High-affinity association and degradation of ^{125}I-labelled low density lipoproteins by human hepatocytes in primary culture

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Catabolism of homologous low density lipoproteins (LDL) was studied in primary culture of human hepatocytes (HH). The cell association and degradation of ^{125}I-labeled LDL (^{125}I-LDL) were curvilinear functions of substrate concentration. Cell association and degradation of ^{125}I-LDL were inhibited by excess unlabeled LDL. Reductive methylation of unlabeled LDL abolished its ability to compete with ^{125}I-LDL for cell association and degradation. Preincubation of HH with unlabeled LDL caused a 63% inhibition of the ^{125}I-LDL degradation. It is concluded that the catabolism of LDL by HH proceeds in part through a receptor-mediated pathway similar to that demonstrated on extrahepatic cells.

Human hepatocyte *LDL* *Cell association* *Degradation* *High affinity association*

1. INTRODUCTION

In man, LDL is the major cholesterol-transporting lipoprotein and has been implicated as an independent risk factor in atherogenesis. The concentration of LDL in the plasma depends upon the rate of LDL synthesis and efficacy of their catabolism by different tissues of the organism. It has been shown that the liver plays an important role in this process. In several species at least one-third of plasma LDL are broken down in the liver [1,2], more than 70% of them being degraded via a high-affinity receptor mechanism [3].

The possible presence of LDL receptor activity has also been tested in human liver. One group of investigators failed to show the presence of specific LDL receptors on membranes of adult human liver [4], whereas another group reported specific LDL receptor activity on membrane fractions prepared from adult human liver biopsies [5]. These data are contradictory, and moreover the methodology of these experiments does not allow study of the intracellular metabolism of LDL by cells of human liver.

This study was undertaken to investigate the mechanism of human hepatic LDL association and degradation using primary culture of hepatocytes.

2. MATERIALS AND METHODS

2.1. *Lipoproteins*

LDL (1.019–1.063 g/ml) were isolated from the plasma of healthy donors by differential ultracentrifugation [6]. The apoprotein composition of LDL fractions was determined by SDS-polyacrylamide gel electrophoresis [7]; they contain almost no protein other than apoprotein B. ^{125}I-LDL (spec. act. 100–300 cpm/ng LDL protein) were prepared by the iodine monochloride method [8]. Reductively methylated LDL (m-LDL) were prepared as described [9].

2.2. *Cells*

Right lobes of the liver were obtained from 4 organ donors aged from 25 to 50 years. None was known to be suffering from any metabolic disease known to influence plasma lipoprotein metabolism. HH were isolated using the two-step hepatic

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portal vein enzymatic perfusion technique described previously [10,11]; the cells were plated in 35-mm plastic dishes at 1.6×10^5 cells/dish in 1 ml RPMI-1640 medium containing 10% fetal calf serum (FCS), insulin (10^{-6} M), hydrocortisone (10^{-8} M), kanamycin (100 μg/ml), and L-glutamine (2×10^{-3} M). Human skin fibroblasts (HF) (6th–10th passages) were obtained from a skin biopsy of healthy donors and cells were plated at 1×10^5 cells/dish in 1 ml EME medium containing 15% FCS, 1% nonessential amino acids, kanamycin (100 μg/ml), L-glutamine (2×10^{-3} M). Cells were maintained at 37°C in a humidified incubator under 5% CO_2/95% air. HH were used within 2–4 days and HF within 3 days after plating.

2.3. *Measurement of LDL cell association and degradation*

18 h before the experiment, the culture medium was replaced with similar medium (1 ml/dish) containing 10% human lipoprotein-deficient serum (LPDS) instead of FCS. Then, 1 ml of fresh media and various amounts of ^{125}I-LDL in the presence or absence of either unlabeled LDL or m-LDL were added to each dish. After incubation at 37°C for the indicated time, dishes were placed on ice and ^{125}I-LDL cell association and degradation were determined [12,13]. K_m and the maximal number of association sites for LDL were calculated as described [14]. Results for 4 studies were quite similar, and here we present the details from one study.

