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Expression of intercellular adhesion molecule-1 in U937 foam cells and inhibitory effect of imperatorin[1]

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YANG Peng-Yuan, RUI Yao-Cheng[2], LI Kai[3] (*Department of Pharmacology, School of Pharmacy*; [3]*Department of Thoracic Cardiac Surgery, Changhai Hospital, The Second Medical Military University, Shanghai 200433, China*); HUANG Xing-Hua, JIANG Jian-Ming, YU Long
(*Institute of Genetics, Fudan University, Shanghai 200433, China*)

KEY WORDS foam cells; intercellular adhesion molecule-1; imperatorin; LDL lipoprotein; Western blotting; Northern blotting

ABSTRACT

AIM: To investigate the expression level of intercellular adhesion molecule-1 (ICAM-1) in a macrophage-derived foam cell model from human U937 cell line and the inhibitory effect of imperatorin (IMP) on the ICAM-1. **METHODS**: U937 cells were incubated with oxidized low density lipoprotein (ox-LDL) 80 mg/L for 48 h and a macrophage-derived foam cell model was established. The medium was pretreated with different concentrations of IMP (0, 25, 50, 100 μmol/L). ICAM-1 protein expression in cells was measured with Western blotting; ICAM-1 mRNA level in cells was measured by Northern blotting. **RESULTS**: After incubated with ox-LDL, ICAM-1 expression level increased greatly. The increase in ICAM-1 protein level and mRNA level was estimated to be about 15-fold and 10-fold. When the cells were pretreated with imperatorin (50, 100 μmol/L), the increase of ICAM-1 in foam cells were remarkably inhibited. Especially when pretreated with IMP 100 μmol/L, the ICAM-1 protein level decreased by 79 % and the mRNA level decreased by 74 % each compared to the level of foam cells. **CONCLUSION**: After incubated with ox-LDL *in vitro*, the U937 foam cells showed an enhanced ICAM-1 expression compared with normal U937 cells. IMP could inhibit the expression of ICAM-1 in U937 foam cells.

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[2] Correspondence to Prof RUI Yao-Cheng. Phn 86-21-2507-0991. Fax 86-21-2507-0991. E-mail Ruiyc@smmu.edu.cn
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INTRODUCTION

Foam cells are characteristic pathological cells in the lesions of atherosclerosis. During the process of atherosclerosis, monocytes seem to play a central role. Once monocytes adhere to the subendothelial space and enter into the intima of the artery, oxidized low density lipoprotein (ox-LDL) and other substances associated with atherogenesis may participate in transformation of the monocytes into macrophage[1,2]. Uptake of ox-LDL by the macrophages through scavenger receptors will lead to foam cells formation[3].

Intercellular adhesion molecule-1 (ICAM-1) is a transmembrane glycoprotein which belongs to the immunoglobulin superfamily. The basic function of ICAM-1 is the induction of specific and reversible cell-cell adhesion, resulting in intercellular communication. The increase of ICAM-1 expression has been implicated in a variety of disorders such as inflammation and immune disorders. It has been reported that the process of atherogenesis is not only a lipid metabolism disorder, but also an inflammatory reaction. The enhanced expression of ICAM-1 in atherosclerotic plaques is observed[4], but few report determined the ICAM-1 expression in foam cells.

Imperatorin (IMP) is an effective pure compounds extracted from the root of *Peucedanum decurisivum* and *Angelica dahurica*. It has been reported that IMP was found to antagonize induced adhesion reaction and had certain effects on cardiovascular system[5]. Through incubated U937 cells with ox-LDL[6], we established a macrophage-derived foam cell model from human U937 cell line. Upon the foam cell model, the study aimed to investigate the expression level of ICAM-1 in foam cells and the inhibitory effect of IMP.

