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Protective effects of 17β-estradiol on endothelial function injured by oxidized low-density lipoproteins[1]

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KEY WORDS LDL lipoproteins; lysophosphatidylcholines; estradiol; vascular endothelium; indomethacin; thoracic aorta; phenylephrine

AIM: To test the protective effects of 17β-estradiol against endothelial cell damages due to oxidized low-density lipoproteins (LDL) and lysophosphatidylcholine (LPC), a principal component of oxidized LDL. **METHODS:** After the tension was increased by phenylephrine, the effects of oxidized LDL or LPC on the vasorelaxation responses to acetylcholine (ACh) were investigated in the isolated rabbit thoracic aortas. **RESULTS:** 17β-Estradiol attenuated the inhibition of vasorelaxation responses to ACh by oxidized LDL or LPC in a concentration-dependent manner. However, the protective effect of 17β-estradiol was partially attenuated by indometacin (10 μmol · L^{-1}), a cyclooxygenase inhibitor. **CONCLUSION:** 17β-Estradiol possesses protective effects on the endothelium against injury elicited by oxidized LDL or LPC, which may be related to its stimulation of epoprostenol production.

Coronary atherosclerosis and other cardiovascular diseases are less commonly seen in women than in men[1], and the cardiovascular mortality rate of postmenopausal women who received estrogens is 30 % - 50 % less than that of their untreated counterparts[2]. Oral estrogen therapy increases blood high-density lipoprotein cholesterol level and decreases low-density lipoproteins (LDL) cholesterol[3]. These suggest that estrogen possess a protective role in cardiovascular events.

Abnormalities of endothelial function may be related, in part, to the oxidatively modified LDL. 17β-Estradiol preserves endothelial vasorelaxation function and limits LDL oxidation in hypercholesterolemic pigs[4]. The present work was to study the effects of 17β-estradiol on the endothelial function injured by oxidized LDL or lysophosphatidylcholine (LPC), a principal component of LDL, in the isolated rabbit aortas. Indometacin was used to explore whether epoprostenol participated in the protective effect of 17β-estradiol.

MATERIALS AND METHODS

Reagents All drugs were purchased from Sigma.

Preparation of oxidized LDL Native LDL (density of 1.019 - 1.063 kg · L^{-1}) were isolated from human plasma[5]. Plasma was obtained from fresh normal human blood, LDL was isolated by sequential ultracentrifugation at 119 000 × *g* in the presence of edetic acid 0.2 mmol · L^{-1}. Then LDL was filtered aseptically (0.2 μm) into dialysis tubing and dialyzed in phosphated-buffered solution (PBS) at 4 ℃ for 24 h. For the preparation of oxidized LDL, native LDL was oxidized by exposure to $CuSO_4$ 10 μmol · L^{-1} at 37 ℃ for 20 h. Oxidized LDL was stored at 4 ℃ in the dark and used within 2 wk. Protein concentrations of LDL were determined[6].

Preparation of aortic rings and tension recording Aortic rings were prepared[7]. Rabbits (2.2 ± 0.2 kg, *n* = 50, ♂) were decapitated, and the thoracic aortas were cut into rings (4 mm in length) Rings were suspended in Krebs solution: NaCl 118, KCl 4.8, $CaCl_2$ 2.5, KH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2, and dextrose 11.5 mmol · L^{-1} (37 ℃, aerated with 95 % O_2 + 5 % CO_2). The tension was recorded by a two-channel physiological recorder (Model MLS-2B). The ring was stretched with 6-g resting tension for 60 min, and then pre-contracted with KCl 40 mmol · L^{-1} After a maximal response to KCl was obtained, the rings were washed repeatedly with Krebs solution and equilibrated again for 30 min. To measure vasorelaxation responses, rings were contracted with phenylephrine (0.3 - 3 μmol · L^{-1}) to 40 % - 50 % of their maximal extent. After the contractions stabilized, cumulative concentration-responses to acetylcholine (ACh) (0.001 - 1 μmol · L^{-1}) were obtained. For oxidized LDL or LPC, rings were exposed for 40 and 30 min, respectively, and these remained in the perfusate for the

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remaining study. To study the effect of 17β-estradiol on the inhibition of vasorelaxation responses to ACh by oxidized LDL or LPC, rings were exposed to 17β-estradiol (0.3 – 3 μmol·L^{-1}) for 10 min and then exposed to oxidized LDL or LPC in the presence of 17β-estradiol for 40 and 30 min, respectively. In the case of indometacin, rings were preincubated with indometacin (10 μmol·L^{-1}) for 30 min and the drug remained in the perfusate for the remaining study.