3. RESULTS AND DISCUSSION

Fig. 1A shows that curves for cell association (surface binding and internalization at ^{125}I-LDL) exhibited a biphasic nature; they did not reach a plateau but continued to increase up to the 100 μg protein/ml tested. In the presence of a 20-fold excess of unlabelled LDL, the saturable component of the curves was abolished and only nonspecific association of ^{125}I-LDL to the cells was observed. The high-affinity receptor-mediated cell association of ^{125}I-LDL was calculated by subtracting the nonspecific cell-associated ^{125}I-LDL from the total (in the absence of unlabeled LDL) and analysed by a Scatchard plot (fig.1B). There was little dif-

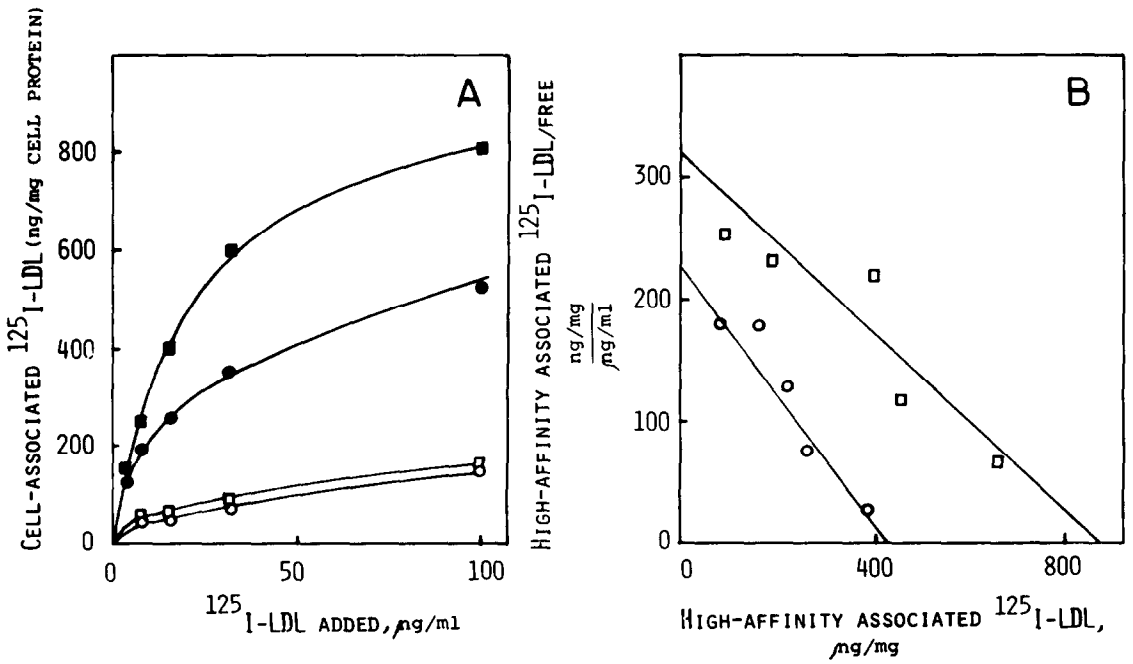

Fig.1. Saturation curves for human ^{125}I-LDL association to HH (○) and HF (□). (A) Cells were prepared as described in section 2 and incubated with increasing concentrations of ^{125}I-LDL in the absence (●,■) or presence (○,□) of 20-fold excess unlabeled LDL. (B) Scatchard plots for HH (○); $K_m = 16.1 \pm 2.8$, $n = 419 \pm 39.5$; FH (□), $K_m = 26.8 \pm 5.8$, $n = 862.4 \pm 78.8$. Values are means of triplicate incubations.

ference in K_m and maximal number of association sites for LDL (n) between the two cell types. The K_m of the HH for cell-associated ^{125}I-LDL was 60% of HF, indicating a small increase in affinity for lipoproteins. The maximal number of sites (n) in HH was 49% of HF; they likely have half the number of HF receptors.

Modification of lysine residues by reductive methylation has been shown to abolish the binding of LDL to receptors in HF [15]. To determine the role of lysine amino groups in human LDL binding to HH receptors, cell monolayers were preincubated with RPMI-1640 medium containing 10% LPDS for 5 h at 37°C. The medium was then changed to RPMI-1640 medium, containing 10% LPDS and ^{125}I-LDL (5 μg protein/ml), and thereafter the cell association of ^{125}I-LDL was measured in the presence of excess of untreated or modified LDL. As shown in fig.2, reductive methylation of LDL completely abolishes its ability to compete with ^{125}I-LDL for association.

