BINDING AND DEGRADATION OF NATIVE AND ACETYLATED LOW DENSITY LIPOPROTEINS BY MONOCYTE DERIVED MACROPHAGES OF NORMAL AND HYPERCHOLESTEROLEMIC RABBITS

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ABSTRACT

Low density lipoprotein (LDL) was isolated from normal rabbits and was modified with acetic anhydride. Blood monocyte derived macrophages from normal and hypercholesterolemic (HC) rabbits were cultured, and on the 8th day the cells were incubated with native and modified LDL to study their binding and degradation. Macrophages from both normal and hypercholesterolemic rabbits express a limited number of receptors for normal plasma LDL whereas they exhibit a large number of receptors for acetyl LDL. There was no significant difference between binding and degradation of acetyl LDL by normal or hypercholesterolemic cells. However, binding and degradation of native LDL by monocytes of hypercholesterolemic cells were significantly less ($p < 0.001$) in comparison to binding and degradation of native LDL by normal cells indicating that there is a feedback inhibitory pathway in the cell that inhibits the synthesis of LDL receptors in the presence of excess LDL.

KEY WORDS: Monocyte, Macrophage, Hypercholesterolemia, Low Density Lipoprotein.

INTRODUCTION

Macrophages are commonly found in early lesions of atherosclerosis as well as in advanced lesions like the fibrous plaque. They are probably the principal cells responsible for advanced lesion formation. In their studies of hypercholesterolemia in non human primates Faggiotto et al (1) found that monocytes derived macrophages were a major component of the fatty streaks in these animals and were found in abundance in fibrous plaques as well. During fatty streak formation, circulating monocytes settle upon the endothelium, invade the intima and begin accumulating cholesterol and cholesterol esters resulting in macrophage transformation to a foam cell (2-7). A study in our lab (8) has demonstrated that rabbits fed 0.1% cholesterol for 3 months had an average of 50 foam cells/mm². The uptake of lipoprotein bound cholesterol in macrophages occurs through the process of receptor mediated endocytosis (9-11). The macrophages express few receptors for normal plasma lipoproteins, whereas they express abundant receptors for chemically modified lipoproteins (9).

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Goldstein and co-workers (9) were the first to describe an acetic anhydride modified form of LDL that could be taken up by macrophages at a rate many times higher than that of native LDL to convert them into foam cells (12). The concentration of LDL in the intima is increased in the presence of high concentration of plasma LDL. If it is oxidized at some ongoing rate, the intimal concentration of oxidised LDL would also be raised, which contributes to the recruitment of circulating monocytes. Initial work done by us (13) has shown that feeding 0.1% cholesterol to rabbits for 16 weeks, increases the lipid peroxidation level by 16%. Within the arterial wall, the return of macrophages to the plasma is inhibited by oxidised LDL. As resident macrophages, these cells express acetyl LDL receptor and take up modified LDL much more rapidly and form foam cells. Hence, an increase in the plasma LDL can accelerate the development of fatty streak lesions. However, the precise mechanism by which LDL promotes the development of lipid laden foam cells in the fatty streak lesion remains to be elucidated (14). The present study focuses upon the capability of monocyte derived macrophages from normal and hypercholesterolemic rabbits towards binding and degradation of native and acetic anhydride modified LDL.

MATERIALS AND METHOD

Male rabbits weighing between 1 to 1.65 kg were divided into two groups: Group A: control group and Group B: hypercholesterolemic group. Animals of group A were given normal diet and those of group B 0.1% cholesterol in addition to the normal diet and were made hypercholesterolemic (total cholesterol: 600-700 mg/dl). Water was given ad-libitum and cholesterol was force-fed.

LDL ISOLATION

LDL was isolated from control rabbits using the sequential floatation technique of Havel (15). Solid potassium bromide was used for density adjustments. Lipoprotein deficient serum was prepared by ultracentrifugation from the infranate solution obtained after isolation of LDL from control rabbits as described previously (16).

