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Oxidized low-density lipoproteins induce apoptosis in vascular smooth muscle cells

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KEY WORDS LDL lipoproteins; vascular smooth muscle; thoracic aorta; cultured cells; DNA fragmentation; apoptosis

AIM: To examine whether oxidized low density lipoproteins (ox-LDL) could induce apoptosis in rabbit aortic smooth muscle cells (VSMC). **METHODS:** Low density lipoproteins (n-LDL) were isolated from healthy human plasma by gradient ultracentrifugation and oxidized by $CuSO_4$ 10 $\mu mol \cdot L^{-1}$. VSMC were exposed to ox-LDL, n-LDL, or phosphate-buffer solution (PBS) as control. Morphological changes were observed under fluorescene microscope after Hoechst 33258 staining. Extracted DNA was electrophoresized on agarose gel. **RESULTS:** Incubation of VSMC with ox-LDL 300 $mg \cdot L^{-1}$, not n-LDL, for 24 h induced morphological apoptosis changes (chromatin condensation, nucleus fragmentation) and DNA fragmentation, which was furthered with the incubation time up to 48 h or at a concentration of 400 $mg \cdot L^{-1}$. Dextran sulfate, a scavenger receptor blocker and butylated hydroxytoluene (BHT), an anti-oxidant, exhibited no effect on DNA fragmentation. Lysophosphatidylcholine (LPC) at a concentration up to 125 $\mu mol \cdot L^{-1}$ (equivalent to ox-LDL 300 $mg \cdot L^{-1}$) did not elicit DNA fragmentation. **CONCLUSION:** Ox-LDL induced apoptosis in VSMC without involving oxygen free radicals and LPC.

Oxidized low-density lipoproteins (ox-LDL) plays a crucial role in atherogenesis[1]. This ox-LDL possessed many potential atherogenic properties, eg, the increased uptake rate by macrophages, toxicity to a variety of cell types, the ability to both stimulation of monocyte chemotactic factors production and the capacity to promote monocyte endothelium adhesion[2].

Toxicity to vascular smooth muscle cells (VSMC) is presumed to be an important atherogenic process because there is VSMC loss from intimal locations during the development of the lipid-rich core region in atherosclerotic plaques[3]. Due to VSMC loss, atheroclerotic plaque becomes unstable and vulnerable to rupture[4].

Apoptosis is a feature of atherosclerosis and restenosis[5]. Apoptotic SMCs were found in advanced atherosclerotic lesions[6]. Ox-LDL triggered apoptosis in macrophages[7] and vascular endothelial cells[8]. This study was aimed to investigate whether ox-LDL could induce apoptosis in VSMC.

MATERIALS AND METHODS

Cell culture VSMC were isolated by outgrowth from explants of New Zealand rabbit thoracic aorta and cultured in M199 medium with 10 % heat-inactivated fetal bovine serum (FBS), benzylpenicillin 100 $kU \cdot L^{-1}$, and streptomycin 100 $mg \cdot L^{-1}$. After confluence, the cultures were passaged at a ratio of 1:3 with 0.1 % trypsin. Cells were characterized as VSMC by morphological appearance of "valley and hill" and by immunohistochemical staining with monoantibody to α-actin. Experiments were performed with confluent monolayer of cells from passage 4 - 10.

Preparation of LDL ox-LDL[9]

Morphological determination of apoptosis VSMC were observed under fluorescence microscope after Hoechst 33258 staining.

DNA electrophoresis[10] At the end of each incubation, cellular DNA was extracted by salting-out and treated with RNase A 10 $mg \cdot L^{-1}$. DNA was electrophoretically fractionated on 1.5 % agarose gel and visualized by ethidium bromide.

Protocol The effect of ox-LDL on DNA fragmentation in VSMC was examined in comparison with n-LDL relative to PBS. To analyze the underlying mechanism: 1) Dextran sulfate as a scavenger receptor antagonist was used to test the role of these receptors in ox-LDL-induced DNA fragmentation; 2) Butylated

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hydroxytoluene (BHT) as an antioxidant was used to test the role of oxygen free radical in this event; 3) Lysophosphatidylcholine (LPC) as an important active component of ox-LDL was used to verify whether LPC could mimick the effect of ox-LDL.

RESULTS

Morphological changes After exposure to ox-LDL 300 mg·L^{-1} for 24 h, VSMC became contracted and partially detached from the culture flask and showed typical morphological changes of apoptosis. The cell volume was reduced, indicating shrinkage of cytoplasm; the chromatin became condensed and nucleus fragmented (Fig 1, Plate 1).

DNA electrophoresis Incubation of VSMC with ox-LDL 300 mg·L^{-1} for 24 h elicited a characteristic "ladder" of DNA fragments representing integer multiples of the internucleosomal DNA length (about 180 – 200 base pair, bp). These changes were furthered with the incubation time up to 48 h or at a concentration of 400 mg·L^{-1}, whereas at lower concentrations (100 and 200 mg·L^{-1}) or with relatively short incubation times (6 or 12 h), no DNA "ladder" was observed. These results indicated that ox-LDL elicited DNA fragmentation in VSMC. However, n-LDL did not elicit a DNA "ladder" when treated VSMC at 400 mg·L^{-1} for 48 h (Fig 2,3).