MATERIALS AND METHODS

Reagents $CuSO_4$ and ethylenediamine tetraacetic

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acid disodium salt (EDTA) were purchased from Sigma Chemical Co. ICAM-1 antibody was obtained from San Fransco Co (USA). ICAM-1 cDNA probe was kindly provided by Dr YUE Tian-Li in Pennsylvania University in USA. IMP was provided by Department of Medical Chemistry of Nature Products in our university.

Preparation of ox-LDL LDL (d = 1.019 to 1.063 kg/L, purchased from Sigma Co in USA) was sterilized by filtration through 0.45-μm Millipore membranes, and stored at 4 ℃ as described previously[7]. After EDTA was removed by dialysis, LDL was oxidized by incubating in $CuSO_4$ 10 μmol/L for 16 h at 37 ℃, and then dialyzed in phosphate buffered saline (PBS) containing EDTA 0.1 mmol/L for 24 h at 4 ℃.

Cell culture The human monocyte line U937 was obtained from Cell Bank in Shanghai Institute of Biological Sciences, Chinese Academy of Sciences. U937 cells were cultured in RPMI-1640 (GIBCO/BRL) containing 10 % fetal bovine serum. For experiments, U937 cells at the confluence in the dishes were pretreated in serum-free RPMI-1640 for 24 h and the medium was treated with different concentrations of IMP (0, 25, 50, 100 μmol/L). Then the U937 cells were incubated with ox-LDL 80 mg/L for 48 h. The foam cell model was established in the cell group without IMP treated. The control group was the normal U937 cells which have not treated with IMP and ox-LDL.

Oil red O dyeing The foam cells were collected and fixed with 4 % paraformaldehyde for 12 h. The cells were then treated with fresh 0.3 % oil red O (AMRESCO, USA) for 20 min.

Western blotting The protein samples were dissolved and boiled in Laemmli buffer for 10 min. The protein concentration was estimated by Lowry method[8]. The samples were then separated by SDS-PAGE using 15 % gels, and transferred to polyvinylglidene fluoride membranes (Bio Rad) at 35 V for 16 h. After blocking with tris(hydroxymethyl) aminomethane buffered solution containing Tween 20 (TBST), the membranes were incubated with the primary antibody in TBST. Secondary antibodies in TBST were added and incubated for 2 h at room temperature. Bound secondary antibodies were detected using an ECL detection system (Amersham Pharmacia Biotech).

Northern blotting Total RNA was isolated from the U937 cells by using Trizol reagent (GIBCO/BRL). RNA (15 μg) was fractionated by denaturing electrophoresis and capillary blotted to nylon membranes (Boehringer Mannheim). The filters were cross-linked with UV light, followed by prehybridization for 4 h at 50 ℃ in prehybridization solution. Hybridization was then performed with 5 mg/L of [α-^{32}P] CTP-labeled ICAM-1 cDNA probe for 18 h at 50 ℃. After hybridization, the filters were washed and exposed to Fuji imaging plate. The relative mRNA level of ICAM-1 was normalized against the signal of GAPDH.

Blot film analysis The Western and Northern blot films were analyzed with smark view software (Furi Co). The corrected density with the software was plotted as a percentage of control value.

Statistics All results are shown as $\bar{x} \pm s$. Statistical analysis was performed with ANOVA.

RESULTS

Morphological form of the U937 foam cells After incubated with ox-LDL, many red pellets were found in the plasma of the U937 foam cells, which showed that U937 cells internalized ox-LDL through scavenger receptor and became foam formation (Fig 1).

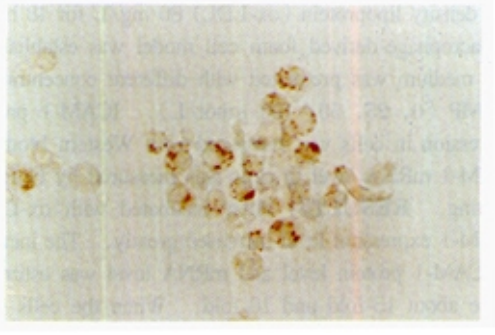

Fig 1. The morphological form of the U937 foam cells dyed with oil red O after incubated with ox-LDL 80 mg/L (×400).

