# Oxidized-LDL Leads to Cholesteryl Ester Accumulation and Apoptosis in Porcine Aortic Smooth Muscle Cells*

YUAN Zhong-Hua[1,2)**], YANG Yong-Zong[2)], YANG Xiao-Yi[2)]

([1)] *Department of Pathphysiology, Xiangya Medical College, Central South University, Changsha* 410078, China;
[2)] *Institute of Cardiovascular Research of Nanhua University, Hengyang, Hunan* 421001, China)

**Abstract** Both cellular cholesterol metabolism imbalance and apoptosis are related to the development of atherosclerosis. To investigate the relationship between the cellular cholesterol metabolism and apoptosis, the porcine aortic smooth muscle cells were cultured with medium 199 containing 15 mg/L oxidized low density lipoprotein (Ox-LDL) for 72 h, the ratio of cellular cholesteryl ester to total cholesterol increased from 26.2% to 64.1%, and Ox-LDL induced accumulation of cellular cholesteryl ester in a concentration dependent manner in the cells. It indicated that the vascular smooth muscle cells had transformed to foam cells. In addition, cells incubated with oxidized low density lipoprotein had characteristic of apoptosis, as determined by fluorescence microscope, laser scanning confocal microscope and flow cytometry. From this findings, it was speculated that the induction of apoptosis may be related to the raise of the ratio of cellular cholesteryl ester to total cholesterol besides oxidation of low density lipoprotein in vascular smooth muscle cells.

**Key words** oxidized low density lipoprotein, cholesteryl ester, apoptosis, vascular smooth muscle cells, atherosclerosis

The foam cells found in the atherosclerotic plaques are derived from blood-born monocytes or vascular smooth muscle cells (VSMCs) that have taken on many of the properties of macrophage. The hallmark of these cells is their high cholesteryl ester content ($\geqslant$50% of total cellular cholesterol)[1,2]. The role of oxidized low density lipoprotein (Ox-LDL) in atherosclerosis has been documented[3,4].

Han *et al*.[5] examined 35 human atherosclerotic lesion samples and identified a substantial number of cells undergoing apoptosis in 25 of the samples. Immunostaining with cell-type-specific markers on the samples revealed that the majority of the apoptotic cells were modulated smooth muscle cells as well as macrophages. It was concluded that apoptosis may have an important role in determining the course of atherogenesis.

In the current experiments, we cultured the porcine aortic smooth muscle cells with Ox-LDL to explore the relationship between accumulation of cellular cholesteryl ester and apoptosis of VSMCs by analyzing the cellular cholesteryl ester (CE) and morphological changes.

## 1 Material and Methods

### 1.1 Materials

Colchicine, Ethidium Bromide, RNase A, Trypsin and Hoechst33342 were purchased from Sigma (St. Louis, MO). Fetal bovine serum (FBS) and Medium 199 were from Gibco (Grand Island Biological, Grand Island, NY). Folin-phenol reagent was from Beijing Honest Co. (Beijing, China). Cellular total cholesterol kit and free cholesterol kit were from Beijing Chemicals Factory (Beijing, China).

### 1.2 Cell culture

VSMCs were obtained by an explant method from the porcine aorta. The media were carefully removed and cut into 1 $mm^2$ sections, then were transferred into tissue culture bottles. The culture medium consisted of Medium 199, antibiotics (penicillin 100 U/ml, streptomycin 100 mg/L) and 10% (*v*/*v*) heat-inactivated FCS. Cells migrating from the tissue culture bottles incubated at 37℃ in a humidified incubator (95% air-5% $CO_2$ atmosphere) were trypsinized and subcultured by repeated passages. VSMCs of 5~10 passages were used for experiments.

### 1.3 Isolation of LDL

Blood from healthy volunteers was collected in tubes and centrifuged at 5 000 r/min for 30 min at 10℃ to remove formed elements. LDL($d$ = 1.018~1.063) was isolated by sequential ultracentrifugation of plasma according to the method of Havel *et al*[6]. The protein concentration of freshly dialyzed LDL

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** Corresponding author.
Tel: 86-734-8281277, E-mail: zhhyuan@cmmail.com
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was determined by the method of Lowry *et al*[7] using bovine serum albumin as the standard. Unless otherwise stated, each concentration of lipoprotein in the paper refer to the concentration of its protein. LDL were stored in the dark at 4 ℃ and used within 3 weeks.

