制备大鼠腹膜炎模型，用放免法测 PGE_2 含量，Folin-酚试剂法测蛋白含量，邻苯三酚－NBT 法测 SOD 活性，竞争性蛋白结合法测 cAMP 含量．结果：组胺致炎时，SG 10－20 mg·kg^{-1} im 减少渗液量；角叉菜胶致炎时，SG 20 mg·kg^{-1} im 使渗液量及其中 PGE_2 含量减少，SOD 活性增加；花生四烯酸致炎时，SG 20 mg·kg^{-1} im 抑制 Neu 中 cAMP 浓度下降，减少渗出液中 PGE_2 含量．SG 也使三种腹膜炎渗出液中 Neu 计数和蛋白含量减少．结论：SG 通过抑制 Neu 游走及 PGE_2 合成、降低毛细血管通透性、清除氧自由基而发挥抗炎作用．

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Quercetin induced apoptosis in human leukemia HL-60 cells[1]

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KEY WORDS quercetin; apoptosis; DNA damage; HL-60 cells; cultured tumor cells

AIM: To examine whether quercetin (Que) might induce apoptosis in human leukemia HL-60 cells. **METHODS**: DNA fragmentation was visualized by agarose gel electrophoresis. Inhibition of proliferation was measured with a colorimetric MTT-assay. The DNA degradation was determined using flow cytometry, and the microscopic changes were observed by an electron microscope. **RESULTS**: Que 15－120 μmol·L^{-1} elicited typical apoptosis morphological changes including condensed chromatin, nuclear fragmentation, and reduction in volume. DNA fragmentation and DNA degradation in a concentration-dependent manner in HL-60 cells. Que inhibited HL-60 cell proliferation. The values of IC_{50} and 95 % confidence limits were 43 (30－61) μmol·L^{-1} after 48-h treatment with Que. **CONCLUSION**: Que induced apoptosis in HL-60 cells.

Quercetin (Que, 3, 3′, 4′, 5, 7-pentahydroxy flavone) is a natural bioflavonoid exerting many biological effects[1-5]. Que exerts growth-inhibitory effects on human breast, ovarian, leukemia, and colon cancer cells[6-8]. It was suggested that Que might be the parent compound of a novel class of anticancer agents. To gain more information on the therapeutic potential of Que we were prompted to examine the apoptotic effect of Que in this study.

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MATERIALS AND METHODS

Reagents Que was purchased from Shanghai Second Chemical Reagent Factory (900905); 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) and RPMI 1640 were the products of Sigma.

Cell culture Human leukemic HL-60 cell line was kindly provided by Assoc Prof CHEN Zi-Xin (Jiangsu Institute of Hematology), and was maintained in RPMI 1640 supplemented with 10 % fetal calf serum, benzylpenicillin/streptomycin 100 kU·L^{-1}, and *L*-glutamine 2 mmol·L^{-1}. Cells were kept in 5 % CO_2 + 95 % air at 37 ℃ and used when in exponential growth. Que was dissolved in Me_2SO and diluted in medium immediately before each experiment with the Me_2SO <0.1 %.

MTT-assay Inhibition of proliferation after incubation with Que was measured in a colorimetric MTT-assay[9]. Single-cell suspensions were prepared, cells were counted using a hemocytometer and then dispersed in replicate 96-well microtiter plates with 2×10^8 cells·L^{-1} (100 μL/well) in RPMI 1640 supplemented as above. Que 3.25－120 μmol·L^{-1} were added immediately after plating. The absorbance was measured on DG-3022A ELISA micro-plate Reader at 540 nm.

Flow cytometry[10] The DNA degradation which often precedes visibly detectable apoptosis was determined directly using flow cytometry. Cells were harvested and fixed. The distribution of DNA contents of the cells in the cell cycle was

determined, and the cells with DNA content less than that in the G_1 phase, indicating apoptosis were detected using an FACS flow cytometer (EPICS XL, Coulter, USA), and the data were processed by multicycle DNA content and cell cycle analysis software (copyright: 1994 University of Washington). The formation of a distinct cell cycle region (A_0) below G_0/G_1 phase after Que-treatment was characteristic of cells undergoing the DNA degradation associated with apoptosis. The % of events in the A_0 region was calculated.

Morphology The ultrastructural appearance were observed under a Hitachi 600 electron microscope. Cells were seeded at a high cell density (5 × 10^6 cells/plate) on 100 mm × 200 mm plates and treated with Que 7.5 – 60 μmol·L^{-1}. After 48-h incubation, the cells were harvested, fixed and viewed[11].

DNA electrophoresis[7] After 48-h incubation with Que, fragmented DNA was analyzed by electrophoresis. The cellular DNA was extracted, dialyzed, electrophoresed in 1.5 % agarose gel, and visualized by ethidium bromide. The gel was photographed under UV light.

Statistics The results were expressed as $\bar{x} \pm s$, and assessed by ANOVA.

RESULTS

Growth-inhibitory effect HL-60 cells exposed to Que 60 μmol·L^{-1} revealed an evident antiproliferative action at 12 h (Tab 1).

