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17β-雌二醇对氧化型低密度脂蛋白损伤的内皮功能的保护作用

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关键词 低密度脂蛋白；溶血磷脂酰胆碱类；雌二醇；血管内皮；吲哚美辛；胸主动脉；苯福林

A 目的：研究 17β-雌二醇对氧化型低密度脂蛋白及其主要成分溶血磷脂酰胆碱(LPC)损伤的内皮功能的保护作用 方法：在兔离体主动脉环用苯福林收缩血管后，观察氧化型 LDL 及 LPC 对血管舒张功能的作用. 结果：17β-雌二醇显著减轻氧化型 LDL 及 LPC 对内皮舒张功能的损伤，并呈剂量依赖性 但吲哚美辛可拮抗 17β-雌二醇的这种保护作用. 结论：17β-雌二醇对氧化型 LDL 或 LPC 损伤的内皮功能具保护作用，其作用可能与刺激依前列醇的产生有关.

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Induction of apoptosis in human leukemia K562 cells by α-anordrin[1]

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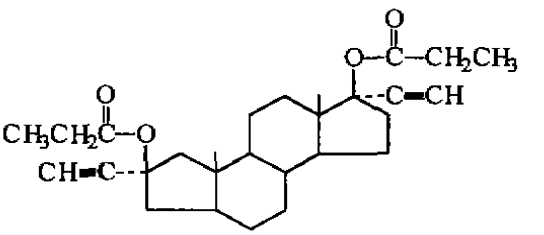
KEY WORDS α-anordrin; apoptosis; leukemia K562; postcoital contraceptives; cultured tumor cells; phase-contrast microscopy; DNA damage

AIM: To study antitumor action of α-anordrin (Ano). **METHODS**: Morphological assessment of apoptosis was performed with light microscope and electron microscope. Membrane integrity was determined by trypan blue exclusion method. Endonucleolysis was assessed by agarose gel electrophoresis and flow cytometric methods. **RESULTS**: Exposure of exponentially growing K562 cells to Ano 2.5 − 50 μmol · L^{-1} for 48 h resulted in growth arrest, Ano 50 μmol · L^{-1} inhibited the growth of K562 cells by 67 %. Cells were mainly blocked to progress through S-phase and arrested at G_1 phase. After treatment of K562 cells with Ano, marked morphological changes including condensed chromatin, nuclear fragmentation, and reduction in volume were observed. Agarose gel electrophoresis of DNA from cells treated with Ano for 24 − 48 h revealed "ladder" pattern, typical features of apoptosis, and near 70 % of cells underwent apoptosis as determined by flow cytometry. The S-phase cells were more susceptible to apoptosis. Despite extensive cleavage of DNA and nuclear fragmentation, the cell membrane of Ano-treated cells remained intact, excluding trypan blue. Apoptotic cells were detected as early as 8 h after Ano (50 μmol · L^{-1}) treatment. **CONCLUSION**: Ano induces apoptosis in K562 cells.

Anordrin is a postcoital contraceptive developed in China[1] and possesses antiestrogenic properties[2]. Our laboratory found that the alpha isomer of anordrin (α-anordrin, Ano) exhibited potent antitumor activities both *in vitro* and *in vivo*[3,4]. In this study we investigated the apoptotic effect of Ano on K562 cells.



α-Anordrin

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MATERIALS AND METHODS

Materials Anordrin was produced by Shanghai 19th Pharmaceutical Factory. It was a mixture of α and β isomers, and the α isomer (Ano) was isolated and purified by low pressure column chromatography[5]. Ano was a crystalline powder, soluble in ethanol, practically insoluble in water. Its melting point was 155 – 156 ℃ A stock solution of Ano was made by dissolving it in absolute ethanol and stored at – 20 ℃. RPMI 1640 was purchased from Gibco. Propidium iodide (PI) was from Sigma Co. DNA molecular marker and RNase A were purchased from Sino-American Biotechnology Co.