### 1.4 Preparation of Ox-LDL

Ox-LDL was produced by incubating LDL [$m$(protein)/$v$ = 168 mg/L, $m$(cholesterol)/$v$ = 30 mg/L] in PBS containing 10 μmol/L $CuSO_4$ for 12 h at 37 ℃ and then stopping the oxidation with 40 μmol/L BHT and 20 μmol/L EDTA. The samples were then dialyzed against three changes of PBS containing 0.01% EDTA for 24 h.

### 1.5 MDA content of LDL and Ox-LDL

The lipid peroxide content of LDL and Ox-LDL was measured by a modification of the thiobarbituric acid-reacting substances (TBARS) assay of Yagi[8]. The average TBARS value of the $n$(Ox-LDL)/$m$(cholesterol) and $n$(LDL)/$m$(cholesterd) were 17.2 μmol/g and 1.5 μmol/g, respectively.

### 1.6 Experimental groups

There were three groups in the experiment. VSMCs in control group were cultured in normal medium as previously described; Ox-LDL group was cultured in normal medium plus with 15 mg/L of Ox-LDL for 72 h. VSMCs in Col group were first incubted with 15 mg/L Ox-LDL for 72 h and then treated by Ox-LDL plus 10 μmol/L colchicine for another 24 h.

### 1.7 Cellular total cholesterol, free cholesterol and cholesteryl ester determination

The cells treated as above were dissociated with trypsin, then were collected by centrifugation (1 000 r/min, 10 min, room temperature) and washed once with 2 ml of phosphate-bufferd saline. After recentrifugation (1 000 r/min, 10 min, room temperature), the supernatant fraction was removed. 100 μl of 0.1 mol/L NaOH and 100 μl of isopropanol were added to the remained cellular component, and the cells were sonicated for 5 min. After centrifugation (1 000 r/min, 10 min, room temperature), cellular total cholesterol and free cholesterol was measured by 50 μl of the supernatant respectively using purchased kit. Cholesteryl ester was determined by the difference of total cholesterol and free cholesterol. The protein content of cells was determined from the pellet by the procedure of Lowry.

### 1.8 Detection of apopotosis by fluorescence microscope and laser scanning confocal microscope

Stock solutions of Hoechst 33342 in PBS (2 mmol/L) was prepared and stored avoid of light. VSMCs were seeded on 24-hole culture plates with a slide in every hole. After treatment, the dye was then added directly to the preparation to effect a 10 μmol/L dilution final concentration and the preparation was incubated for 15 min at 37 ℃. Then the medium was changed with suspension medium which is free of serum. The slides were removed from the culture plate and washed with PBS for two times. The slides, were put into acetone for 2 s, then fixed at carrier slides with colourless nail enamel. The preparations were analyzed with UV-visible fluorescence microscope (Nicon Co. Japan) and laser scanning confocal microscope (LSCM) (Opton Co. Germany).

### 1.9 Flow cytometric analysis

Exponentially growing VSMCs were synchronized to $G_0$ phase by removing serum and replenishing[9]. Cells were maintained in Medium 199 with 1% serum for 2 days and were refected with complete medium.

For flow cytometric analysis of DNA content, Cells ($1 \times 10^6 \sim 2 \times 10^6$) which had been treated as described in section **1.4** were fixed in 70% ethanol at 4 ℃ for 24 h. The fixed cells were treated with 1 g/L RNase A in PBS for 30 min at 37 ℃ and then placed in an ice bath to stop the reaction. The cells were stained with 5 g/L ethidium bromide at 0 ℃ for 30 min in the dark. The samples were analyzed by FACS402 (Becton Dickinson Co.). For each sample, 10 000 cells in the gate region (the distribution region of cycling cells) were collected for data analysis and repeated three times.

### 1.10 Statistical analysis

Data are presented as $\bar{x} \pm s$. Significance at the $P < 0.05$ level was determined, where applicable, by the student's $t$-test.