Statistics Statistical analyses were performed using one-way ANOVA, and Tukey's test was used for multiple comparisons when ANOVA indicated significant differences between groups.

RESULTS

Effect of 17β-estradiol on vasorelaxation responses to ACh After the tension was increased by phenylephrine (0.3 – 3 μmol·L^{-1}), ACh caused a concentration-dependent relaxation in the isolated rabbit thoracic aortas. After preincubation with 17β-estradiol (1 μmol·L^{-1}) for 30 min, phenylephrine-induced contraction in the presence of 17β-estradiol was stable ($n = 3$). Exposure to oxidized LDL (500 mg protein·L^{-1}) or LPC (5 mg·L^{-1}) reduced vasorelaxation responses to ACh. However, pretreatment with 17β-estradiol markedly attenuated the inhibition of vasorelaxation responses to ACh by oxidized LDL or LPC in a concentration-dependent manner (Tab 1, 2).

Influence of indometacin on the protective effect of 17β-estradiol Preparations were pretreated with indometacin (10 μmol·L^{-1}). 17β-Estradiol markedly attenuated the inhibition of vasorelaxation responses to ACh by LPC. The effect of 17β-estradiol was significantly attenuated in the presence of indomethacin (Tab 3). However, indometacin itself had no effect on the vasorelaxation responses to ACh on the aortas ($n = 3$).

Tab 1. Effects of 17β-estradiol (Est) on inhibition of vasorelaxation responses to acetylcholine by oxidized LDL. $n = 5$, $\bar{x} \pm s$. $^bP < 0.05$ *vs* control; $^dP > 0.05$, $^eP < 0.05$ *vs* oxidized LDL.

ACh/ $-\lg$ mol·L^{-1}	Relaxation of isolated rabbit aorta/ %				
	Control	Oxidized LDL 0.5 mg·L^{-1}	LDL + Est 0.3 μmol·L^{-1}	LDL + Est 1 μmol·L^{-1}	LDL + Est 3 μmol·L^{-1}
9	4.0 ± 0.6	0 ± 0^b	0 ± 0^d	3.2 ± 1.5^d	3.6 ± 1.2^d
8.5	14 ± 4	0 ± 0^b	0 ± 0^d	11 ± 4^d	15.3 ± 1.5^e
8	37 ± 5	0 ± 0^b	3.4 ± 1.6^d	18 ± 7^e	28 ± 3^e
7.5	47 ± 6	6.2 ± 1.4^b	10.3 ± 2.9^d	32 ± 6^e	38 ± 6^e
7	61 ± 5	10.8 ± 2.1^b	19 ± 4^d	39 ± 8^e	44 ± 8^e
6.5	69 ± 4	18.2 ± 1.9^b	28 ± 3^d	47 ± 6^e	55 ± 5^e
6	70 ± 3	25.7 ± 2.1^b	34 ± 4^d	50 ± 5^e	60 ± 4^e

Tab 2. Effects of 17β-estradiol (Est) on inhibition of vasorelaxation responses to acetylcholine by LPC. $n = 5$, $\bar{x} \pm s$. $^bP < 0.05$ *vs* control; $^dP > 0.05$, $^eP < 0.05$ *vs* LPC.

