Oxidized low-density lipoprotein is chemotactic for arterial smooth muscle cells in culture

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The effects of human native and Cu^{2+}-oxidized low-density lipoprotein (LDL) were tested on the migration of cultured bovine aortic smooth muscle cells (SMCs) in blind-well chambers. LDL oxidation was controlled by measuring the formation of conjugated dienes and lipid hydroperoxides, and by agarose gel electrophoresis. Oxidized LDL stimulated SMC migration, and the effect was dose-dependent up to 200 μg/ml. The stimulation was chemotactic in nature. Native LDL was without significant activity. The results suggest that oxidized LDL may contribute to the migration of medial SMCs into the intima during atherogenesis.

Atherosclerosis; Oxidized low-density lipoprotein; Vascular smooth muscle cell; Chemotaxis; Migration

1. INTRODUCTION

During atherogenesis smooth muscle cells (SMCs) accumulate into the arterial intima by cell proliferation and migration [1]. The factors that attract SMCs from the media to the intima remain unknown. Possible attractants include the factors known to be chemotactic for cultured SMCs, such as the platelet-derived growth factor (PDGF) [2].

Oxidatively modified low-density lipoprotein (LDL) has been shown to possess atherogenic properties by causing lipid accumulation in macrophages [3,4]. Oxidized LDL has also been shown to be chemotactic for blood monocytes [5].

We studied the effect of oxidized LDL on the migration of cultured arterial SMCs.

2. MATERIALS AND METHODS

2.1. *Isolation, oxidation, and characterization of LDL*

LDL was isolated from human plasma with sequential ultracentrifugation between densities 1.020 and 1.063 g/l as described [6]. LDL was dialyzed against 0.15 M NaCl containing 0.01% EDTA, pH 7.40 for 48 h with several buffer changes. LDL protein was determined by the method of Lowry et al. using bovine serum albumin (BSA; Sigma Chemical Co., USA) as a standard. LDL was filter-sterilized (0.22 μm, Millipore, USA), stored in dark at 4°C, and used for experiments within two weeks.

Before LDL oxidation, EDTA was removed by gel filtration on a Sephadex G-25M column (PD-10, Pharmacia, Sweden) equilibrated with phosphate-buffered saline, pH 7.40. LDL (1.0 μg protein/ml) was oxidized at room temperature in the presence of 40 μM $CuCl_2$. The formation of conjugated dienes was monitored according to Esterbauer et al. [7]. Oxidation of LDL was stopped at the diene peak (5-6 h) by addition of EDTA (1 mg/ml). The solution was dialyzed overnight at 4°C against 0.15 M NaCl containing 0.01% EDTA and filter-sterilized. Oxidation was further characterized by measuring the presence of lipid hydroperoxides according to El-Saadani et al. [8] and by agarose gel electrophoresis [9]. Native LDL was prepared similarly, except that $CuCl_2$ addition was omitted and EDTA was added immediately after buffer exchange.

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Abbreviations: LDL, low-density lipoprotein; SMC, smooth muscle cell; PDGF, platelet-derived growth factor

2.2. *Cell culture*

Bovine aortic SMCs were cultured from explants of intima-media as described [10]. The cells were grown in Dulbecco's modification of Eagle's medium (DMEM; Flow Laboratories, UK) supplemented with 2 mM L-glutamine, 50 U/ml penicillin-streptomycin, 1% nonessential amino acids (all from NordVacc, Sweden), and 10% fetal bovine serum (FBS; Gibco Bio-Cult, USA). The cells showed typical 'hills and valleys' growth pattern of SMCs and were used for experiments between passages 3 and 6.

2.3. *Cell migration assay*

SMC migration was measured as described [10] in blind-well chambers using gelatin-coated polycarbonate filters (both from Nuclepore Co., USA). The upper compartments were loaded with trypsinized cell suspension [4×10^4 cells/chamber) in DMEM with supplements and 10% FBS. After 1 h incubation in a tissue culture incubator the filters with the adhering SMCs were transferred to chambers containing serum-free DMEM with supplements and 0.2% BSA, into which the components to be tested were added. After 7 h of incubation the filters were removed, fixed with ethanol, and stained with Giemsa. The cells on the lower side of the filters were counted by light microscopy out of a total of 5-10 fields using 400-fold magnification. The results were expressed as percentages of migration as compared to chambers containing the control medium. PDGF (Collaborative Res. Inc., USA) was used as a positive control.