## 2 Results

### 2.1 Cholesteryl ester accumulation in VSMCs

Addition of different concentrations of Ox-LDL to the culture medium of VSMCs for 72 h, The content of cellular total cholesterol, free cholesterol and cholesteryl ester were gradually increasing. As shown in Figure 1, the ratio of cholesteryl ester to total cholesterol (CE/TC) was also gradually increasing. At an approximate Ox-LDL concentration of 12 mg/L, the ratio was 50%. When the concentration of Ox-LDL was 15 mg/L, CE/TC had been close to maxima (64.1%).

When adding 15 mg/L Ox-LDL to medium for 72 h. The ratio of CE/TC of Ox-LDL group increased significantly ($P < 0.05$) as compared to control group (Table 1).

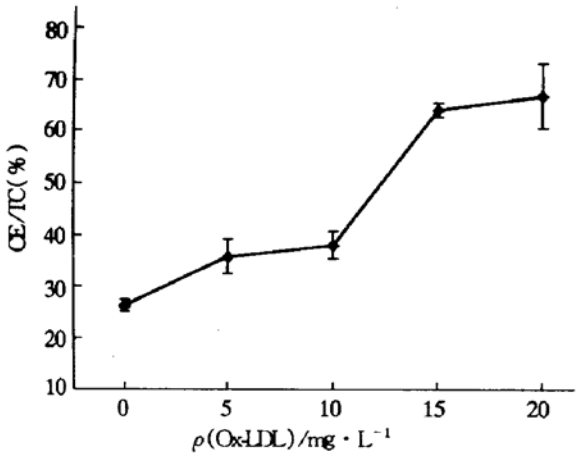


**Fig. 1 Effect of accumulation of cholesteryl ester in VSMCs by Ox-LDL**

VSMCs were incubated with various concentration of Ox-LDL for 72 h. Cellular total cholesterol and cholesteryl ester were determined as described in methods. $\bar{x} \pm s$, $n = 10$.

**Table 1 Ratio of cholesteryl ester to total cholesterol in VSMCs incubated with Ox-LDL**

| Group | TC | FC | CE | CE/TC(%) |
|---|---|---|---|---|
| control | 171.9±10.1 | 126.6±3.6 | 45.1±2.9 | 26.2±1.2 |
| Ox-LDL | 292.3±8.5 | 105.0±17.1 | 187.3±9.2 | 64.1±1.4* |

Values are expressed as $\bar{x} \pm s$, $n = 10$. * $P < 0.05$ *vs*. control group.

Finally, we attempted to analyze the time course of CE/TC in VSMCs incubated with Ox-LDL. When VSMCs incubated with 15 mg/L of Ox-LDL for different incubation time, significant CE/TC in Ox-LDL group was observed after 48 h of incubation and the value was more than 50% (Figure 2). The number of death cells increased rapidly after 96 h and the variance of the ratio of CE/TC was much greater.

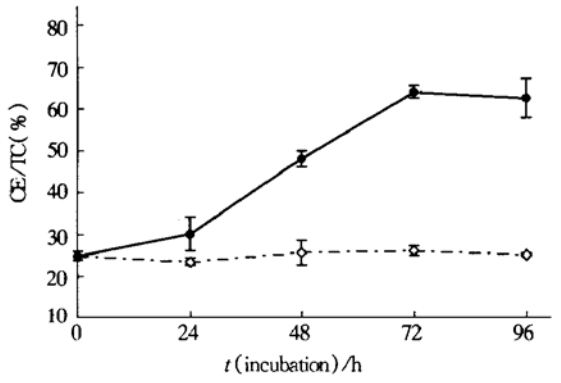


**Fig. 2 Time course of CE/TC in VSMCs incubated with Ox-LDL**

VSMCs were incubated with 15 mg/L of Ox-LDL for different space. The results are expressed as $\bar{x} \pm s$, $n = 10$. * $P < 0.05$ when compared to incubation without Ox-LDL. ◇——◇: control; ●——●: Ox-LDL.

## 2.2 Morphological changes of apoptotic cells

The nuclear events of apoptosis begin with collapse of the chromatin against the nuclear periphery and into one or a few large clumps within the nucleus[10]. The nuclear envelope remains morphologically intact. The chromatin becomes progressively more condensed, eventually adopting one of several characteristic morphologies. In many cases the entire nucleus condenses into a single dense ball, whereas in others the chromatin buds outward into smaller balls resembling acluster of grapes, with each 'grape' surrounded by nuclear envelope. In yet other cases, the chromatin adopts a striking half-moon morphology as it condenses against only one side of the nucleus. Whatever the specific pattern, the final level of condensation is dramatic, apparently exceeding even that seen at mitosis.

