510630；2. 山东大学医学院 生物医学工程教研室，山东 济南 250012；3. 山东大学齐鲁医院 血液肿瘤中心，山东 济南 250012)

摘 要：目的 探讨冬凌草甲素对白血病 NB4 细胞的诱导凋亡作用及其机制。**方法** 以不同质量浓度 (8、16、24 和 32 μmol/L) 的冬凌草甲素作用于体外培养的 NB4 细胞。应用四氮唑蓝 (MTT) 比色法检测细胞生长抑制率，流式细胞术 (FCM) 检测细胞凋亡率；采用 Hoechst 33258 荧光染色法和琼脂糖凝胶电泳观察细胞凋亡；采用蛋白印迹 (Western blot) 和比色法测定细胞凋亡前后 caspase-3 表达水平及其活性的变化。**结果** 冬凌草甲素 (16 μmol/L 以上浓度) 对白血病 NB4 细胞具有显著的增殖抑制及诱导凋亡作用，并且呈现出一定的时间-效应和剂量-效应关系。Hoechst 33258 荧光染色观察到典型的核浓缩、核碎裂等细胞凋亡的形态学变化，琼脂糖凝胶电泳观察到细胞凋亡时的 DNA "梯形"条带；Western blot 检测结果表明 32 kDa 的 caspase-3 酶原被激活，出现 20 kDa 的亚单位活化片段，同时在细胞凋亡过程中，caspase-3 活性显著增高。**结论** 冬凌草甲素能够通过激活 caspase-3 的表达而诱导 NB4 细胞发生凋亡，可为冬凌草甲素进一步应用于临床治疗急性白血病提供实验依据。

关键词：冬凌草甲素；caspase-3；细胞凋亡；NB4 细胞

中图分类号：R286.91 **文献标识码**：A **文章编号**：0253-2670(2005)08-1188-06

1 Introduction

Oridonin, a diterpenoid compound, is extracted and purified from traditional Chinese medicinal herb, *Rabdosia rubescens* (Hemsl.) Hara or *Isodon japonicus* (Burm. f.) Hara[1,2]. It is one of the most important traditional Chinese herbs used in clinical treatment nowadays. More than a half century ago, oridonin showed a variety of biological effects such as immunoregulatory and anti-inflammatory functions as well as antiviral functions especially in the upper respiratory tract infection. Recent laboratory and clinical data suggest that oridonin is a very effective antitumor reagent with profound effects on a number of malignant diseases such as prostate, breast, non-small cell lung cancers[3].

Apoptosis is a form of cell death defined by a characteristic set of morphological and biochemical changes, and many recent studies have begun to identify the role of caspases in apoptotic death especially in cancer cells[4]. Different members of the caspase family mediate apoptosis in different cell types, and even within a given cell type, distinct caspases have been found to mediate apoptosis depending upon the apoptotic stimulus received by the cells[5]. Some caspases, such as caspase-3 and caspase-9, are more and more important in the caspases mediated apoptosis and the variation of their activity is correlated to a large variety of cancer apoptosis[6].

Though oridonin has been proved to be very effctive in a variety of malignancies, many of its antitumor mechanisms have not been demonstrated. Up to date, there is no detailed laboratory evidence about the mechanisms of oridonin on leukemic NB4 cells. In order to clarify some of its anti-leukemic mechanisms, the apoptotic effect of various concentrations of oridonin (8—32 μmol/L) on NB4 cells *in vitro* was investigated in this study, and the variation of caspase-3 expression was detected to provide laboratory evidence of oridonin for the clinical treatment of acute leukemia.

2 Materials and methods

2.1 Reagents: Oridonin was presented by Prof. Pan Xiang-lin, and Hoechst 33258 was purchased from Sigma Company, NB4 cell strain was purchased from Shanghai Ruijin Hospital, anti-caspase-3 antibody was purchased from Pharmingen, and caspase-3 detecting kit was purchased from Promiga Company.

2.2 Cell culture: Human leukemia cell line NB4 cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated calf serum, and 100 U/mL Penicillin in a humidified 5% incubator

at 37 ℃. Cells were passaged twice weekly and routinely examined for mycoplasma contamination.