ICAM-1 protein levels in foam cells and the effects of IMP Before the samples were subjected to SDS-PAGE, the protein concentrations were adjusted at a same level, and the result was expressed as a ratio relative to the control expression level. As shown in Fig 2, after incubated with ox-LDL 80 mg/L, the increase in ICAM-1 was estimated to be about 15-fold, which indicated that foam cells could give a strong ICAM-1 protein expression. However, the protein level in IMP treated cells decreased greatly. Especially when treated with IMP 100 μmol/L, the ICAM-1 protein level decreased by 79 % compared to that of foam cells.

ICAM-1 mRNA levels in foam cells and the

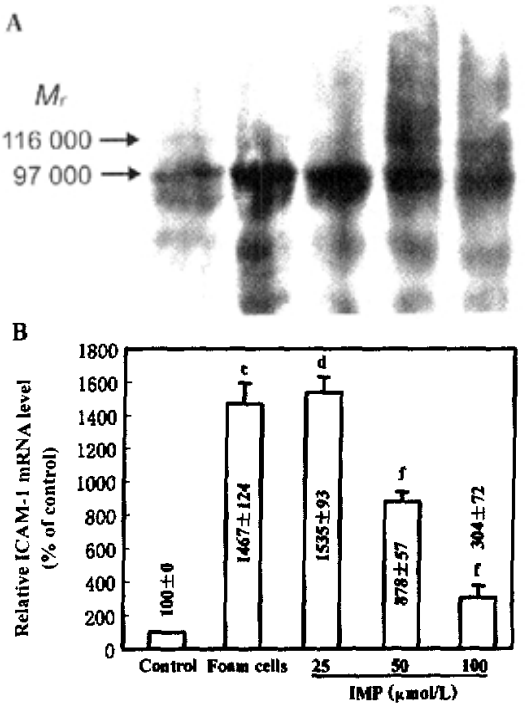

Fig 2. Western blotting analysis of the expression of ICAM-1 in U937 foam cells and the effect of IMP. A) protein was analyzed by Western blotting. B) corrected density with smark view software was plotted as a percentage of control value. $n = 3$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control group. $^dP > 0.05$, $^fP < 0.01$ *vs* foam cell group.

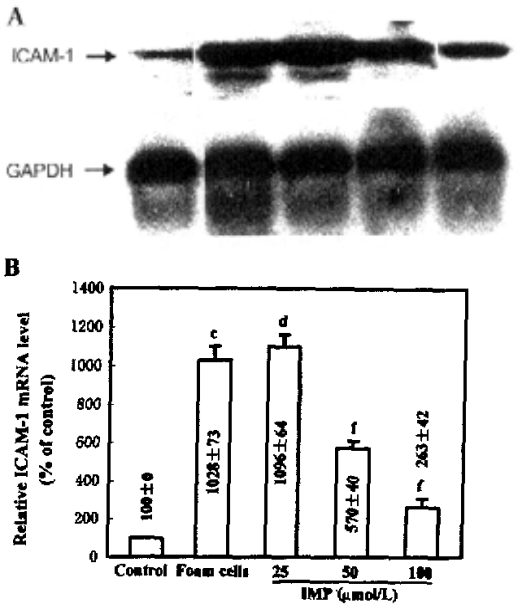

Fig 3. Northern blotting analysis of the expression of ICAM-1 in U937 foam cells and the effect of IMP. A) mRNA was analyzed by Northern blotting. B) corrected density with smark view software was plotted as a percentage of control value. $n = 3$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* control group. $^dP > 0.05$, $^fP < 0.01$ *vs* foam cell group.

effects of IMP The relative mRNA level of ICAM-1 was normalized against the signal of GAPDH and the result was expressed as a ratio relative to the control expression level. As shown in Fig 3, after incubated with ox-LDL 80 mg/L, the increase in ICAM-1 was estimated to be about 10-fold, which indicated that foam cells could give a strong ICAM-1 mRNA expression. But the mRNA level in IMP treated cells decreased greatly. When treated with IMP 100 μmol/L and incubated with ox-LDL, the ICAM-1 mRNA level decreased by 74 % compared to that of foam cells.

