BIBLID: ISSN 0253-9756 Acta Pharmacologica Sinica 中国药理学报 1997 Mar; 18 (2): 169 - 172

α-Anordrin-induced apoptosis of leukemia K562 cells is not prevented by cicloheximide[1]

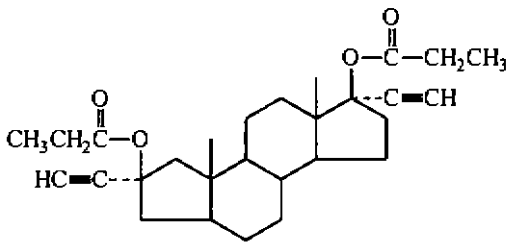
LOU Li-Guang, XU Bin
(*Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China*)

KEY WORDS α-anordrin; cicloheximide; leukemia K562; DNA damage; cultured tumor cells

AIM: To study effect of protein synthesis inhibitor cicloheximide (Cic) on the apoptosis induced by α-anordrin (Ano) in leukemia K562 cells. **METHODS:** Morphological changes were observed by fluorescent microscopy. DNA content was measured by flow cytometry. DNA fragmentation was analyzed by agarose gel electrophoresis. **RESULTS:** Exposure of K562 cells to Ano 50 μmol·L^{-1} for 24 h induced apoptotic cell death. Cic 1 μmol·L^{-1} did not abrogate or delay this effect. Indeed, Ano-induced apoptosis was augmented by Cic. Cic 100 μmol·L^{-1} itself stimulated 25 % K562 cell apoptosis after 24-h culture. **CONCLUSION:** Ano-induced apoptosis was independent of *de novo* protein synthesis.

Apoptosis was often abrogated or delayed by inhibitors of macromolecular synthesis, such as cicloheximide (Cic)[1,2]. Suppression of apoptosis by inhibitor of protein synthesis, however, was not universal. Systems in which macromolecular synthesis blocker such as Cic or dactinomycin had either no effect on cell death or even triggered apoptosis themselves have been reported[3,4].

Anordrin (2α,17α-diethynyl-A-nor-5α-androstane-2α,17α-diol 2,17-dipropionate), a postcoital contraceptive first developed in China, stimulated apoptosis of human leukemia K562 cells[5]. In the present study we sought to determine the protein synthetic requirements of apoptosis in this model using Cic.



α-Anordrin

MATERIALS AND METHODS

Materials Anordrin was produced by Shanghai No 19 Pharmaceutical Factory. Its α isomer (α-anordrin, Ano) was prepared in our Institute[5]. A stock solution of Ano 50 mmol·L^{-1} was made in absolute ethanol. The final concentration of ethanol for all treatments was <0.1 % which had no significant effect on the measured parameters. Cic, propidium iodide (PI), and diamidino-2-phenylindole (DAPI) were purchased from Sigma Chemical Co.

Cell culture and drug treatment The K562 cells were maintained in RPMI 1640 (Gibco Lab) supplemented with 10 % calf serum, benzylpenicillin 100 kIU·L^{-1}, streptomycin 100 mg·L^{-1}, at 37 ℃ in a 5 % CO_2 + 95 % air atmosphere. K562 cells (2×10^8 cells·L^{-1}) were exposed to Ano 50 μmol·L^{-1}, Cic 1 or 100 μmol·L^{-1}, alone or combined for 24 h, then harvested by centrifugation for analysis.

Morphological examination Apoptotic cells were identified under a fluorescent microscope after staining with DAPI.

DNA content Quantitative detection of apoptotic cells and analysis of cell cycle distribution in cultures by flow cytometry was a modification of [6]. The "sub-G_1" peak formed by cells having reduced DNA content in DNA content frequency histogram represents the presence of apoptotic cells[7], which allowed quantitative comparison of the degree of apoptosis induced by different treatments. Following treatment with phosphate-citrate buffer, the cells were stained with PI 50 mg·L^{-1} dissolved in PBS containing RNAase 100 mg·L^{-1}, and 0.1 % Triton X-100. The fluorescence of individual cells was measured with FACStar^{plus} flow cytometer.

