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

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KEY WORDS securinine; apoptosis; HL-60 cells; DNA fragmentation; flow cytometry; cultured tumor cells

ABSTRACT

AIM: To study whether securinine might induce apoptosis in human leukemia HL-60 cells. **METHODS**: Inhibition of proliferation was measured using MTT assay. The amount of apoptotic cells was measured by flow cytometry. DNA fragmentation was visualized by DNA agarose gel electrophoresis and the cellular changes were observed by electron microscope. **RESULTS**: Securinine 5 - 80 mg·L^{-1} elicited typical apoptosis morphological changes and DNA fragmentation in a concentration-dependent manner in HL-60 cells. Securinine inhibited HL-60 cell proliferation in a time- and concentration-dependent manner. The IC_{50} and 95 % confidence limits were 27 (15 - 47) mg·L^{-1} after 12-h treatment with securinine. **CONCLUSION**: Securinine induced apoptosis in HL-60 cells.

INTRODUCTION

Securinine (Sec) is an alkaloid extracted from *Securinega suffruticosa* (Pall) Rehd. It is one of central nervous stimulants and clinically applied to treat sequela of poliomyelitis and aplastic anemia. Our experiments discovered that Sec inhibited the growth of human leukemia HL-60 cells. It was suggested that Sec might be a potential anti-tumor drug. This study was to investigate whether Sec could cause HL-60 cells death primarily by apoptosis.

MATERIALS AND METHODS

Reagents Sec was purchased from Yixing Pharmacentical Factory of Jiangsu Province. 3-[4, 5-Dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) and 4′,6-diamide-2-phenglindole hydrochloride (DAPI) were the products of Sigma, and RPMI-1640 was obtained from Gibco.

Cell culture The human leukemic cell line HL-60 was obtained from Shanghai Institute of Cell Biology. The cells were cultured at 37 ℃ in RPMI-1640 medium containing 10 % fetal calf serum and glutamine 2 mmol·L^{-1} in a 5 % CO_2 atmosphere. Cells were used when in exponential growth. Sec was dissovled in ethanol and the final concentration of ethanol was < 0.5 %.

Viability assay Cell viability was measured by the MTT tetrazolium salt assay[1]. Cells were placed in replicated 96-well microtiter plate at a dencity of 25 × 10^7 cells·L^{-1} in a volume of 0.1 mL. Sec 5 - 80 mg·L^{-1} were added immediately. The absorbance (A) was measured on DG-3022A ELISA micro-plate Reader at 570 nm.

Flow cytometry Flow cytometry was made to identify apoptotic cells[2]. Briefly, cells were harvested and fixed in 70 % ethanol at 4 ℃

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overnight. Subsequently, cells were treated with Tris-HCl buffer (pH 7.4) containing 1 % RNase A and were stained with propidium iodide (PI) 5 mg·L^{-1}. Distribution of cells with different DNA contents was determined on flow cytometry (EPICS XL; Coulter, USA) and the data were analyzed by multicycle DNA content and cell cycle analysis software (copyright: 1994 University of Washington).

DNA fragmentation After 48-h incubation with Sec, fragamented DNA was analyzed by eletrophoresis. The cellular DNA was extracted, dialyzed, eletrophoresed in 1.5 % agarose gel, and visualized under UV light after staining with ethidium bromide[3].

Electron microscopy[4] Control and treated cells (2×10^7) were collected and washed twice with PBS. The cell pellets were processed for fixation and stained with osmium tetroxide, and then were observed with electron microscopy (Hitachi 600).

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

RESULTS

Growth-inhibitory effect A concentration-dependent inhibition of cell growth was seen after 12-h treatment with Sec ($r = 0.99$), IC_{50} (95 % confidence limits) were 27 (15 – 47) mg·L^{-1} (Tab 1).

Tab 1. Antiproliferation action of securinine on HL-60 cells at 12 h. $n = 6$ experiments (25×10^3 cells/well). $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Drug/mg·L^{-1}	A_{570}	Inhibitory rate/%
Sec 0	1.30 ± 0.05	
5	1.07 ± 0.04^c	17.69
10	0.97 ± 0.05^c	25.39
20	0.692 ± 0.023^c	46.77
40	0.571 ± 0.019^c	56.08
80	0.342 ± 0.023^c	73.69
0.5 % ethanol	1.25 ± 0.04^b	3.85

Exposure of HL-60 cells to Sec 20 mg·L^{-1} revealed that cell proliferation was inhibited in a time-dependent manner (Tab 2).

