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THE CONTRIBUTION OF THE MACROPHAGE RECEPTOR FOR OXIDIZED LDL TO ITS CELLULAR UPTAKE

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SUMMARY: Oxidized LDL (Ox-LDL) was shown to be taken up by macrophages via several receptors including the acetyl-LDL(Ac-LDL), the LDL, and the Ox-LDL receptors. Cellular uptake and degradation of Ox-LDL could be dissociated from that of LDL and Ac-LDL as demonstrated by using macrophages that lack the LDL or the Ac-LDL receptors. In J-774 A.1 macrophage-like cell line unlabeled Ox-LDL reduced the ^{125}I-Ox-LDL by up to degradation of 91% whereas unlabeled Ac-LDL and native LDL reduced ^{125}I-Ox-LDL degradation by only 51% and 23%, respectively. Analysis of macrophage degradation of ^{125}I-Ox-LDL in the presence of 30-fold excess concentration of LDL + Ac-LDL(to block uptake of ^{125}I-Ox-LDL via the LDL and the Ac-LDL receptors) revealed that cellular degradation via the Ox-LDL receptor could account for 45% of the macrophage uptake of Ox-LDL.

Oxidized low density lipoprotein (Ox-LDL), unlike native LDL was shown to induce macrophage cholesterol accumulation and foam cell formation (1-3). Macrophage possesses, in addition to the LDL receptor, several scavenger receptors (1,4). Ox-LDL and acetyl-LDL (Ac-LDL) interact with similar as well as with different receptors (5,6). Furthermore, recently we have shown that Ox-LDL can also bind to the LDL receptor on macrophages(7). The present study was undertaken to assess the relative contribution of the LDL, Ac-LDL and Ox-LDL receptors on macrophages to the cellular uptake and degradation of Ox-LDL.

Abbreviations: LDL, low density lipoproteins(s); Ox-LDL, Oxidized-LDL; Ac-LDL, acetyl-LDL; DMEM, Dulbecco's Modified Eagle's Medium.

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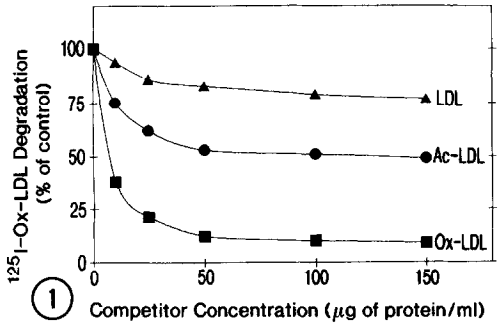
METHODS

Cells. J-774 A.1 murine macrophage-like cell line and U-937 macrophage histiocytes were purchased from American Tissue Culture Collection (ATTC), Rockville, MD. Cells were plated at 2.5X10 cells/16-mm dish in DMEM supplemented with 10% fetal calf serum (FCS). The cells were fed every 3 days and were used for experiments within 7 days of plating. Human monocyte-derived macrophages (HMDM) were prepared as previously described (8), from healthy volunteers as well as from one patient with homozygous familial hypercholesterolemia (HFH).
Lipoproteins. LDL was prepared from human plasma derived from fasted normolipidemic volunteers. LDL was prepared by discontinuous density gradient ultracentrifugation as described previously (9). The lipoprotein was washed at d = 1.063 g/ml and dialyzed against 150 mM NaCl, 1mM EDTA (pH 7.4) under nitrogen in the dark at 4C. LDL was then sterillized by filtration (0.22 nm) and used within 2 weeks.LDL was iodinated by the method of McFarlane as modified for lipoproteins (10). LDL was oxidized in the presence of 10μM CuSO4 (5). LDL was acetylated by repeated additions of acetic anhydride to 4mg/ml LDL diluted 1:1 (v:v) with saturated ammonium acetate (11).
Cellular degradation of lipoproteins. LDL degradation was measured following incubation of the iodinated lipoprotein with cells for 5h at 37°C. The hydrolysis of LDL protein was assayed in the incubation medium by measurment of tricholoroacetic acid soluble, non-iodide radioactivity(12). Cell-free LDL degradation was minimal and was subtracted from total degradation. The cell layer was washed three times with PBS and extracted by a 1-h incubation at room temperature with 0.5 ml of 0.1N NaOH for measurement of cell-associated radioactivity and of cellular protein content by the method of Lowry et al. (13). Competition studies were performed using a constant amount of ^{125}I-Ox-LDL (5μg of protein/ml) and increasing concentration of unlabeled lipoproteins (LDL, Ac-LDL, Ox-LDL).