Hoechst 33342 has been widely used in the past as an intravital fluorescent stain which will allow separation of cells on the basis of their DNA content and which is not deleterious to the cells themselves[11]. This fluorescent stain is known not to intercalate in the DNA helix and to preferentially bind to AT-rich regions in the DNA[12].

Cells were stained intravitally with the DNA dyes Hoechst 33342 as described previously. Under ultraviolet (UV) epi-illumination ($\lambda_{ex}$, 330 ~ 380 nm; $\lambda_{em}$ > 430 nm), cells emit blue fluorescence due to Hoechst 33342. Apoptotic cells can be distinguished by their nuclear morphology with nuclear condensation and fragmentation, as well as by the higher intensity of blue fluorescence of the nuclei. As seen in Figure 3, the chromatin of the nuclei in control group cell was uniform. The margin of nuclei was smooth. The chromatin in Ox-LDL group had condensed and there were some dense gobbets in the nucleus. Colchicine, as a positive drug, made the cells apoptosis apparently[13]. The chromatin in Col group intensively condensed and the margin of nuclei was very rough.

Confocal microscopy produces "optical sections" through semitransparent tissue without the need for cutting thin slices. It eliminates the blur and flare of out-of-focus planes in an object, has improved axial resolution that enables accurate three-dimensional reconstruction, and has rapid speed of image acquisition. In addition, LSCM images had a better resolution and allowed individual nuclei to be more clearly defined. With LSCM, we could see the chromatin in control group being distributed equably (Figure 4). To cutting the Ox-LDL group cells, we attained 9 "optical sections". Figure 4b was the photo of the third section. There were some chromatin mass which was not near margin of the nuclear envelope (marked with "+ ") in nucleus. The nucleus in the Col group had large chromatin mass and progressively shrank.


**Fig. 3 Detection of apoptosis induced by Ox-LDL in VSMCs**

(a) Control group; (b) Ox-LDL group; (c) Col group. After treatment as described in methods, cells were stained with Hoechst 33342 and photographed under fluorescence microscopy using Kodak type 100 film (bar= 100 μm). Apoptotic cells show condensation of chromatin in nucleus.

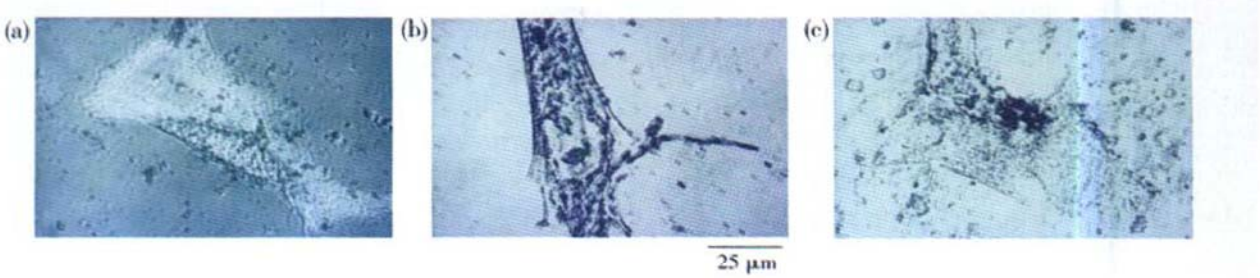


**Fig. 4 Detection of apoptosis induced by Ox-LDL in VSMCs**

(a) Control group; (b) Ox-LDL group; (c) Col group. After treatment as described in methods, cells were stained with Hoechst 33342 and photographed under LSCM using Kodak type 100 film (bar= 25 μm). Apoptotic cells show condensation of chromatin in nucleus.