Cell culture and drug treatment K562 cells were maintained in RPMI 1640 supplemented with 10 % heat-inactivated calf serum, penicillin 100 kU·L^{-1}, streptomycin 100 mg·L^{-1} in a humidified atmosphere containing 5 % CO_2 at 37 ℃. Exponentially growing cells (2×10^8 cells·L^{-1}) were exposed to Ano 2.5 – 50 μmol·L^{-1} for 24 – 48 h. After treatment, cells were harvested by centrifugation and washed twice with cold Ca^{2+}, Mg^{2+}-free PBS.

Cell growth and morphological assessment Cell growth and membrane integrity was determined by trypan blue dye exclusion method. For assessing morphological changes, the cells were collected by centrifugation for electron microscopic examination.

DNA content DNA content was assayed[6] with minor modification. The cellular DNA was stained with PI instead of diamidino-2-phenylindole. PI 50 mg·L^{-1} was dissoved in PBS containing Triton X-100 0.1 % and RNase A 100 mg·L^{-1}. The fluorescence of individual K562 cells was measured by FACS flow cytometer.

DNA gel electrophoresis The fragmentation of K562 cell DNA was assessed[7]. DNA extracted from untreated and treated cells were loaded onto a 1.8 % agarose gel in TBE (Tris 45 mmol·L^{-1}-borate buffer, edetic acid 1 mmol·L^{-1}, pH 8.0), and electrophoresed at 40 V for 5 h. The DNA in gels was visualized under uv light after staining with ethidium bromide 5 mg·L^{-1}.

Minimal time of cell exposure to Ano resulting in apoptosis To study the minimal duration of Ano treatment leading to initiation of DNA degradation, the cells were treated with Ano 50.0 μmol·L^{-1} for 7, 8, 12, 24 h. The percentage of apoptotic cells was determined by flow cytometry.

Statistical analysis Data were analyzed by t-test.

RESULTS

Cell growth and morphological assessment After exposure of K562 cells to Ano 2.5 – 50 μmol·L^{-1} for 24 – 48 h, the cell growth was inhibited (Fig 1).

Phase-contrast microscopy showed that these cells were multinucleated with micronuclei. The

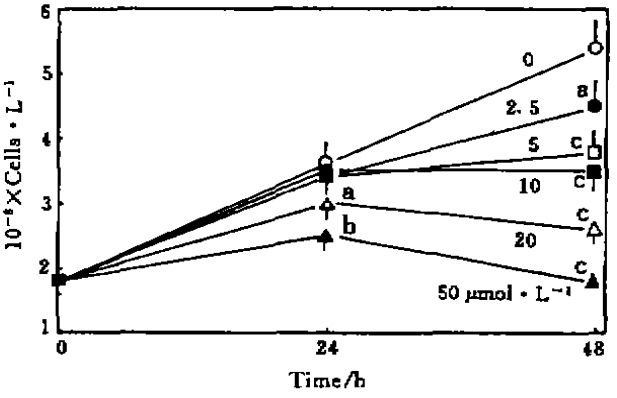

Fig 1. Effect of Ano 2.5 – 50 μmol·L^{-1} for 48 h on growth of K562 cells. $n = 6$ samples, $\bar{x} \pm s$. $^aP > 0.01$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

cell shrinkage, membrane blebbing and reduction in volume were seen (Fig 2, Plate 1).

Electron microscopy revealed a marked chromatin condensation forming circumscribed masses along the nuclear membrane (Fig 3).

Membrane integrity More than 95 % of cells treated with Ano 50 μmol·L^{-1} for 48 h excluded trypan blue, indicating the membrane integrity was preserved.

DNA fragmentation Agarose gel electrophoresis of DNA extracted from cells treated with Ano revealed a "ladder" pattern (Fig 4), suggesting that the preferential DNA degradation occured at internucleosomal, linker DNA regions.