Incubation of HH for 18 h at 37°C with increasing concentrations of ^{125}I-LDL demonstrated that, similar to cell association, degradation was a curvilinear function of substrate concentration (fig.3). The degradation of LDL by HH at 200 μg/ml was 0.8 μg/mg cell protein per day which corresponds

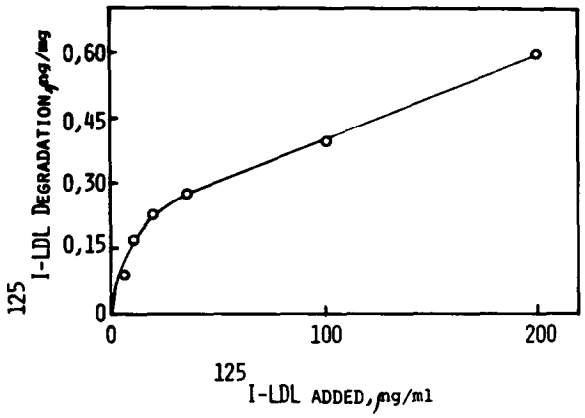

Fig.3. Degradation of ^{125}I-LDL by HH as a function of concentration. Values are means of triplicate incubations.

to 0.121 mg LDL protein/g liver per day. From studies utilizing sucrose-[^{14}C]LDL, the rate of degradation of LDL by pig liver in vivo has been estimated to be 0.23 mg/g of liver per day [16]. Thus, the capacity of HH to degrade LDL is of the same order of magnitude as that found for the hepatic contribution to LDL degradation in vivo.

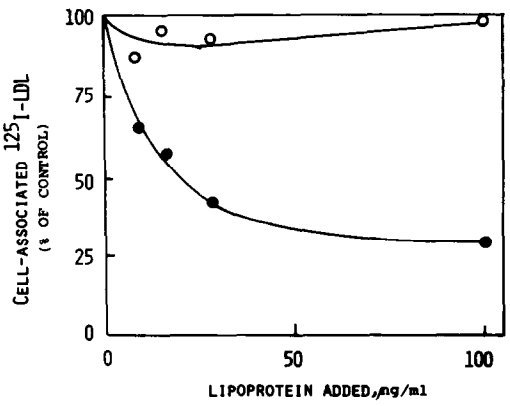

Fig.2. Effect of LDL (○) and m-LDL (●) on ^{125}I-LDL association with HH. HH monolayers were incubated for 5 h in medium containing 5 μg/ml ^{125}I-LDL and unlabelled lipoproteins at the indicated protein concentrations. The '100% control value' for cell-associated ^{125}I-LDL in the absence of unlabeled lipoproteins was 92 ng/mg cell protein per 5 h. Values are means of triplicate incubations.

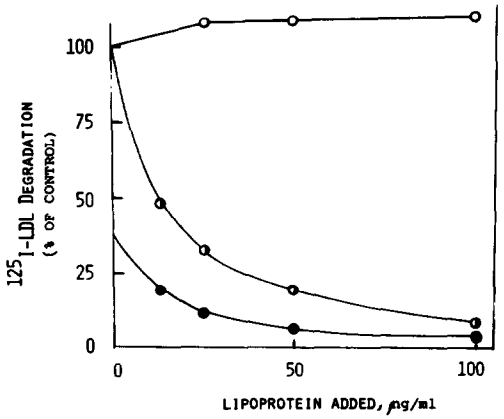

Fig.4. Effect of LDL (◑), m-LDL (○), and preincubation with LDL (●) on ^{125}I-LDL degradation by HH. During the experiments, cells were incubated with 5 μg/ml ^{125}I-LDL and in the presence or absence of increasing concentration of either unlabeled LDL or m-LDL. The 100% control value for ^{125}I-LDL degraded in the absence of unlabeled lipoproteins was 90 ng/mg cell protein per 18 h. Values are means of triplicate incubations.

The LDL receptors in HF and other extrahepatic cells are down-regulated according to the uptake of LDL cholesterol [17]. To determine whether HH receptors are similarly regulated, cells were preincubated in the presence of 10% LPDS with or without a high concentration of unlabeled LDL (100 μg protein/ml). After 18 h preincubation at 37°C, the medium was changed to RPMI-1640 medium containing 10% LPDS, ^{125}I-LDL (5 μg protein/ml) and increasing concentrations of unlabeled LDL, and degradation was measured during the next 18 h. As shown in fig.4, this degradation by cells previously incubated with LDL was only about 37% of that in cells previously incubated with LPDS. Most of the degradation occurred by the high-affinity receptor-mediated mechanism since unlabeled LDL inhibit this degradation. Reductive methylation of LDL completely abolished their ability to compete with ^{125}I-LDL for degradation (fig.4).

Thus, these data demonstrate the presence of a high-affinity receptor-dependent mechanism of LDL uptake and degradation in cultured human hepatocytes which is similar to the mechanism described for the extra hepatic cells.

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