RESULTS

The ability of unlabeled LDL, acetyl LDL (Ac-LDL) and copper incuced-oxidized LDL (Ox-LDL) to compete with ^{125}I-Ox-LDL for its uptake and degradation by J-774 A.1 macrophages-like cell line was tested following 5 hours of cell incubation with 5μg of ^{125}I-Ox-LDL protein/ml in the presence of increasing concentrations of the unlabeled lipoproteins (Fig 1). In the presence of 30 fold excess concentration of unlabeled Ox-LDL, Ac-LDL or native LDL, cellular degradation of ^{125}I-Ox-LDL was reduced by 91%, 51% and 23%, respectively (Fig 1). Similar results were obtained for ^{125}I-Ox-LDL cell-association (data not shown). To assess the contribution of the macrophage receptor for Ox-LDL to the cellular uptake of Ox-LDL, cells were



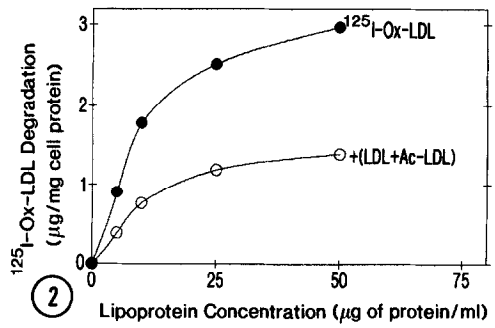


Fig 1. Competition for macrophage degradation of ^{125}I-Ox-LDL by unlabeled LDL, Ac-LDL and Ox-LDL. Competition studies were performed using 5μg of ^{125}I-Ox-LDL protein/ml that was incubated with J-774 A.1 macrophages in the presence of the indicated concentrations of unlabeled lipoproteins for 5 hours at 37°C prior to analysis of lipoprotein degradation. Each point is the mean of duplicate determinations (each performed in triplicates).

Fig 2. Saturation curves for degradation of ^{125}I-Ox-LDL by J-774 A.1 macrophages. Macrophage degradation of ^{125}I-Ox-LDL was determined using increasing concentrations of ^{125}I-Ox-LDL in the absence or presence of 30 fold excess concentrations of unlabeled LDL + Ac-LDL. Each point is the mean of duplicate determination (each performed in triplicates).

incubated with increasing concentrations of ^{125}I-Ox-LDL in the absence (control) or presence of 30 fold concentration of both unlabeled LDL and Ac-LDL (Fig 2). The contribution of the macrophage receptor for Ox-LDL to the degradation of Ox-LDL was about 45% of the total degradation (obtained in the absence of excess concentrations of unlabeled LDL+Ac-LDL, that were used to block the uptake of Ox-LDL via the LDL and via the acetyl LDL receptors respectively). Similar results were found for cell-association of ^{125}I-Ox-LDL (data not shown). Fucoidin binds to the Ac-LDL receptor on macrophages(4). The degradation of ^{125}I-Ac-LDL (5μg of protein/ml) by J-774 A.1 macrophages was inhibited by 81% in the presences of 50μg/ml fucoidin. To find out whether the Ox-LDL receptor is also blocked by fucoidin, the effect of this compound on cellular degradation of ^{125}I-Ox-LDL was tested in the absence or presence of 30 fold concentration of LDL + Ac-LDL. Fucoidin (50μg/ml) inhibited the degradation of ^{125}I-Ox-LDL in both cases by

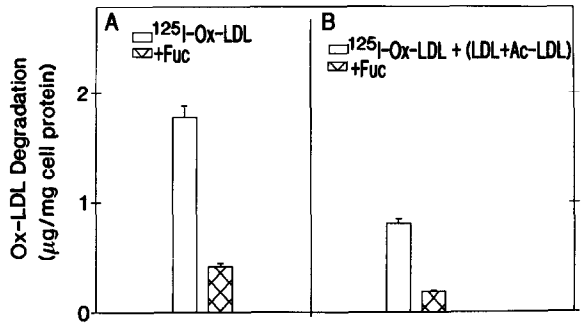


Fig 3. The effect of fucoidin on macrophage degradation of Ox-LDL. J-774 A.1 macrophages were incubated with 10μg of ^{125}I-Ox-LDL/ml in the absence or presence of 50μg/ml fucoidin(A). Similar study was carried out using 10μg of ^{125}I-Ox-LDL/ml+300μg of protein/ml of LDL and Ac-LDL (B). Lipoprotein degradation rates were studied as described under Methods.