Cell cycle After treatment of K562 cells with Ano 2.5 – 50 μmol·L^{-1} for 48 h, the G_1-phase cells increased from 39 % to 55 %, and S-phase cells decreased from 55 % to 36 %, suggesting that Ano mainly arrested K562 cells at G_1-phase. The exposure of K562 cells to Ano resulted in the appearance of cells with a fractional DNA content, typical of apoptosis. The cell cycle analysis of the unaffected cell population revealed that the G_1-phase cells were increased from 38 % to 48 % and G_2/M-phase cells from 5.4 % to 20 %, whereas S-phase cells were decreased from 56 % to 32 %, it was coincident with increase of apoptotic cells from 2.1 % to 70 %, indicating that S-phase cells were preferentially undergoing apoptosis (Tab 1).

An 8-h incubation of Ano resulted in 5.6 % K562 cell apoptosis. Eight hour later, along with the S-phase cells diminished, the percentage of

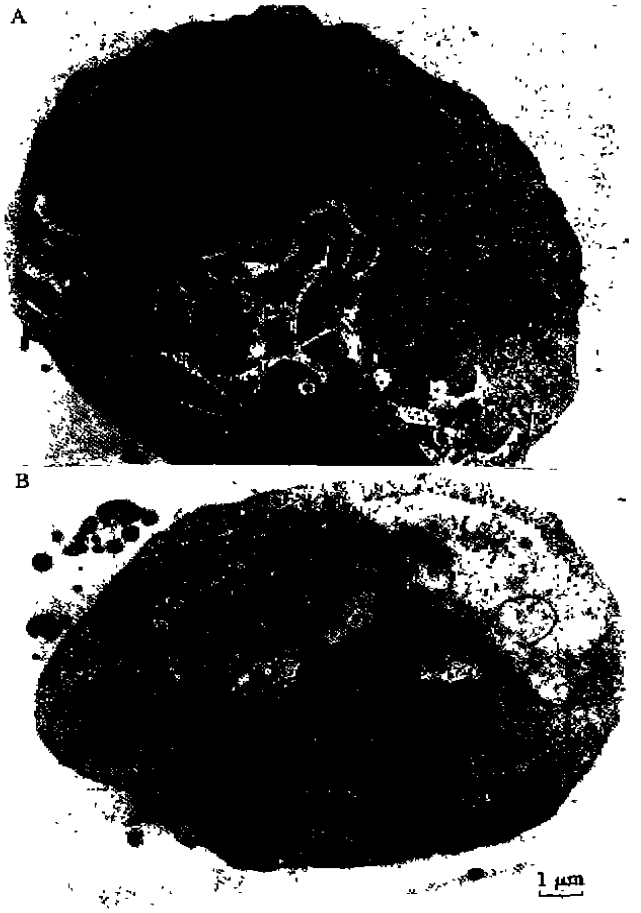

Fig 3. Electron microscopy of K562 cells. (A) control, ×5000. (B) treated with Ano 50 μmol·L^{-1} for 48 h, ×6000.

apoptotic cells were increased in a dose-dependent manner.

Tab 1. Effect of Ano 2.5 – 50 μmol·L^{-1} for 48 h on cell cycle distribution of the nonapoptotic K562 cells. n = 3 samples (10000 cells were measured in each sample). $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ vs control.

Ano/ μmol·L^{-1}	Cell cycle distribution/% G_1	S	G_2/M	Apototic cells/%
0	38 ± 5	56 ± 6	5.4 ± 1.9	2.1 ± 1.0
2.5	42 ± 5^a	52 ± 6^a	5.8 ± 2.0^a	10 ± 4^b
5	43 ± 5^a	49 ± 5^a	8 ± 3^a	15 ± 4^c
10	44 ± 5^a	45 ± 5^c	11 ± 4^a	22 ± 5^c
20	48 ± 5^a	37 ± 4^c	15 ± 4^b	34 ± 6^c
50	48 ± 5^a	32 ± 5^c	20 ± 5^c	70 ± 10^c