### 2.3 Changes of DNA content

To further confirm that Ox-LDL induced apoptosis of VSMCs, the cell DNA content was assessed by flow cytometry. Ox-LDL treatment was associated with the appearance of a cell population with a reduced DNA content (hypodiploid), usually referred to as sub $G_1$ or Ap, characteristic of apoptosis (Figure 5). Most dramatic reductions of DNA content were evident in floating cells (not shown), reflecting the effects of chromatin fragmentation. In Figure 5, horizontal axis represents relative DNA content and vertical axis represents cell number. The result from control group was shown in Figure 5a. The first peak (broken line) shows G1 phase and the second peak shows G2+ M phase. S phase can be seen between the first and the second peak. The ratio of apoptotic cells (apoptotic cell number/total cell number) was 1.2%. There was a low Ap peak in Ox-LDL group (Figure 5b) and the ratio of apoptotic cells was 17.6%. But the Ap peak in Col group was very high and the ratio of apoptotic cells was 62.3%. There were a few cells in other cell cycles phases. The ratio of apoptotic cells of Ox-LDL group and Col group increased significantly ($P< 0.05$) as compared to control group.

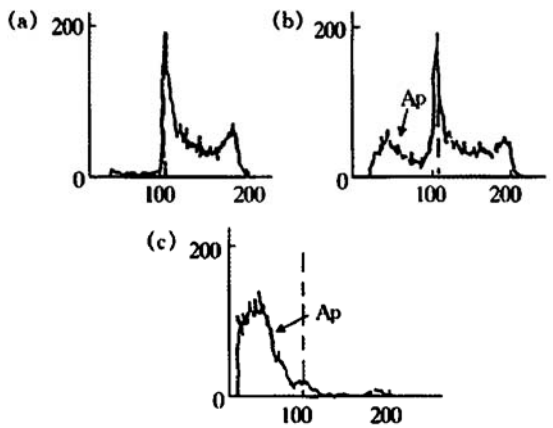


**Fig. 5 DNA histogram of VSMCs**

(a) Control group; (b) Ox-LDL group; (c) Col group. Horizontal axis represents relative content of DNA. Vertical axis represents cell number. Ap peak which is characteristic of apoptosis mirrors the effects on chromatin fragmentation.

## 3 Discussion

The cells of the arterial wall were shown to contain lipoprotein lipase, cholesterol esterase, phospholipase $A_2$, phospholipase C, phospholipase D and also lipoxygenase. It had been demonstrated that LDL, the major cholesterol carrier in human plasma, can be modified by enzymatic as well as by nonenzymatic processes[14] and the atherogenicity of LDL could be attributed to these modifications, especially oxidized modification. The early lesion in human atherosclerosis contain monocyte-derived macrophages which accumulate cholesterol mostly from plasma LDL. The native LDL has to undergo some modification in order to contribute to macrophage cholesterol accumulation *in vitro*[15]. Whereas macrophages predominate in

the early lesion, in the advanced atherosclerotic plaque, arterial smooth muscle cells play a major role by their proliferation, migration and cholesterol accumulation. In the advanced atherosclerotic lesion, cholesterol accumulates not only in macrophages, but also in arterial smooth muscle cell and thus contributes to the formation of the complicated fibrous plaque. In our experiments, when VSMCs incubated with native LDL, the ratio of cellular CE/TC increased from 26.2% to 30.1%, but the total cholesterol did not increase obviously which was consistent with the previous reports, it may be the reaction of porcine vascular smooth muscle cells to LDL being different from the other cells. However, when the cells incubated with Ox-LDL, the ratio of CE/TC could increased from 26.2% to 64.1% and the total cholesterol increased obviously. Foam cells in atherosclerotic plaques mainly derived from macrophages and arterial smooth muscle cells. Compared with original cells, the hallmark in foam cell was the accumulation of cholesteryl ester. Apparently, the VSMCs incubating with Ox-LDL had translated to foam cell.

Escargueil-Blanc *et al*.[16] demonstrated that apoptosis could been induced by Ox-LDL and accompanied with cellular calcium increased in lymphoblastoid cells. de Bono *et al*.[17] also reported that hydrogen peroxide caused bovine endothelial cells apopotosis. Geng *et al*.[18] found that apoptotic vascular smooth muscle cells in advanced human atheroma, mostly located in the fibrous cap of plaque. All of these findings confirmed the importance of apoptosis in atherogenesis and injury of blood vessels.