Tab 2. Effect of securinine on proliferation in HL-60 cells. $n = 6$ experiments (25×10^3 cells/well). $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Time /h	A_{570} Control	A_{570} Sec 20 mg·L^{-1}	Inhibitory rate/%
0	0.971 ± 0.017	0.977 ± 0.011^a	
6	1.01 ± 0.05	0.873 ± 0.009^c	13.56
12	1.05 ± 0.04	0.72 ± 0.03^c	31.43
24	1.33 ± 0.05	0.32 ± 0.03^c	75.26

DNA degradation Apoptotic cells were distinguished by their fractional DNA content, while the nonapoptotic cells were classified as in G_1, S, and G_2/M phases of the cell cycle. The % of apoptotic cells with fractional DNA content increased from 3.5 % to 44.6 %. Changes in cell cycle distribution of the nonapoptotic cell population occurred in the cells treated with Sec. Sec decreased the proportion of G_1 phase cells, concomitant with an increase in the proportion of S and G_2 + M phase cells. (Fig 1)

DNA fragmentation HL-60 cells were incubated with Sec (5 – 80 mg·L^{-1}) for 48 h. DNA electrophoresis showed a typical "ladder" of DNA. No DNA fragmentation of HL-60 cells was seen in the control group. (Fig 2)

Electron microscopy HL-60 cells treated with HL-60 cells showed typical cell changes of apoptosis. The cell volume reduced indicating that shrinkage of cytoplasm, but the plasma membrane remained intact. The chromation became condensed, and nucleus with the formation of apoptotic body was seen. (Fig 3)

DISCUSSION

The present study demonstrated that Sec suppressed HL-60 cell growth in a concentration-

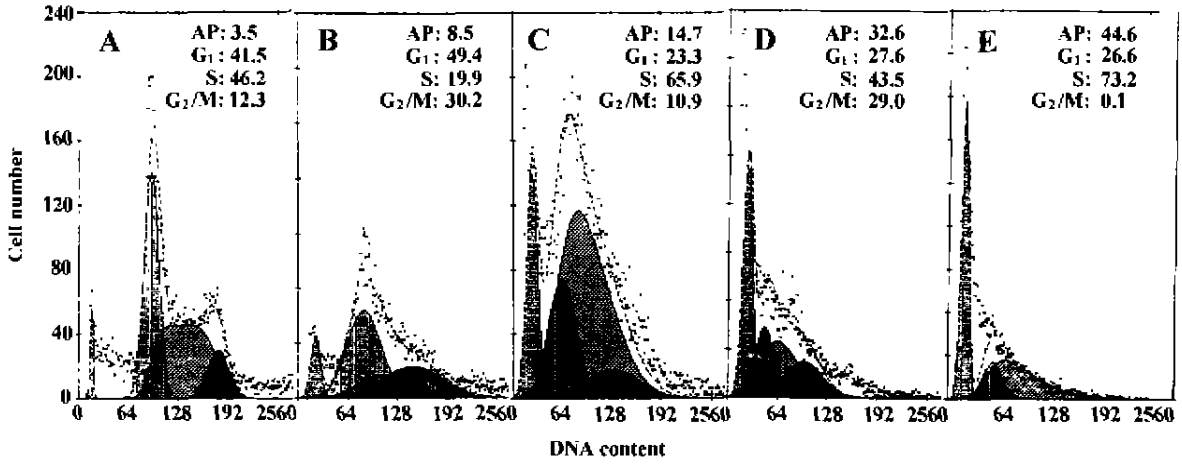

Fig 1. DNA contents of HL-60 cells treated with securinine for 48 h.
Control (A); securinine 10 mg·L^{-1}(B); 20 mg·L^{-1}(C); 40 mg·L^{-1}(D); 80 mg·L^{-1}(E)

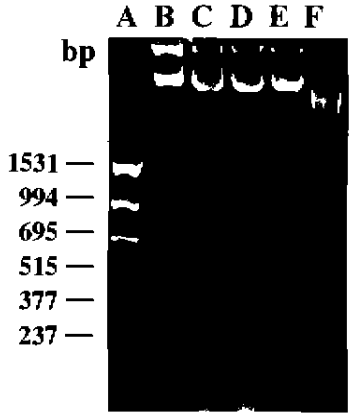

Fig 2. Agarose gel electrophoresis of DNA extracted from HL-60 cells treated with Sec for 48 h. MG3161 DNA marker (A); control (B); Sec 5 mg·L^{-1}(C); 20 mg·L^{-1}(D); 40 mg·L^{-1}(E); 80 mg·L^{-1}(F).

and time-dependent manner using the MTT assay. Further more, our study revealed the occurrence of apoptosis in HL-60 cells treated with Sec. Apoptosis, also termed programmed cell death, is a common mode of eukaryotic cell death characterized by distinct ultrastructural features and a ladder-like DNA fragmentation pattern[6,7]. Recent evidence suggests that the failure of cells to undergo apoptosis be involved in the pathogenesis of many human diseases including cancer[5]. On the other hand, apoptosis may also shed light on cancer therapy[8].