ACh/ $-\lg$ mol·L^{-1}	Relaxation of isolated rabbit aorta/ %				
	Control	LPC 5 μg·L^{-1}	LPC + Est 0.3 μmol·L^{-1}	LPC + Est 1 μmol·L^{-1}	LPC + Est 3 μmol·L^{-1}
9	3.5 ± 1.3	0 ± 0^b	0 ± 0^c	1.6 ± 0.7^d	0.9 ± 0.5^d
8.5	11.4 ± 1.8	0 ± 0^b	0 ± 0^d	4.4 ± 1.0^d	3.4 ± 0.7^e
8	39.3 ± 2.9	0 ± 0^b	0.9 ± 0.5^d	16 ± 5^d	23 ± 8^e
7.5	50.7 ± 2.6	0.8 ± 0.8^b	5.5 ± 0.8^d	30 ± 6^e	37 ± 6^e
7	69 ± 5	9.4 ± 2.3^b	21 ± 3^d	46 ± 6^e	59 ± 4^e
6.5	79 ± 5	24 ± 5^b	36 ± 5^d	66 ± 5^e	72.2 ± 1.5^e
6	81 ± 4	38 ± 9^b	49 ± 5^d	70.6 ± 2.7^e	72.2 ± 1.5^e

Tab 3. Influence of indometacin (Ind) on protective effect of 17β-estradiol (Est). $n = 5$, $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* LPC; $^dP > 0.05$, $^eP < 0.05$ *vs* LPC + Est.

ACh/ $-\lg$ mol·L^{-1}	Relaxation of isolated rabbit aorta/ %			
	Control	LPC 5 μg·L^{-1}	LPC + Est 1 μmol·L^{-1}	LPC + Ind + Est 1 μmol·L^{-1}
9	4.0 ± 0.6	0 ± 0	3.2 ± 2.5^a	0 ± 0^d
8.5	15 ± 3	0 ± 0	11 ± 4^b	2.4 ± 1.6^d
8	38 ± 4	0 ± 0	20 ± 7^b	4.4 ± 1.9^e
7.5	54 ± 6	6.5 ± 1.2	32 ± 6^b	16 ± 4^e
7	66 ± 3	12.1 ± 2.2	39 ± 9^b	21.1 ± 2.5^e
6.5	68.5 ± 2.6	18.4 ± 1.7	47 ± 6^b	33 ± 5^e
6	68.6 ± 2.6	24.8 ± 1.9	50 ± 5^b	37 ± 8^e

DISCUSSION

We demonstrated that 17β-estradiol attenuated the impairment of endothelium-dependent relaxation due to oxidized LDL or LPC, a principal component of oxidized LDL. These results suggest that the anti-atherogenic effect of estrogen, besides inhibiting low-density lipoprotein oxidation, may be related to the reduction of endothelial cell damages due to oxidized LDL.

LPC increases the intracellular free calcium concentration in vascular smooth muscle cells[8]. LPC increases vascular superoxide anion production and impairs release of the EDRF via activation of protein kinase C[9]. Estrogens act as natural antioxidants of membrane phospholipid peroxidation[10]. In the present study, 17β-estradiol attenuated the impairment of endothelium-dependent relaxation elicited by oxidized LDL or LPC in isolated rabbit thoracic aortas. It is probable that the protective effect of 17β-estradiol is related to its anti-oxygen free radical and anti-lipid peroxidation.

Epoprostenol as well as calcium-channel blocking agents which stimulate the production and/ or release of epoprostenol protect the endothelial cells via anti-oxygen free radical and anti-lipid peroxidation[11,12]. The 17β-estradiol stimulates the secretion of epoprostenol in the cultured piglet endothelial cells[13]. In the present study, the protective effects of 17β-estradiol was partially reversed by indometacin, a cyclooxygenase inhibitor, suggesting that the protective role played by 17β-estradiol may be correlated with its stimulation of epoprostenol production.

In conclusion, 17β-estradiol possesses a protective effect on endothelium against injury elicited by oxidized LDL, and this effect may be due to its stimulation of epoprostenol production.

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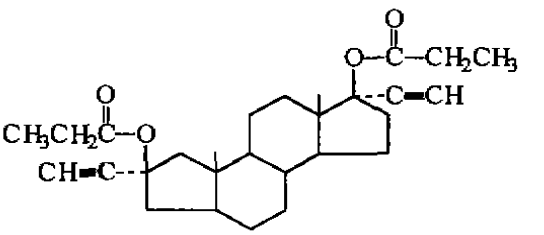
KEY WORDS α-anordrin; apoptosis; leukemia K562; postcoital contraceptives; cultured tumor cells; phase-contrast microscopy; DNA damage

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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