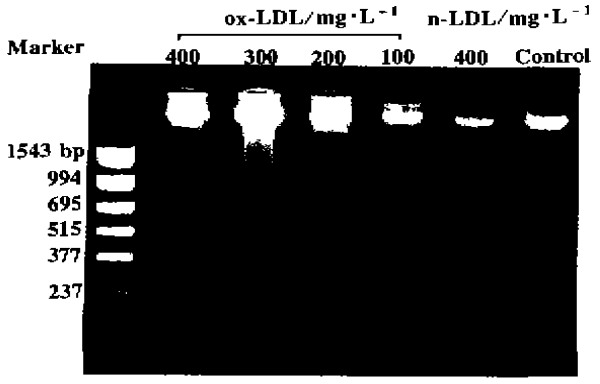

Fig 2. Agarose gel electrophoresis of ox-LDL-induced DNA fragmentation in VSMC.

Effect of lysophosphatidylcholine (LPC) Incubation of VSMC with LPC at a concentration even up to 125 μmol·L^{-1} (equivalent to ox-LDL

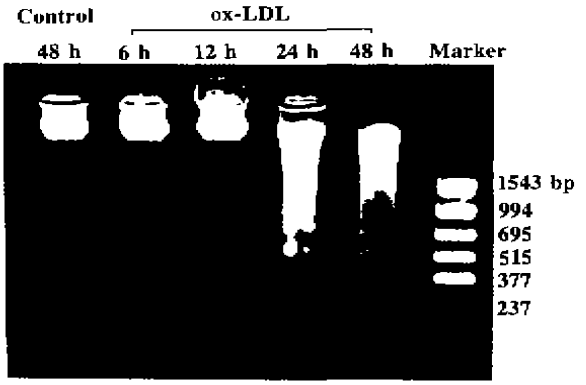

Fig 3. DNA fragmentation induced by ox-LDL in VSMC.

Effect of dextran sulfate Pretreatment of VSMC with a scavenger receptor blocker, dextran sulfate (DS) 20 mg·L^{-1}, for 30 min before adding ox-LDL exhibited no effect on DNA fragmentation induced by ox-LDL 300 mg·L^{-1} (Fig 4).

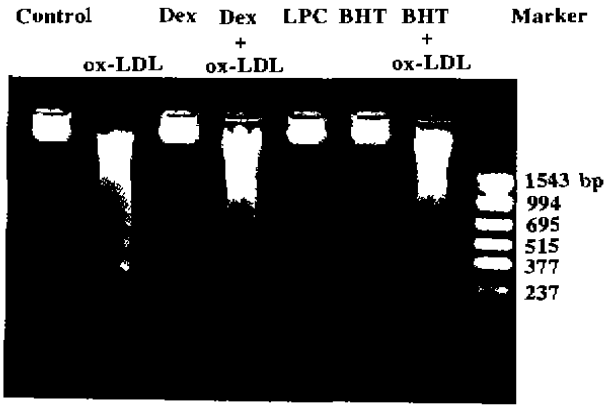

Fig 4. Effect of LPC, DS, and BHT on DNA framentation in VSMC.

300 mg·L^{-1}) for 24 h did not elicit DNA fragmentation (Fig 4).

Effect of butylated hydroxytoluene (BHT) Coincubation of VSMC with BHT 50 μmol·L^{-1} and ox-LDL 300 mg·L^{-1} for 24 h did not abolish the DNA fragmentation-inducing effect of ox-LDL (Fig 4).

DISCUSSION

In atherosclerosis, there is excessive accumulation of cells in the intima mainly due to increased migration and/or proliferation of VSMC

and monocytes/macrophages[11]. However, there is a low density of cells within advanced atherosclerotic plaque. We have previously demonstrated that ox-LDL caused the death of macrophages and vascular endothelial cells by an apoptotic process[7, 8]. The present study indicated that ox-LDL induced apoptosis in VSMC, which seemed to be time and dose related.

Scavenger receptor was recently demonstrated to be expressed in VSMC and mediate its uptake of modified LDL to form foam cells[12]. The fact that scavenger receptor blocker exhibited no effect on ox-LDL-induced DNA fragmentation suggested that scavenger receptor was not involved in the apoptotic process. This was compatible with our previous finding in macrophages[7].

Lysophosphatidylcholine (LPC) is formed during the oxidation of LDL due to the activation of phopholipase A_2. LPC could mimick many of the potential pro-atherogenic effects of ox-LDL[13]. However, the present study showed that LPC at a concentration even up to 125 μmol $\cdot L^{-1}$ did not cause DNA fragmentation. Along with our previous similar result in macrophages, this further indicated that LPC was not an apoptosis-inducing component of ox-LDL.