Apoptosis has been characterized biochemically by activation of an endonuclease that cleaves the DNA of the cell at the linker regions between nucleosomes, yielding small double-stranded fragments of DNA 180 bp to 200 bp long. These fragments can be visualized as a series of bands ("DNA ladder") by agarose gel electrophoresis; such a ladder pattern has in fact been regarded as the most characteristic hallmark for apoptosis. Several laboratories, however, have now established that the classic DNA ladder may be delayed or absent in cell death that appears by other criteria to be apoptotic[19~21]. In our experiments, we did not find the "DNA ladder" (data not shown).

Observing nuclear changes in apoptosis is a more reliable method, but its sensitivity for detecting apoptotic cells is very low. In current study paper, apoptotic VSMCs were identified by fluorescence microscope, LSCM and flow cytometry for improving the sensitivity. The result showed typical nuclear changes in apoptosis of VSMCs induced by Ox-LDL. Especially, with LSCM, we could see the changes of nucleus from the collapse of chromatin against the nuclear periphery, a few large clumps within the nucleus to the dense ball. Analysis by flow cytometry, apoptotic cells number was related to the height of Ap peak. As shown in Figure 5, Ox-LDL apparently induced VSMCs apoptosis.

Apoptosis can be triggered by various stress agents, including cytokines, ionizing and UV radiation, anticancer drugs, heat shock, and exposure to hydrogen peroxide. In recent years, the fashionable mechanism of apoptosis is about the changes of gene, cellular calcium ion and proteinase, but it was not clear. Bennett *et al*.[22] studied the human vascular smooth muscle cells derived from coronary plaques and normal coronary arteries. Their data confirmed that in apoptotic cells derived from coronary plaques were easier to be induced than that from normal coronary arteries. In addition, the expression of bcl-2 was not different. In 2000, Yin *et al*.[23] discovered that cholesterol oxides, but not cholesterol, were found to inhibit proliferation and induce apoptosis of vascular smooth muscle cells in tissue culture. In the current experiments, oxidation of LDL may be one of the causes for apoptosis, but we speculate the more important cause may be related to the cholesteryl ester loading to cells, since in foam cell formation was the elevation of cholesterol ester. Many enzymes of hydrolysis and re-esterification of cholerseryl ester (e.g. acyl-CoA: cholesterol acyltranferase, and neutral cholesteryl ester hydrolase) may involved in this process. From the results, we speculate that the apoptosis induced by Ox-LDL in VSMCs be triggered by some enzymes in cholesterol metabolism, but this needs to be confirmed and investigated in further studies.

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## References

1 Wurster N B, Zilversmit D B. The role of phagocytosis in the development of atherosclerotic lesions in therabbit. Atherosclerosis, 1971, **14** (3): 309~322

2 Small D M. Cellular mechanisms for lipid deposition in atherosclerosis (first of two parts). N Engl J Med, 1977, **297** (16): 873~877

3 Steinberg D, Parthasarathy S, Carew T E, *et al*. Beyond cholesterol: modifications of low-density-lipoprotein that increase