Tab 1. Antiproliferative action of quercetin on HL-60 cells. n = 6 experiments (2 × 10^8 cells·L^{-1}, 100 μL/well), $\bar{x} \pm s$. $^aP>0.05$, $^cP<0.01$ *vs* control.

Time/h	MTT/A_{540} Control	MTT/A_{540} Que 60 μmol·L^{-1}
0	0.22±0.04	0.22±0.05^a
4	0.29±0.05	0.27±0.08^a
8	0.46±0.04	0.46±0.08^a
12	0.79±0.09	0.50±0.05^c
24	0.82±0.05	0.58±0.03^c
48	0.93±0.07	0.50±0.12^c
72	0.84±0.12	0.44±0.09^c

Que produced a dose-dependent inhibition of cell growth after 48-h treatment (r = 0.9844, IC_{50} and 95 % confidence limits were 43 (30 – 61) μmol·L^{-1} (Tab 2).

DNA fragmentation Internucleosomal DNA damage in HL-60 cells was readily detected 48 h after treatment with Que 15 – 60 μmol·L^{-1}, in which a typical ladder of DNA was seen. In comparison, no obvious DNA fragmentation from HL-60 cells treated with Que 7.5 μmol·L^{-1} and Me_2SO (0.1 %) were observed (Fig 1).

Tab 2. Effect of Que on proliferation and apoptosis in HL-60 cells at 48 h. n = 6 experiments (2 × 10^8 cells·L^{-1}, 100 μL/well), $\bar{x} \pm s$. $^aP>0.05$, $^bP<0.05$, $^cP<0.01$ *vs* control.

Quercetin/ μmol·L^{-1}	MTT/ A_{540}	Inhibition/ %	Apoptotic cells/%
0	0.93±0.07		1.6±0.4
3.75	0.88±0.10^a	5.37	1.4±0.4^a
7.5	0.83±0.07^b	10.75	1.8±0.6^a
15.	0.69±0.08^c	25.81	27.1±2.9^c
30.	0.58±0.11^c	37.63	40.5±3.0^c
60.	0.50±0.12^c	46.24	44.2±1.6^c
120.	0.47±0.05^c	49.46	58.9±8.6^c

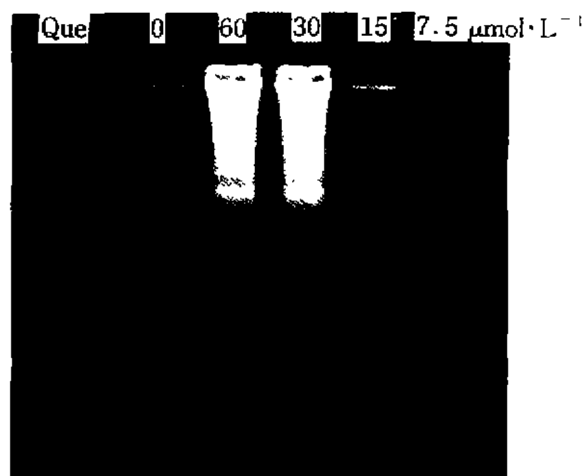

Fig 1. Internucleosomal DNA fragmentation in HL-60 cells treated with Que for 48 h.

DNA degradation by flow cytometry Control and Que (3.75 – 120 μmol·L^{-1}) treated HL-60 cells for 48 h were analyzed by flow cytometry. The apoptotic cell population was calculated by determing the % of cells with a DNA content less than that of G_1 phase. At 48 h after adding Que 15 – 120 μmol·L^{-1}, 27.1 % – 58.9 % collected events were located in a sub-G_1-phase position (A_0), consistent with cells undergoing DNA degradation during apoptosis (Tab 2). The HL-60 cells exposed to Que 60 μmol·L^{-1} showed a time-

dependent increase of apoptotic cells, starting after 24 h of Que treatment (Tab 3).

Tab 3. Effect of Que (60 $\mu mol \cdot L^{-1}$) on apoptosis in HL-60 cells $n = 4$ experiments (2×10^{8} cells $\cdot L^{-1}$, 100 μL/well). $\bar{x} \pm s$. [a]$P>0.05$, [b]$P<0.05$, [c]$P<0.01$ vs control.

Time/h	Apoptotic cells/%	
	Control	Quercetin
0	1.6±0.4	1.4±0.5[a]
6	1.6±0.5	1.6±0.4[a]
12	1.8±0.6	1.9±0.7[a]
24	1.4±0.4	3.6±0.4[b]
48	2.1±1.0	41.6±3.5[c]
72	7.5±3.2	65.2±9.2[c]

Morphological changes The ultrastructural features of a representative apoptotic HL-60 cells treated with Que 30 $\mu mol \cdot L^{-1}$ for 48 h were shown in Fig 2. The cell volume reduced, indicating shrinkage of cytoplasm, and the plasma membrane remained well defined. The chromation became condensed, and nucleus with the formation of apoptotic body was seen. Thus morphological data strongly suggest the occurrence of apoptosis in HL-60 cells treated with Que.