2.3 Cell inhibitory rate (MTT Assay): Cell inhibitory rate was assayed using the microculture tetrazolium method. Briefly, NB4 cells in logarithmic growth-phase were collected and 2×10^5 cells/well were dispensed within 96-well culture plates in 100 mL volumes. Then different concentrations of oridonin (8, 16, 24, and 32 μmol/L) were put in different wells. Every one of the concentrations above was regarded as one treated group while there was no oridonin in the control group. Each of the treated or control groups contained six parallel wells. Culture plates were then incubated for 0, 24, 48, and 72 h prior to the addition of tetrazolium reagent. MTT working solution was prepared as follows: 5 mg MTT/mL phosphate buffered saline (PBS) was sterile by being filtered with 0.45 μm filter units. Each of the above cultured wells was added 20 μL of MTT working solution and then incubated continuously for 4 h. All culture medium supernatant was removed from wells after each of the plates was centrifuged (1 000 r/min, 15 min) and replaced with 100 μL of DMSO. Following thorough solublization, the absorbance (*A* value) of each well was measured using a microculture plate reader at 570 nm. Cell inhibitory rate was calculated according to the formula as follows: inhibitory rate = (*A* value of control group − *A* value of treated group)/*A* value of control group × 100%.

2.4 Flow cytometry detection: For DNA content analysis, cells treated with different concentrations of oridonin were collected, pelleted, washed with PBS, and resuspended in PBS containing 20 mg/L PI and 1 g/L ribonuclease A. 2×10^6 fixed cells were examined per experimental condition by flow cytometry (FCM), and percentage of degraded DNA was determined by the number of cells dispaying subdiploid (sub-G_1) DNA divided by the total number of cells examined.

2.5 Hoechst 33258 staining: The morphology of NB4 cells when exposed to oridonin for different time was observed firstly under inverted microscope, then Hoechst 33258 staining was used to observe the apoptotic morphology of NB4 cells especially treated with oridonin for 48 h. Cells were fixed with 4% formaldehyde in PBS for 10 min, stained by Hoechst 33258 (10 mg/L) for 1 h, and then subjected to fluorescence microscopic analysis. After treatment with oridonin (16, 24, and 32 μmol/L), the morphologic changes including reduction in the volume and nuclear chromatin condensation were observed.

2.6 DNA Fragmentation assay: Apoptosis was confirmed by detection of fragmentation of chromosomal DNA with the classic DNA ladder method. Briefly, 2×10^6 cells were immersed in cytolysis buffer (Tris-HCl 1 mmol/L, pH 8.0, edetic acid 10 mmol/L, pH 8.0, proteinase K 200 mg/L, 0.5% SDS) and incubated for 3 h at 50 ℃. DNA was extracted with phenol-chloroform, precipitated in 1/10 volume of NaAc 2 mol/L and 2 volumes of ethanol at −20 ℃ overnight, recovered by centrifugation at 1 000 × *g* for 30 min at 4 ℃, and then resuspended in TE buffer. RNase A was added at a concentration of 200 mg/L, then the treated extract was incubated for 30 min at 37 ℃ and electrophoresed on a 1.2% agarose gel.

2.7 Western blot analysis: For Western bloting, 2×10^6 cells were washed with ice-cold PBS twice and lysed for 30 min at 4 ℃, then debris was removed by centrifugation for 15 min at 15 000 × *g* at 4 ℃, and equivalent amounts of protein were separated by 10% SDS-PAGE and transferred onto nitrocellulose filter. The filters were first stained to confirm uniform transfer of all samples and then incubated in blocking solution for 2 h at room temperature. The filters were reacted firstly with monoclonal antibody at a dilution of 1 ∶ 1 000 for 2 h, followed by extensive washes with PBS twice and TBST twice. Filters were then incubated with horseradish peroxidase-conjugated secondary antibody of 1 ∶ 1 000 for 1 h, washed with TBST and immunoreactive proteins were detected using an ECL Western blotting detection system.

2.8 Caspase-3 activity assay: Caspase-3 activity was assayed with an Apoalert caspase-3 Colorimet-

ric Assay Kit (Calbiochem, San Diego, CA, USA) according to the manufacturer's instructions. Briefly, control and treated cells were washed with cold PBS and lysed in lysis buffer containing 50 mmol/L HEPES, pH 7.4, 100 mmol/L NaCl, 0.1% CHAPS, 1 mmol/L dithiothreitol (DTT), and 0.1 mmol/L EDTA for 10 min and then centrifuged at 10 000×g for 20 min before collecting the supernatants. Fifty micrograms of each lysate were added to the assay buffer (50 mmol/L HEPES, pH 7.4, 100 mmol/L NaCl, 0.1% CHAPS, 1 mmol/L DTT, 0.1 mmol/L EDTA, and 10% glycerol) to make a total volume of 90 μL which was then incubated for 10 min at 37 ℃. Ten microliters of colorimetric substrate for caspase-3 (final concentration, 200 μmol/L) were added to the mixtures. A_{405} was recorded for each samples after incubation for 2 h at 37 ℃.