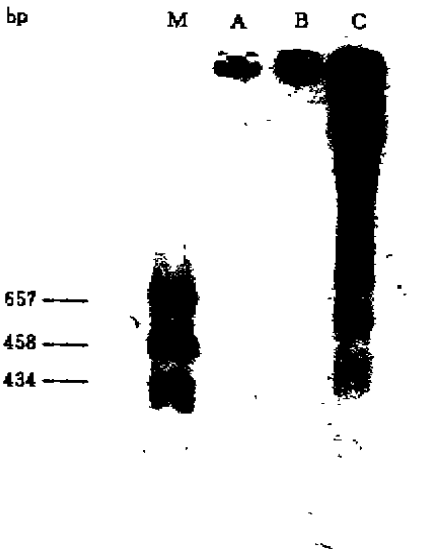

Fig 4. Agarose gel electrophoresis of DNA extracted from the untreated K562 cells (A) and cells treated with Ano 50 μmol·L^{-1} for 24 h (B) and 48 h (C). M: molecular weight markers.

DISCUSSION

In this work, we studied the apoptosis-inducing effect of Ano on K562 cells. At low concentrations, Ano mainly blocked the progression of K562 cells through S-phase and arrested them at G_1-phase. After treatment with Ano at higher concentration (50 μmol·L^{-1}), the cells exhibited marked morphological and biochemical changes related to apoptosis such as cell shrinkage; membrane blebbing; reduction in cell volume; fractional DNA content; and internucleosomal DNA fragmentation. Exposure of K562 cells to Ano for 8 h was enough to induce apoptosis which was assessed by flow cytometric method. However, simultaneous agarose gel electrophoresis of DNA extracted from these cells did not reveal "ladder" pattern which is characteristic of apoptosis. It suggested that flow cytometric measurement of cell DNA content be a more sensitive method to determine apoptosis.

During apoptotic process, the integrity of cell plasma membrane is still preserved, and the functions of membrane remain unchanged. This is

in contrast to cell necrosis, where one of the earliest changes is loss of membrane function and its structural integrity. In this experiment, we found that more than 95 % of cells were trypan blue-exclusive, but about 70 % cells were idenified by flow cytometry as apoptotic cells. These results are coincident with the fact that DNA degradation occurs prior to the loss of membrane integrity during the process of apoptosis[8].

Steroid receptors can act as nuclear transcription factors, and when it is bound to its respective ligand, estrogen receptors may directly regulate steroid-dependent gene transcription[9]. Ano possesses antiestrogen activity and might prevent estrogen from binding to estrogen-receptor, thus, it is likly that Ano alters estrogen receptor-mediated expression of specific genes associated with apoptosis.

In summary, our experiment demonstrated that Ano at appropriate concentration 50 $\mu mol \cdot L^{-1}$ cleaved the chromatin DNA at linker section of nucleosomes and triggered apoptosis of K562 cells. It is, at least in part, related to its antitumor activity.

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α-双炔失碳酯诱导人白血病 K562 细胞凋亡

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关键词 α-双炔失碳酯; 细胞凋亡; 白血病 K562; 事后避孕药; 培养的肿瘤细胞; 相差显微镜检查; DNA 损伤 R965 R73-36

目的: 研究 α-双炔失碳酯(Ano)的抗肿瘤作用. **方法**: 形态学研究分别应用光学显微镜和电子显微镜. 细胞膜完整性检测用台盼蓝排除法. 用流式细胞仪及琼脂糖凝胶电泳检测 DNA 断裂. **结果**: Ano 2.5 - 50 μmol·L^{-1}处理 K562 细胞 48 h, 细胞生长被明显抑制, 50 μmol·L^{-1}时抑制率达 67 %. 细胞主要阻滞在 G_1 期. 处理后的细胞染色质凝缩, 核断裂, 体积缩小, 呈典型的凋亡改变. K562 细胞经 Ano 50 μmol·L^{-1}处理 24 - 48 h 后, 提取细胞 DNA 进行电泳, 呈现典型的"ladder"带型 流式细胞仪检测约有 70 %细胞发生凋亡, S 期细胞比较敏感 此时, 虽然发生广泛的 DNA 断裂, 但细胞膜仍然完整, 排除台盼蓝. Ano 50 μmol·L^{-1}处理 K562 细胞 8 h 即能诱导部分细胞发生凋亡. **结论**: Ano 能诱导 K562 细胞凋亡.