3. RESULTS

During oxidation of LDL there were clear increases in the amount of conjugated dienes by 760 nmol/mg

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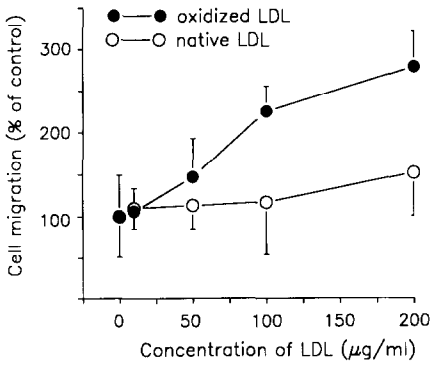

Fig. 1. Dose/response curves of the oxidized and native LDLs on the SMC migration. Each symbol represents the mean of 10 observations. Vertical bars are SDs. The effects of oxidized LDL at 100 μg/ml and 200 μg/ml are statistically significant (at 100 μ/ml, $P<0.005$ vs control, $P<0.05$ vs native LDL; at 200 μg/ml $P<0.001$ vs control, $P<0.01$ vs native LDL, Student's t-test).

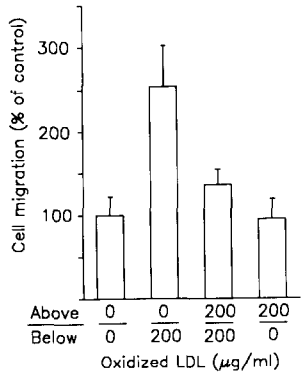

Fig. 2. Modified checkerboard analysis, where the oxidized LDL (200 μg/ml) was added above or below the filter (n = 8). Vertical bars are SDs. Only the effect of oxidized LDL below the filter is statistically significant ($P<0.001$ vs control, $P<0.01$ vs oxidized LDL on both sides of the filter, Student's t-test).

LDL (SD 287, n = 3), in lipid hydroperoxides (native LDL 24.3 nmol/mg LDL, SD 5.0; oxidized LDL 269 nmol/mg LDL, SD 123; n = 3), and in electrophoretic mobility (relative mobility versus native LDL 1.28, n = 2). The oxidized LDL caused a dose-dependent stimulation in SMC migration with the maximum (279%) at the highest protein concentration tested (200 μg/ml) (Fig. 1). Native LDL did not significantly stimulate SMC migration. In modified checkerboard analysis, the presence of positive concentration gradients of the oxidized LDL across the filter resulted in greater stimulation of SMC migration than in the presence of equal concentrations, which indicates that the migration-stimulating effect was chemotactic in nature (Fig. 2). Furthermore, the increased SMC migration was not due to adhesion gradients in the filters (haptotaxis), as no stimulation occurred in filters preincubated with oxidized LDL (Table I).

4. DISCUSSION

SMC migration from the media into the arterial intima during atherogenesis is considered to result from chemotactic reaction towards factor(s) present in the intima [1]. Possible attractants include factors chemotactic for cultured SMCs, such as PDGF [2], leukotriene B_4 [11], interleukin-1 [12], fibrinogen [13], and transforming growth factor β [14]. In this study we demonstrate that also oxidized LDL is chemotactic for vascular SMCs in culture. It remains to be shown whether these factors are active also in vivo in the arterial wall.

Numerous different modified LDLs, including chemical modifications and LDL oxidized by transition metal ions or by cells, have been reported to have atherogenic properties by causing lipid accumulation in arterial cells [3]. One of the chemical modifications, acetoacetylated LDL, has recently been shown to be chemotactic for arterial SMCs [15]. Chemically modified LDLs have not been found in vivo, but recent evidence suggests the presence of oxidatively modified LDL in the intima [3,16]. Oxidation of LDL in the intima may, according to our results, render it chemotactic for medial SMCs, thus contributing to plaque formation and atherogenesis.

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Table I
Lack of haptotactic effect of oxidized LDL on SMC migration (n = 6)

Medium below filter	Filter preincubation	Cell migration (% of control)	Statistical[a] significance	
			vs A	vs B
(A) Control	no[b]	100 (26)[c]		$P<0.01$
(B) Oxidized LDL	no	243 (47)	$P<0.01$	
(C) Control	5 h	106 (18)	NS	$P<0.01$
(D) Control	2 h	94 (31)	NS	$P<0.01$
(E) Control	1 h	110 (33)	NS	$P<0.02$

[a]Student's t-test. NS = not significant
[b]Filters were preincubated for the indicated time periods without cells in chambers containing oxidized LDL in lower compartments (200 μg/ml). The filters were then transferred to clean chambers and the SMCs were added.
[c]SD in parentheses.

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