DISCUSSION

The present study revealed the occurrence of apoptosis in HL-60 cells treated with Que. Ultrastructural studies of the fragmented HL-60 cells revealed that the cytoplasm was extensively condensed with the cellular organelles remaining intact. The preservation of cellular membranes and organelles is one of the most reliable markers of apoptosis[11]. The morphological evidence presented here is consistent with an apoptotic mode of death of HL-60 cells treated with Que.

The internucleosomal fragmentation of DNA, which resulted in a ladder type pattern comprising 180 base pair intervals in gel electrophoresis, was a key molecular event reported in apoptosis. In our experiment, a ladder of fragmented DNA from Que-treated HL-60 cells was seen. DNA degradation characteristic of apoptosis may be determined rapidly in single cells using flow cytometry by monitoring the loss of nuclear DNA, subsequent to endonuclease activity resulting in apoptosis. Our results showed that 48 h after Que treatment, 27.1 % - 58.9 % of collected HL-60 cells were located in a sub-G_1-

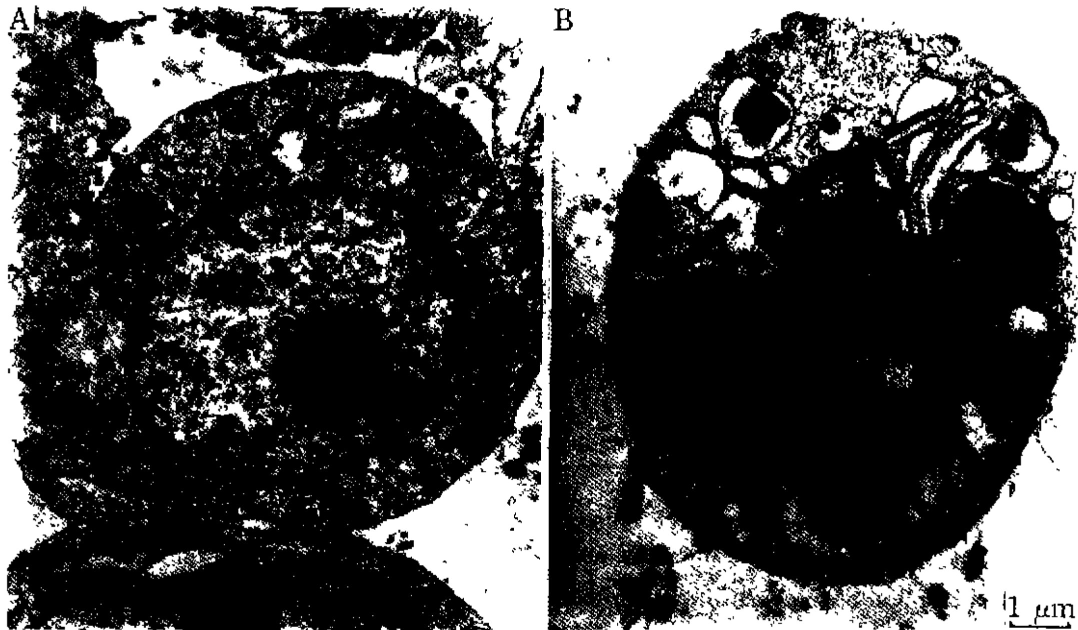

Fig 2. Ultrastructure of HL-60 cells treated with Que for 48 h. (A) Control, ×8000; (B) Que 30 $\mu mol \cdot L^{-1}$, ×8000.

phase position, consistent with cells undergoing DNA degradation during apoptosis. Taken together, our data provide strong evidence that Que induced HL-60 cells apoptosis.

The MTT assay quantifies metabolically viable cells by their ability to reduce MTT[9]. Our study also demonstrated that Que suppressed HL-60 cell growth in a dose-dependent manner using the MTT assay. Recent data indicated that Que increased DNA fragmentation induced by dexamethason in Nb_2 lymphoma cells[12] and by hyperthermia in K562 and KU812 cells[7]. Our results confirmed for the first time that Que elicited apoptosis in HL-60 cells. That Que initiated whether directly by DNA damage or by signal from glucocorticoids, calcium, ionophore, growth factor withdrawal or aging, remains to be clarified.

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槲皮素诱导人白血病 HL-60 细胞凋亡[1]

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关键词　槲皮素；细胞凋亡；DNA 损伤；HL-60 细胞；培养的肿瘤细胞

目的：研究槲皮素是否能诱导人白血病 HL-60 细胞凋亡．方法：应用琼脂糖凝胶电泳法观察 DNA 碎片；采用流式细胞仪检测 DNA 断裂；电镜技术观察凋亡的形态学改变，用 MTT 测定法测定细胞增殖．结果：槲皮素 15 - 120 $\mu mol \cdot L^{-1}$ 诱导 HL-60 细胞凋亡，电镜观察到典型的形态学改变，电泳显示梯状条带，槲皮素能剂量依赖性地触发 DNA 降解及抑制细胞增生(IC_{50}和 95 % 可信区间分别为 43 (30 - 61) $\mu mol \cdot L^{-1}$．结论：槲皮素诱导人白血病 HL-60 细胞凋亡．