The morphological evidence presented here, including condensed chromatin, nuclear fragmentation, apoptotic bodies, and complete cellular membrane was one of the most reliable markers of apoptosis[4]. Our data also showed that Sec induced DNA "ladder" and apoptotic DNA peak in HL-60 cells treated with Sec. In DNA histograme after flow cytometric analysis, 3.5 % – 44.6 % of HL-60 cells treated with Sec after 48 h were located in sub-G_1-phase position. The elements of apoptosis measured here suggested that Sec killed HL-60 cells primarily by the mechanism of apoptosis.

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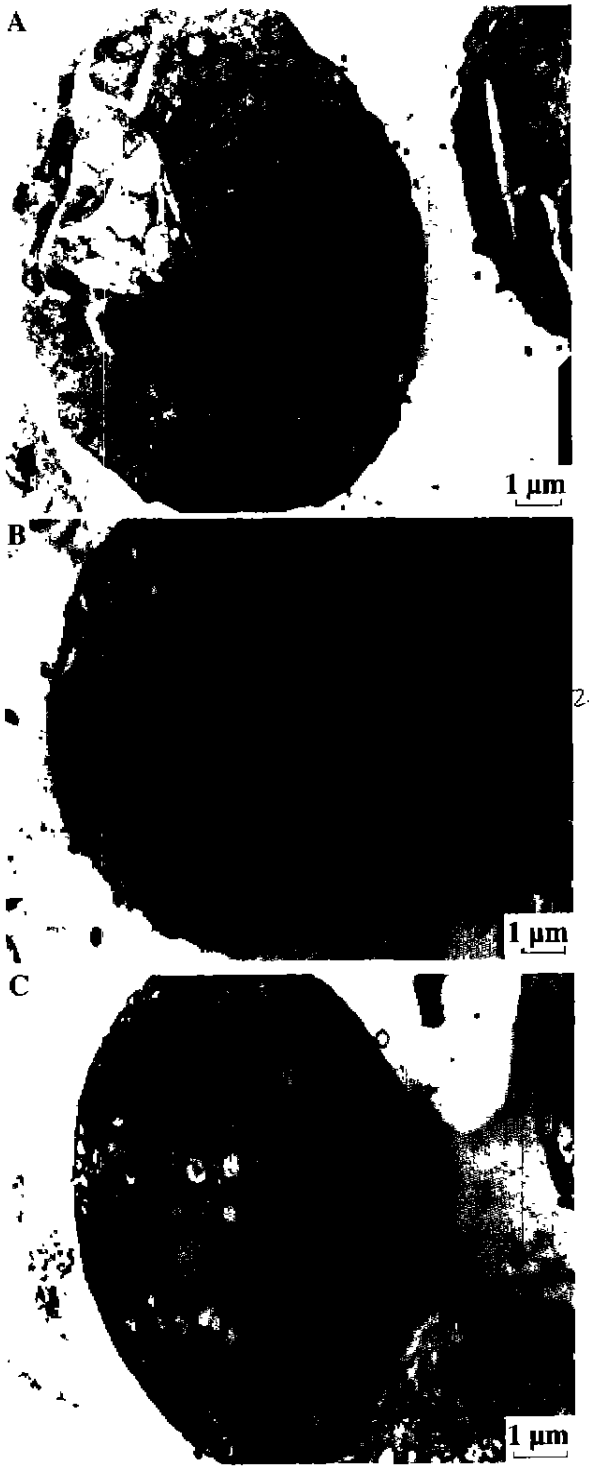

Fig 3. Ultrastructure of HL-60 cells treated with Sec for 48 h, osmium tetroxide stain. A) Control, ×6000; B) Sec 10 mg·L^{-1}, ×6000; C) Sec 10 mg·L^{-1}, ×8000.

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一叶秋碱诱导人白血病 HL-60 细胞凋亡[1]

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关键词 一叶秋碱；细胞凋亡；HL-60 细胞；DNA 断片；流动血细胞计数；培养的肿瘤细胞

目的：研究一叶秋碱能否诱导 HL-60 细胞凋亡。**方法**：用 MTT 法检测一叶秋碱对细胞增殖影响；应用流式细胞仪检测凋亡细胞数；采用琼脂糖凝胶电泳法观测 DNA 碎片；透射电镜观察凋亡的形态改变。**结果**：一叶秋碱 5 – 80 mg·L^{-1}能诱导 HL-60 细胞凋亡。电镜观察到典型的凋亡形态学改变，电泳呈现出阶梯状条带，流式细胞仪检测到凋亡率随剂量的增高而升高。MTT 法示一叶秋碱抑制 HL-60 细胞增殖，并且呈时间、剂量依赖性，药物作用 12 h 的 IC_{50}（95 %可信区间）分别为 27（15 – 47）mg·L^{-1}。**结论**：一叶秋碱诱导 HL-60细胞凋亡。

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