77% (Fig 3), suggesting that both scavenger receptors (the Ac-LDL receptor as well as the Ox-LDL receptor) are similary inhibited by fucoidin. U-937 macrophages, unlike J-774 A.1 cells, were shown to possess relatively small number of receptors for Ac-LDL (14). In the present study we found that in U-937 cells as opposed to J-774 A.1 macrophages, both Ac-LDL and Ox-LDL degraded to only 1/4 of the cellular degradation of native LDL (Table 1). In human monocyte-derived macrophages from healthy subjects (HMDM-Normaol), Ox-LDL degradation was 3 fold higher than that of native LDL, but only 1/3 the degradation of Ac-LDL (Table 1). In HMDM from a patient with

TABLE 1. Lipoprotein degradation in various types of macrophages

Cell type	Lipoprotein Degradation (25μg of protein/ml) (ng/mg cell protein/ 5h)		
	Ox-LDL	LDL	Ac-LDL
J-774 A.1	4635±224	2135±158	7039±339
U-937	1418±61	5478±239	1407±58
HMDM-Normal	1003±57	305±23	3015±166
HMDM-HFH	911±42	95±17	3068±195

HMDM, Human monocyte-derived macrophages
Results are the mean ± S.D. of 3 experiments.

homogygous familial hypercholesterolemia (HMDM-HFH) which lack the LDL receptor, Ox-LDL degradation was also 30% the degradation of Ac-LDL (Table 1).

DISCUSSION

Oxidized LDL can lead to macrophage cholesterol accumulation following its uptake into the cells via several receptors (1). Unlike the LDL receptor which is regulated by the cellular cholesterol content (15), the Ac-LDL receptors (16,17) are not similarly regulated and thus Ac-LDL can cause macrophage cholesterol accumulation and foam cell formation (1-4). Oxidized LDL consists of heterogeneous populations, thus different receptors may recognize different epitopes on Ox-LDL. Ac-LDL mimics only a fraction of the various epitopes on Ox-LDL (5). The specific macrophage receptor for Ox-LDL which is not shared by Ac-LDL or native LDL (5,6) was shown in the present study to substantially contribute to the cellular uptake and degradation of Ox-LDL. In U-937 macrophages, unlike J-774 A.1 cells and HMDM, the addition to cells incubated with ^{125}I-Ox-LDL of excess concentrations of unlabeled LDL+Ac-LDL did not affect the degradation of ^{125}I-Ox-LDL (data not shown), suggesting that Ox-LDL might be taken up in U-937 cells only by the Ox-LDL receptor and not by the Ac-LDL or the LDL receptors. By using various types of macrophages it could be shown that cellular degradation of Ox-LDL could be dissociate from that of LDL and Ac-LDL and thus further support the existence of specific macrophage receptor(s) for Ox-LDL. Macrophage degradation of Ox-LDL was shown (18) to be about half the degradation of Ac-LDL. Thus, it might be that lipoprotein uptake via the Ox-LDL receptor is followed by different intracellular pathway than that of Ac-LDL. Ox-LDL is

differently targeting to perinuclear or peripheral vesicles than LDL or B-VLDL (19,20).

It is not known whether the Ox-LDL receptor is regulated by cellular cholesterol content like the LDL receptor. If so, than the reduced cellular uptake of Ox-LDL in comparison to the uptake of Ac-LDL may be related to the fact that only about half of the degraded Ox-LDL is taken up via the Ac-LDL receptor (which is not regulated by cellular cholesterol content). In U-937 macrophage-like cell line, the number of receptors to both Ac-LDL and Ox-LDL were found to be low in comparison to native LDL, suggesting that these receptors may share similar properties. Furthermore, fucoidin which blocks the uptake of Ac-LDL by macrophages,similarly inhibited cellular uptake of Ox-LDL. Unlike the Ac-LDL receptor however that was recently purified (16,17),the macrophage receptor for Ox-LDL was not purified yet to allow a better understanding of its contribution to macrophage cholesterol metabolism.

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