DNA gel electrophoresis DNA extracted from control and drug-treated cells (2×10^6 cells)[8] was electrophoresed on horizontal 1.8 % agarose gel at 50 V for 5 h. The DNA was visualized under uv light after staining with ethidium bromide 5 mg·L^{-1}. All experiments were repeated 3 times, and representative data were reported.

[1] Project supported by the National Natural Science Foundation of China, No 39320003.
Received 1996-04-12 Accepted 1996-09-10

RESULTS

Cell morphology All of the dead cells present in cultures treated with Ano or / and Cic exhibited cell shrinkage, chromatin condensation, and nuclear fragmentation, typical of apoptosis[9] (Fig 1). Both Ano and Cic induced apoptosis of K562 cells. Ano-induced apoptosis was not blocked by Cic, but increased after Ano + Cic treatment.

DNA content K562 cells underwent apoptosis in the presence of Ano 50 $\mu mol \cdot L^{-1}$. Ano + Cic led to remarkable increase in the level of apoptosis. Cic 100 $\mu mol \cdot L^{-1}$ in itself induced K562 cell apoptosis up to 25 % after 24-h incubation (Fig 2).

Cell cycle analysis of nonapoptotic cells showed that Ano led to the accumulation of cells in G_1 phase. Cic reduced G_1 and G_2/M phase cells, suggesting that the cells in G_1 and G_2/M phase preferentially underwent apoptosis. The pattern of cell cycle distribution of unaffected cells as a result of treatment with Ano + Cic was similar to that seen in the presence of Ano alone, despite the fact that a comparable number of apoptotic cells were present in these cultures, indicating that apoptosis induced by Ano + Cic occurred in all phases in the cell cycle (Fig 2).

Agarose gel electrophoresis DNA isolated from K562 cells cultured with Ano 50 $\mu mol \cdot L^{-1}$ for 24 h showed the "ladder" pattern characteristics of apoptosis. A comparison with molecular weight

Fig 1. K562 cells: untreated (A), and treated with Ano 50 $\mu mol \cdot L^{-1}$ (B), Cic 1 $\mu mol \cdot L^{-1}$ (C), Ano 50 $\mu mol \cdot L^{-1}$ + Cic 1 $\mu mol \cdot L^{-1}$ (D), and Cic 100 $\mu mol \cdot L^{-1}$ (E) for 24 h. Fluorochrome DAPI stain, ×400.