The finding that antioxidant butylated hydroxytoluene (BHT) did not affect the effect of DNA fragmentation suggested that oxygen free radical generation was not responsible for the effects of ox-LDL, which was in accordance with Diane's observation that the combined addition of BHT and ox-LDL to the culture medium did not alleviate the cytotoxicity of ox-LDL to fibroblasts, although the oxidation of LDL was completely prevented by BHT 20 μmol $\cdot L^{-1}$[14].

In conclusion, ox-LDL induced apoptosis in VSMC. Scavenger receptor, LPC, and oxygen free radicals were not involved in this event. This may have implications in advanced atherosclerotic plaque rupture due to VSMC death.

REFERENCES

1 Steinbrecher UP, Zhang H, Lougheed M. Role of oxidatively modified LDL in atherosclerosis. Free Rad Bio Med 1990; 9: 155 – 68

2 Penn MS, Chilsolm GM. Oxidized lipoproteins, altered cell function and atherosclerosis. Atherosclerosis 1994; 108 (Suppl): S21 – 9

3 Bocan TMA, Schifani TA, Guyton JR. Ultrastructure of the human aortic fibrolipid lesion: formation of the atherosclerotic lipid-rich core. Am J Pathol 1986; 123: 413 – 24

4 Schroeder AP, Falk E. Vulnerable and dangerous coronary plaques. Atherosclerosis 1995; 118 (Suppl): S141 – 9

5 Isner MJ, Kearney M, Bortman S, Passeri J. Apoptosis in human atherosclerosis and restenosis. Circulation 1995; 91: 2703 – 11.

6 Han DKM, Haudenschild CC, Hong MK, Tinkle BT, Leon MB, Liau G. Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. Am J Pathol 1995; 147: 267 – 77.

7 Niu XL, Zhang XW, Guo ZG. Oxidized low-density lipoproteins induce apoptosis in macrophages. Acta Pharmacol Sin 1996; 17: 467 – 70.

8 Guo ZG, Niu XL, Guo X. Oxidized low density lipoproteins induce apoptosis in aortic and endocardial endothelial cells. Acta Pharmacol Sin 1997; 18: 525 – 8.

9 Li YJ, Li YJ, Wu JX, Yu XJ, Yan YF. Protective effect of tetramethylpyrazine against damages of aortic endothelial cells elicited by low density lipoproteins. Acta Pharmacol Sin 1994; 15: 407 – 10.

10 Tanaka M, Ito H, adachi S, Akimoto H, Nishikawa T, Kasajima T, *et al*. Hypoxia induces apoptosis with enhanced expression of Fas antigen messenger RNA in cultured neonatal rat cardiomyocytes. Circ Res 1994; 75: 426 – 33.

11 Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990's. Nature 1993; 362: 301 – 9.

12 Pitas RE, Friera A, McGuire J, Dejager S. Further characterization of the acetyl LDL (scavenger) receptor expressed by rabbit smooth muscle cells and fibroblasts. Arteriosclerosis Thromb 1992; 12: 1235 – 44.

13 Cox DA, Cohen ML. Effects of oxidized low-density lipoprotein on vascular contraction and relaxation: clinical and pharmacological implications in atherosclerosis. Pharmacol Rev 1996; 48: 3 – 19.

14 Morel DW, Hessler JR, Chisolm GM. Low density lipoprotein cytotoxicity induced by free radical peroxidation of lipid. J Lipid Res 1984; 24: 1070 – 6.

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氧化型低密度脂蛋白诱导血管平滑肌细胞凋亡

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关键词 低密度脂蛋白类; 血管平滑肌; 胸主动脉; 培养的细胞; DNA 断片; 细胞凋亡

目的: 研究氧化型低密度脂蛋白(ox-LDL)诱导血管平滑肌细胞凋亡. **方法**: 梯度超速离心分离血浆 LDL, 以 $CuSO_4$ 10 μmol $\cdot L^{-1}$氧化, 观察 ox-LDL 对培养兔胸主动脉平滑肌细胞的损伤作用. Hoechst 33258 荧光染色观察形态学改变, 抽提细胞 DNA 进行琼脂糖凝胶电泳. **结果**: ox-LDL 300 mg $\cdot L^{-1}$与 VSMC 共温育 24 h 诱导典型的凋亡形态学变化和 DNA 降解, 但天然低密度脂蛋白无此作用. 当 ox-LDL 为 400 mg $\cdot L^{-1}$或温育时间延至 48 h, 上述变化更加明显. 硫酸葡聚糖 20 mg $\cdot L^{-1}$和 BHT 50 μmol $\cdot L^{-1}$对此作用无影响. LPC 125 μmol $\cdot L^{-1}$无诱导凋亡作用. **结论**: ox-LDL 诱导血管平滑肌细胞凋亡, 氧自由基和 LPC 不参与这一过程.