2.9 Statistical analysis: All experiments were performed in triplicate and the results were expressed as mean ± SD. Statistical analysis were performed with t-test using SAS 6.12 software.

3 Results

3.1 Cell inhibitory rate caused by oridonin: Oridonin below 8 μmol/L had little inhibitory rate on NB4 cells, but it could inhibit the proliferation of NB4 cells significantly at a higher concentration (between 16—32 μmol/L oridonin), especially the concentration of 32 μmol/L. The inhibitory rate of oridonin between 24—32 μmol/L is much higher than that of lower concentrations of oridonin ($P<0.01$) (Fig. 1).

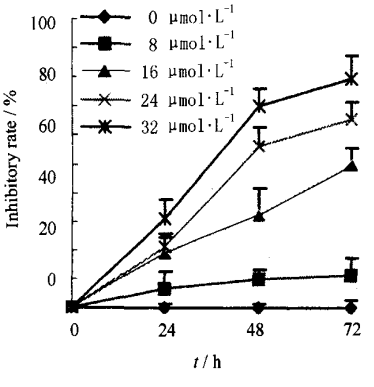


Fig. 1 Cell inhibitory rate caused by oridonin

3.2 Cell apoptotic rate detected by FCM: Oridonin (over 16 μmol/L) could induce apoptosis when cultured with NB4 cells after 24—72 h, the apoptotic rate was very high and over 50% when cells cultured with oridonin for 48 h (oridonin>24 μmol/L) (Fig. 2)

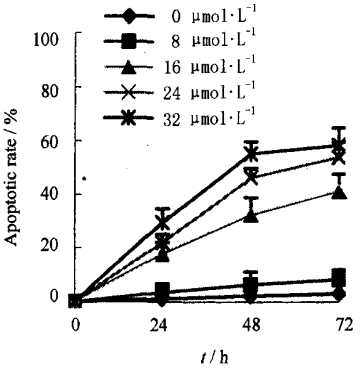
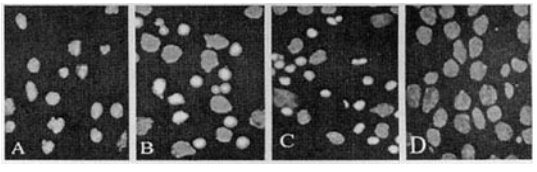


Fig. 2 Cell apoptotic rate caused by oridonin

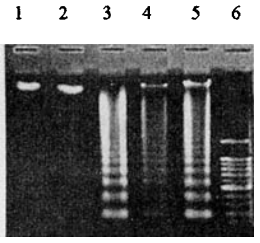
3.3 Morphology of cell apoptosis: Apoptotic cells gradually increased in both dose- and time-dependent manners when exposed to oridonin (over 16 μmol/L). After the cells exposed to oridonin for 48 h, marked morphological changes of cell apoptosis including condensation of chromatin and nuclear fragmentation were found using Hoechst 33258 staining (Fig 3). Oridonin below 16 μmol/L had little apoptotic effect on NB4 cells (Fig. 3-A), but apoptotic cells were then gradually increased along with the enhancement of concentration (Fig. 3-B and -C), the apoptotic cells were between 40%-50% when the NB4 exposed to 32 μmol/L oridonin for 48 h, and no obvious apoptosis was found in the control group (0 μmol/L oridonin) (Fig. 3-D)



A-16 μmol/L oridonin B-24 μmol/L oridonin
C-32 μmol/L oridonin D-control

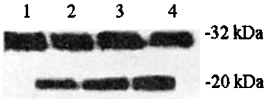
Fig. 3 Apoptotic cells observed by Hoechst 33258 staining (200 ×) after cells exposed to oridonin for 48 h

3.4 DNA Fragmentation: The integrity of DNA was assessed by agarose gel electrophoresis. Incubation of NB4 cells with oridonin 16—32 μmol/L



1-control 2—5-treated groups of 8, 16, 24, and 32 μmol/L oridonin 6-DNA Marker

Fig. 4 DNA fragmentation of cells exposed to different concentrations of oridonin for 48 h



1-control 2—4-treated groups of 16, 24, and 32 μmol/L oridonin

Fig. 5 Western blot analysis of caspase-3

for 48 h elicited a characteristic "ladder" of DNA fragments representing integer multiples of the internucleosomal DNA length (about 180—200 bp) (Fig. 4).