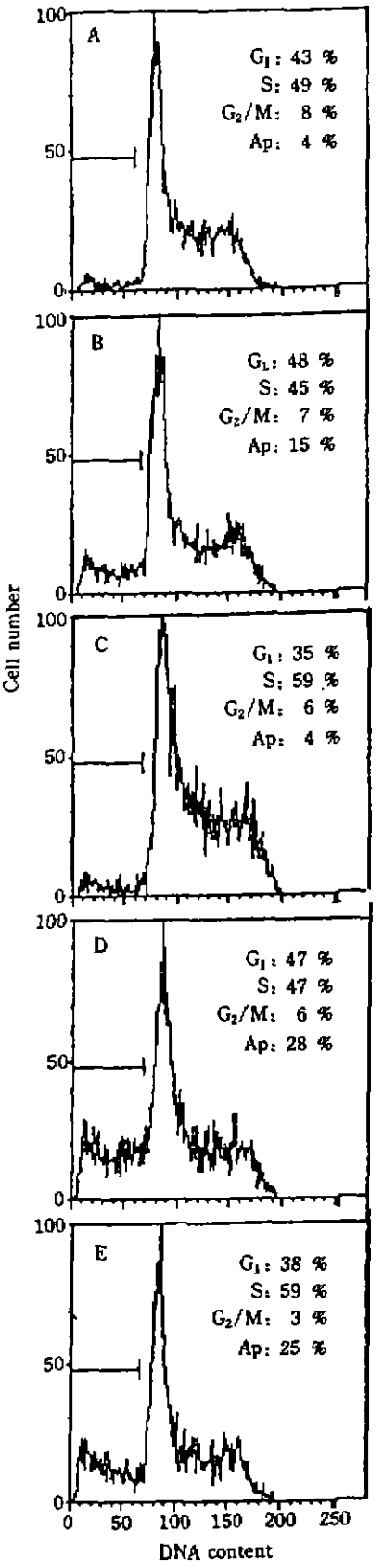


Fig 2. Cell cycle distribution of K562 cells treated with Ano 50 μmol·L^{-1}(B), Cic 1 μmol·L^{-1}(C), Ano 50 μmol·L^{-1} + Cic 1 μmol·L^{-1}(D), and Cic 100 μmol·L^{-1}(E) for 24 h. Control (A). Ap = Apoptotic cells.

markers indicated that the fragments were multiple of approximately 180 – 200 base pairs, indicating cleavage of chromatin at the internucleosomal region. Ano + Cic resulted in a greater extent of DNA fragmentation than Ano did (Fig 3). Cic (100 μmol · L^{-1}) treatment also produced typical "ladder" pattern on th gel. The amount of DNA fragmentation was consistent with the results obtained by morphological estimation and flow cytometric analysis.

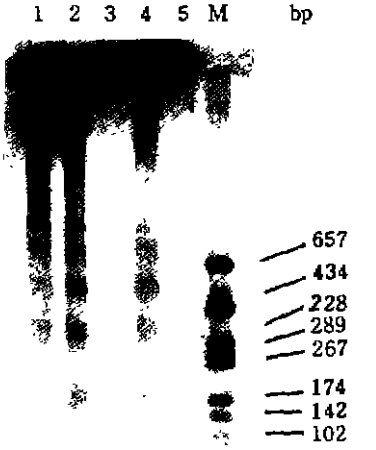


Fig 3. Agarose gel electrophoresis of DNA extracted from cultured K562 cells. 1) Cic 100 μmol·L^{-1}; 2) Ano 50 μmol·L^{-1} + Cic 1 μmol·L^{-1}; 3) Cic 1 μmol·L^{-1}; 4) Ano 50 μmol·L^{-1}; 5) control; M) molecular weight marker.

DISCUSSION

The view that apoptosis is a tightly regulated cell self-destruction mechanism is widely accepted. Numerous studies showed that apoptosis ocurring in a number of circumstances was either delayed or abrogated by RNA or protein synthesis inhibitors[10,11], suggesting synthesis of new protein be required in this process. In this work, however, Cic did not inhibit the Ano-induced apoptosis in K562 cells. In contrast, treatment with Cic markedly augmented apoptosis induced by Ano. The results indicated that apoptosis might be stimulated by other mechanisms. Our resluts were consistent with the findings[12,13].

Why Cic inhibits apoptosis in some experimental systems but not in others is still unknown. Based on the different effects of Cic on the process, Cohen[14] has suggested that conditions in which apoptosis is triggered by the presence of inhibitors of RNA and protein synthesis would represent a "release" mechanism, because these cells behave as though the suicide program is constitutively expressed but inhibited by factors with short half-lives. Ano inhibits RNA and protein synthesis[15], thus, it was likely that Ano induced apoptosis by "release" mechanism. So, it was not surprising that combination of Ano and Cic resulted in greater extent of apoptosis. From our findings, it was concluded that apoptosis induced by Ano was not prevented by protein synthesis inhibitor Cic, ie, Ano-induced apoptosis of K562 cells was independent of new protein synthesis.

REFERENCES

1 Wyllie AH, Morris RG, Smith AL, Dunlop D. Chromatin cleavage in apoptosis: association with condensed chromatin morphology and dependence on macromolecular synthesis. J Pathol 1984; **142**: 67 - 77.

2 Ling YH, Priebe W, Perez-Soler R. Apoptosis induced by anthracycline antibiotics in P388 parent and multidrug-resistant cells. Cancer Res 1993; **53**: 1845 - 52.