its atherogenicity. N Eng J Med, 1989, **320** (14): 915~ 924
4 Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature, 1993, **362** (6423): 801~ 809
5 Han D K, Haudenschild C C, Hong M K, *et al*. Evidence for apoptosis in human atherogenesis and in a rat vascular injury model. Am J Pathol, 1995, **147** (2): 267~ 277
6 Havel R J, Eder H A, Bragdon J H. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest, 1995, **34** (5): 1354~ 1353
7 Lowry O H, Rosebrough N J, Farr A L, *et al*. Protein measurement with the Folin phenol reagent. J Biol Chem, 1951, **193** (23): 265~ 275
8 Yagi K. Simple flurometric assay for lipoperoxide in blood plasma. Biochem Med, 1976, **15** (2): 212~ 216
9 Ormerod M G, Kubbies M. Cell cycle analysis of asynchronous cell populations by flow cytometry using bromodeoxyuridine label and Hoechst-propidium iodide stain. Cytometry, 1992, **13** (7): 678 ~ 685
10 Wyllie A H, Kerr J F R, Currie A R. Cell death: the significance of apoptosis. Int Rev Cytol, 1980, **68** (21): 251~ 305
11 Arndt-Jovin D J, Jovin T M. Analysis and sorting of living cells according to deoxyribonucleic acid content. J Histochem Cytochem, 1977, **25** (7): 585~ 589
12 Murray V, Martin R F. Sequence specificity of $^{125}$I-labelled Hoechst 33258 in intact human cells. J Mol Biol, 1988, **201** (2): 437~ 442
13 Escargueil-Blanc I, Salvayre R, Negre-Salvayre A. Necrosis and apoptosis induced by oxidized low density lipoproteins occur through two calcium-dependent pathways in lymphoblastoid cells. FASEB J, 1994, **8** (13): 1075~ 1080
14 Steinberg D, Parthasarathy S, Carew T E, *et al*. Beyond cholesterol: modification of low density lipopreotein that increase its atherogenicity. N Engl J Med, 1989, **320** (14): 915~ 924
15 Witztum J L, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest, 1991, **88** (6): 1785~ 1792
16 Escargueil-Blanc I, Salvayre R, Negre-Salvayre A. Necrosis and apoptosis induced by oxidized low density lipoproteins occur through two calcium-dependent pathways in lymphoblastoid cells. FASEB J, 1994, **8** (13): 1075~ 1080
17 De Bono D P, Yang W D. Exposure to low concentration of hydrogen peroxide caused delayed endothelial cell death and inhibits proliferation of surviving cell. Atherosclerosis, 1995, **114** (2): 235~ 245
18 Geng Y J, Libby P. Evidence for apoptosis in advanced human atheroma. Am J Pathol, 1995, **147** (2): 251~ 266
19 Oberhammer F, Wilson J W, Dive C, *et al*. Apoptotic death in epithelial cells: cleavage of DNA to 300 and/or 59 kb fragments prior to or in the absence of intracellular fragmentation. EMBO J, 1993, **12** (9): 3679~ 3684
20 Leszczynske D, Zhao Y, Luokkamaki M, *et al*. Apoptosis of vascular smooth muscle cells: protein kinase C and oncoprotein Bcl-2 are involved in regulation of apoptosis in non-transformed rat vascular smooth muscle cells. Am J Pathol, 1994, **145** (6): 1265 ~ 1270
21 Schultze-Osthoff K, Waleczak H, Droge W, *et al*. Cell nucleus and DNA fragmentation are not required for apoptosis. J Cell Biol, 1994, **127** (1): 15~ 20
22 Bennett M R, Evan G I, Schwartz S M. Apoptosis of human vascular smooth muscle cells derived from normal vessels and coronary atherosclerotic plaques. J Clin Incest, 1995, **95** (5): 2266~ 2274
23 Yin J, Chaufour X, McLachlan C, *et al*. Apoptosis of vascular smooth muscle cells induced by cholesterol and its oxides *in vitro* and *in vivo*. Atherosclerosis, 2000, **148** (2): 365~ 374

# 氧化低密度脂蛋白诱导主动脉平滑肌细胞胆固醇酯聚集和凋亡*

袁中华[1,2)]** 杨永宗[2)] 杨小毅[2)]

([1)]中南大学湘雅医学院病理生理教研室，长沙 410078; [2)]南华大学心血管病研究所，衡阳 421001)

**摘要** 细胞内胆固醇代谢的失衡和细胞凋亡都与动脉粥样硬化的发生有关. 为了研究两者之间的关系，我们把猪的主动脉平滑肌细胞与 15 mg/L 氧化低密度脂蛋白共同孵育 72 h，发现细胞内胆固醇酯与总胆固醇的比值由 26.2% 增加到 64.1%，并且细胞内胆固醇酯的积聚有剂量依赖关系，表明细胞已经转化为平滑肌源性的泡沫细胞. 另外，使用荧光显微镜、激光共聚焦显微镜和流式细胞仪分别发现，与氧化低密度脂蛋白共孵育的细胞有典型的凋亡形态改变. 从实验可以推测，由氧化低密度脂蛋白诱导的平滑肌细胞凋亡，除了低密度脂蛋白氧化的因素外，也可能与细胞内胆固醇酯与总胆固醇的比值升高有关.

**关键词** 氧化低密度脂蛋白，胆固醇酯，凋亡，平滑肌细胞，动脉粥样硬化

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** 通讯联系人. Tel: 0734-8281277, E-mail: zhhyuan@cmmai.com

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