DISCUSSION

Atherosclerosis is not merely a disease in its own right, but a process that is the principal contributor to the pathogenesis of myocardial and cerebral infarction, gangrene, and loss of function in the extremities. The active stages of the lession of atherosclerosis are characterized by the extensive infiltration of blood-derived monocyte/macrophages through the endothelium into the arterial intima. The migration of momocytes into the arterial intima where they internalize ox-LDL through scavenger receptor[9] and become macrophage-derived foam cells results in formation of fatty streaks, which are believed to represent the earliest type of atherosclerotic plaque. The foam cells also play a role in the fibroproliferative process by their capacity to form numerous growth factors in particular platelet-derived growth factor[10,11] as well as IL-1[12], TNFα[13], and vascular endothelial growth factor[14].

Poston *et al*[4] have found a strong expression of ICAM-1 on endothelial cells, microphages, and smooth muscle cells of the atherosclerosis plaques, while normal arterial cells outside plaques give weaker or negative reactions. The basic function of ICAM-1 is the induction of specific and reversible cell-cell adhesion, resulting in intercellular communication. In this study, we proved *in vitro* that after incubated with ox-LDL, the U937 foam cells showed an increased expression compared with normal U937 cells. The enhanced ICAM-1 expression in foam cells indicated that ox-LDL might promote the

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activation of an immunological process and the interactions of microphage and other cells, which may aggravate the process of atherosclerosis.

IMP, extracted from the root of *Peucedanum decurisivum* and *Angelica dahurica*, is a kind of coumarin, which has tranquilization, anti-inflammatory, and antibiosis effects. Recent studies[5] have proved that IMP could inhibit the expression of ICAM-1 induced by brain isochemia-reperfusion and also could inhibit the stimulated monocyte adhesion to isochemic-reperfusion tissue. The present foundings suggested that IMP could inhibit the expression of ICAM-1 in U937 foam cells, which indicated that IMP might have potentiality to be clinically applied for atherosclerosis.

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U937泡沫细胞中细胞间粘附分子-1的表达及欧芹素乙的抑制作用[1]

杨鹏远，芮耀诚[2]，李 凯[3]
(第二军医大学药学院药理教研室，[3]长海医院胸心外科，上海 200433，中国)；
黄兴华，蒋建明，余 龙
(复旦大学遗传学研究所，上海 200433，中国)

关键词 泡沫细胞；细胞间粘附分子-1；欧芹素乙；低密度脂蛋白类；蛋白质印迹法；RNA印迹法

目的：研究在人类单核细胞系U937泡沫细胞中，细胞间粘附分子-1 (ICAM-1)的表达水平，观测欧芹素乙(欧前胡内酯，imperatorin，IMP)对ICAM-1表达的抑制作用. **方法**：将U937细胞与80 mg/L氧化低密度脂蛋白孵育48 h，建立U937泡沫细胞模型. 在培养基中预加入不同浓度的IMP(0，25，50，100 μmol/L). 采用Western blotting检测ICAM-1的蛋白表达；采用Northern blotting检测ICAM-1的mRNA水平. **结果**：泡沫细胞中ICAM-1的表达显著高于正常U937细胞. ICAM-1的蛋白和mRNA水平分别是正常U937的15和10倍. 经IMP 50和100 μmol/L预处理后，泡沫细胞中ICAM-1的高表达被显著抑制. 当IMP浓度达到100 μmol/L时，ICAM-1的蛋白水平降低了79 %，mRNA水平降低了74 %. **结论**：经氧化低密度脂蛋白孵育后，U937泡沫细胞中ICAM-1呈现高表达，IMP能显著抑制这种表达.

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