3 Tsuchida H, Takeda Y, Takei H, Shinzawa H, Takahashi T, Sendo F. *In vivo* regulation of rat neutrophil apoptosis occuring spontaneously or induced with TNF-α or cycloheximide. J Immunol 1995; **154**: 2403 - 12

4 Takano YS, Harmon BV, Kerr JFR. Apoptosis induced by mild hyperthermia in human and murine tumour cell lines: a study using electron microscopy and DNA gel eletrophoresis. J Pathol 1991; **163**: 329 - 36.

5 Lou LG, Xu B. Induction of apoptosis in human leukemia K562 cells by α-anordrin. Acta Pharmacol Sin 1996; **17**: 255 - 8.

6 Gorczyca W, Gong J, Ardelt B, Traganos F, Darzynkiewicz Z. The cell cycle related differences in susceptibility of HL-60 cells to apoptosis induced by various antitumor agents. Cancer Res 1993; **53**: 3186 - 92.

7 Kalemkerian GP, Slusher R, Ramalingam S, Gadgeel S, Mabry M Growth inhibition and induction of apoptosis by fenretinide in small-cell lung cancer cell lines. J Natl Cancer Inst 1995; **87**: 1674 - 80

8 Kamesaki S, Kamesaki H, Jorgensen TJ, Tanizawa A, Pommier Y, Cossman J. bcl-2 Protein inhibits etoposide-induced apoptosis through its effects on events subsequent to topoisomerase II-induced DNA strand breaks and their repair. Cancer Res 1993; **53**: 4251 - 6

9 Piazza GA, Rahm ALK, Krutzsch M, Sperl G, Paranka NS, Gross PH, *et al*. Antineoplastic drugs sulindac sulfide and sulfone inhibit cell growth by inducing apoptosis. Cancer Res 1995; **55**: 3110 - 6.

10 Sellins KS, Cohen JJ. Gene induction by γ-irradiation leads to DNA fragmentation on lymphocytes. J Immunol 1987; **139**: 3199 - 206.

11 McConkey DJ, Hårtzell P, Duddy SK, Håkansson H, Orrenius S. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin kills immature thymocytes by Ca^{2+}-mediated endonuclease activation. Science 1988; **242**: 256 - 9.

12 Martin SJ, Lennon SV, Bonham AM, Cotter TG. Induction of apoptosis (programmed cell death) in human leukemic HL-60 cells by inhibition of RNA or protein synthesis. J Immunol 1990; **145**: 1859 - 67.

13 Collins RJ, Harmon BV, Souvlis T, Pope JH, Kerr JFR Effects of cycloheximide on B-chronic lymphocytic leukaemic and normal lymphocytes *in vitro*: induction of apoptosis. Br J Cancer 1991; **64**: 518 - 22.

14 Cohen JJ. Apoptosis. Immunol Today 1993; **14**: 126 - 30.

15 Weng SM, Xu YP, Xu B. Antitumor effect of alpha isomer of anordrin *in vitro* and cell cycle arrest at G_1 phase. Acta Pharmacol Sin 1994; **15**: 47 - 50.

169-172

环己米特不能阻止 α-双炔失碳酯诱导的白血病 K562 细胞凋亡

R977.6
R979.1

楼丽广，胥 彬
（中国科学院上海药物研究所，上海 200031，中国）

关键词 α-双炔失碳酯；环己米特；白血病 K562；DNA 损伤；培养的肿瘤细胞

目的：研究蛋白质合成抑制剂环己米特（cicloheximide，Cic）对 α-双炔失碳酯（α-anordrin，Ano）诱导的白血病 K562 细胞凋亡的影响. 方法：荧光显微镜观察形态学变化；流式细胞仪检测 DNA 含量；琼脂糖凝胶电泳分析 DNA 断裂. 结果：Ano 50 μmol·L^{-1}处理 K562 细胞 24 小时诱导细胞凋亡. Cic 1 μmol·L^{-1}共同处理不能减弱或延缓 Ano 的这一作用. 相反，Cic 明显增强 Ano 诱导的细胞凋亡. Cic 100 μmol·L^{-1}本身诱导 25%K562 细胞凋亡. 结论：Ano 诱导的 K562 细胞凋亡不依赖于新的蛋白质合成.