3.5 Western blot analysis: Western blot analysis revealed that caspase-3 was activated by the loss of caspase-3 proenzyme (32-kDa) and the appearance of its 20-kDa subunit after the cells exposed to 16-32 μmol/L oridonin for 72 h (Fig. 5).

3.6 Variation of caspase-3 activity: Caspase-3 activity of NB4 cells were increased remarkably when exposed to oridonin for 24—72 h, the higher the oridonin concentration, the more increased expression of caspase-3 activity of NB4 cells, and caspase-3 activity was enhanced to its peak at 72 h (Fig. 6).

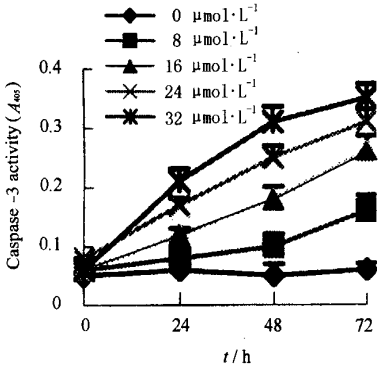


Fig. 6 Caspase-3 activity caused by oridonin

4 Discussion

There are many compounds extracted from *R. rubescens* and oridonin is one of its most effective derivatives, which is recently proved to have activity against a number of cancer cells, and the main antitumor ingredient, oridonin, chemically belongs to ent-kaurane diterpenoid. Recent data have shown that oridonin can inhibit the growth of many cancer cells and inducing apoptosis may be one of its antitumor mechanisms, TUNEL assay and cell cycle analysis showed that oridonin induced apoptosis and G_0/G_1 cell cycle arrested in some cancer cells such as in prostate cancer cell. Furthermore, detailed references indicated that oridonin inhibited the proliferation of cancer cells via apoptosis and cell cycle arrested with p53 playing a central role in several cancer types which expressed the wild-type p53 gene. Oridonin may be a novel, adjunctive therapy for a large variety of malignancies[2,3].

Our previous studies[7] demonstrated that oridonin had considerable anti-proliferation effects on some leukemic cells *in vitro* and down-regulating the telomerase activity of leukemic cells may be its important anti-leukemic mechanism. In this study, that oridonin could inhibit the proliferation and induce apoptosis on leukemic NB4 cells *in vitro* in a time- and dose-dependent manner has been found. Oridonin showed a significant apoptosis inducing effects at the doses ranging from 16 to 32 μmol/L. Marked morphological changes of cell apoptosis were observed very clearly by Hoechst 33258 staining as well as electronic microscopy, and DNA fragmentation was observed by agarose gel electrophoresis after the cells exposed to oridonin for 48 h; Western blotting showed cleavage of the caspase-3 zymogen protein (32 kDa) with the appearance of its 20-kDa subunit; along with the apoptotic process caspase-3 activity was increased remarkably. The apoptosis induced by oridonin on acute leukemic NB4 cells via activation of caspase-3 could be concluded.

Among all of the caspases, caspase 3 is one of the most important effector or executor, and caspase 3 activity is the common effector of the most of the apoptotic pathways[6]. Once activated, caspase 3 is capable of cleaving many important cellular substrates, such as ICAD (inhibitor of caspase-activated DNase), poly (ADP-ribose) polymerase (PARP, a DNA repair enzyme), actin, fodrin, and lamin. Many laboratory data have proved that

active caspase-3 can cause membrane blebbing, disassembly of the cell structure and DNA fragmentation, which eventually lead to cell death. Some initiator caspases, such as caspase 9, can activate procaspase 3, which then can cleave the cellular substrates needed for the orchestration of apoptosis and form a "wheel of death"[8-10]. Recent data have shown that apoptosis, especially the caspase-mediated cell death, plays an important role in the etiology, pathogenesis, and therapy of a variety of hematological malignancies such as acute leukemia, and cytotoxic effects of most anti-leukemia drugs are based on induction of apoptosis[11]. All these studies indicate that induction of apoptosis may be a index for new antitumor drug selection and an important method of assesement for the clinical effectiveness of many anti-leukemia drugs[12].

In summary, the results demonstrate that oridonin can induce apoptosis in NB4 cells via activation of caspase-3. This indicates that oridonin may be an important potential anti-leukemia reagents.

Acknowledgments: We thank the members of our laboratories for their insight and technical support.

References:

[1] Wang N, Xie G X, Shi X H, *et al*. Study on extracting and separating of oridonin in *Rabdosia rubescens* [J]. *Chin Tradit Herb Drugs* (中草药), 2003, 34(10): 901-902.

[2] Han Q B, Li M L, Li S H, *et al*. *Ent*-kaurane diterpenoids from *Isodon rubescens* var. *lushanensis* [J]. *Chem Pharm Bull*, 2003, 51(7): 790-793.

[3] Kezoe T, Chen S S, Tong X J, *et al*. Oridonin induces growth inhibition and apoptosis of a variety of human cancer cells [J]. *Int J Oncol*, 2003, 23(4): 1187-1189.

[4] Beauparlant P, Shore G C. Therapeutic activation of caspases in cancer: a question of selectivity [J]. *Curr Opin Drug Discov Devel*, 2003, 6(2): 179-187.

[5] Thorburn A. Death receptor-induced cell killing [J]. *Cell Signal*, 2004, 16(2): 139-144.

[6] Qi S N, Yoshida A, Wang Z R, *et al*. GP7 can induce apoptotic DNA fragmentation of human leukemia cells through caspase-3-dependent and -independent pathways [J]. *Int J Mol Med*, 2004, 13(1): 163-167.

[7] Liu J J, Pan X L, Wu X Y, *et al*. Inhibitory effect and mechanism of oridonin on Raji cells proliferation [J]. *Chin Tradit Herb Drugs* (中草药), 2004, 35(7): 287-290.

[8] Denault J B, Salvesen G S. Caspases: keys in the ignition of cell death [J]. *Chem Rev*, 2002, 102 (12): 4489-4500.

[9] Boatright K M, Salvesen G S. Caspase activation [J]. *Biochem Soc Symp*, 2003, (70): 233-242.

[10] Philchenkov A A. Caspases as regulators of apoptosis and other cell functions [J]. *Biochemistry*, 2003, 68(4): 365-376.

[11] Smolewski P, Darzynkiewicz Z, Robak T. Caspase-mediated cell death in hematological malignancies: theoretical considerations, methods of assessment, and clinical implications [J]. *Leuk Lymphoma*, 2003, 44(7): 1089-1104.

[12] Beauparlant P, Shore G C. Therapeutic activation of caspases in cancer: a question of selectivity [J]. *Curr Opin Drug Discov Devel*, 2003, 6(2): 179-187.

雷公藤甲素对胶原诱导的关节炎大鼠关节局部热休克蛋白和主要组织相容性复合体 I 类分子表达的影响

涂胜豪，胡永红，曾克勤，张明敏，赖先阳，张玮琛

（华中科技大学同济医学院附属同济医院 中西医结合科，湖北 武汉 430030）

摘 要：目的 观察雷公藤甲素对胶原诱导的关节炎大鼠关节局部热休克蛋白（HSPs）和主要组织相容性复合体 I 类分子（MHC-I）表达的影响。**方法** 建立胶原诱导的关节炎大鼠模型，雷公藤甲素按 40 μg/kg im 给药，运用逆转录聚合酶链反应（RT-PCR）和免疫酶组织化学染色的方法，观察大鼠关节滑膜细胞和软骨细胞 HSP60、HSP70 和 MHC-I 类分子的表达情况。**结果** 与正常对照组相比，模型组大鼠关节滑膜细胞及软骨细胞 HSP60、HSP70 和 MHC-I 类分子的表达均显著增高（$P<0.05$），与模型组相比，雷公藤甲素可以下调关节炎大鼠关节局部 HSP60、HSP70 和 MHC-I 类分子的表达（$P<0.05$）。**结论** 降低关节炎大鼠关节局部软骨细胞和滑膜细胞异常表达的 HSPs 与 MHC-I 类分子的表达，可能是雷公藤甲素治疗类风湿关节炎（RA）的作用机制之一。

收稿日期：2004-12-29
基金项目：国家自然科学基金资助项目（30070961）
作者简介：涂胜豪（1965—），男，湖北武汉人，硕士，华中科技大学同济医学院附属同济医院副教授，硕士生导师，主要从事中西医结合治疗风湿病研究。 Tel：（027）83663379 E-mail：shtu@tjh.tjmu.edu.cn