ACETYL LDL PREPARATION

LDL was acetylated with repeated additions of acetic anhydride (16). After acetylation the reaction solution was dialyzed for 24 hours at 4°C against 0.15 M NaCl and 0.3 mM EDTA (pH 7.4).

RADIOIODINATION OF LDL

Radioiodination of native and acetyl LDL was done by the iodine monochloride method as described by Van Tol et al (17). After iodination the reaction mixture was passed through a sephadex column.

ISOLATION OF MONOCYTE DERIVED MACROPHAGES

Mononuclear cells were isolated from peripheral blood of normal and hypercholesterolemic rabbits by the method given in current protocols in immunology (18). Monocytes and lymphocytes were separated by differential adherence. On the day of the experiment, the cells were washed twice with RPMI-1640 medium and medium containing lipoprotein deficient serum was added. Radiolabelled native and acetyl LDL were added as required.

BINDING AND DEGRADATION OF ^{125}I -LDL and ^{125}I -ACETYL LDL BY MONOCYTE DERIVED MACROPHAGES OF CONTROL AND HYPERCHOLESTEROLEMIC RABBITS

Binding experiments were performed at 4°C (19). Monocyte derived macrophages from group A and group B were incubated with 200 cpm/ng protein of ^{125}I -LDL and 192 cpm/ng protein of ^{125}I -acetyl LDL. The amount of ^{125}I bound was determined in a γ counter in triplicate dishes.

For degradation assays, each monolayer received medium containing lipoprotein deficient serum and the indicated concentration of either ^{125}I -LDL (200 cpm/ng protein) or ^{125}I acetyl LDL (192 cpm/ng protein). After incubation for 5 hours at 37°C the amount of ^{125}I labelled acid soluble material released into the medium was measured by the method of Goldstein et al (19).

RESULTS AND DISCUSSION

Blood monocytes from normal and hypercholesterolemic rabbits were maintained for 8 days in a medium containing 20% (v/v) autologous serum and then incubated in lipoprotein free medium to which were added different concentrations of ^{125}I -LDL and ^{125}I -acetyl LDL. Experiments were performed on the 8th day because by this time the adherent cells increase in size and assume characteristic features of cultured macrophages (20) and in between 7th and 9th day the cultures exhibit a 3 fold increase in the Specific activities of the lysosomal enzymes N-acetyl-B-glucosaminidase and acid phosphatase.

Monocytes of controls expressed the binding of native and acetyl LDL as 8 and 20% respectively.

similarly, the binding of native and acetyl LDL by monocytes of hypercholesterolemic rabbits was 1.5 and 22% respectively (Fig. 1).

The percentage of degradation was 3.8 and 7.8% respectively for native and acetyl LDL by monocytes of controls and 0.4% and 12% respectively, by monocytes of hypercholesterolemic rabbits (Fig. 2).

*Significant difference between normal and HC cells, $p < 0.001$
Fig. 2. Values are Mean $\pm$ SD

Although cells from hypercholesterolemic rabbits appeared to bind and degrade more acetyl LDL than cells from normal rabbits, no statistically significant difference between these two groups was observed. However binding (1.5%) and degradation (0.4%) of native LDL by hypercholesterolemic cells was significantly less ($p < 0.001$) in comparison to the binding (8%) and degradation (3.8%) of native LDL by normal cells. This may be attributed to a relatively limited number of native LDL receptor expression by macrophages. In the present study, we have documented that the receptor for native LDL expressed by macrophages is down regulated in the presence of high concentration of LDL in hypercholesterolemic rabbits. However, the monocytes cultured from both control and hypercholesterolemic rabbits display similar acetyl LDL receptor activity. Hence, it can be inferred that, on exposure to excess LDL, a feedback inhibitory pathway operates in the cell that inhibits the synthesis of LDL receptors in rabbits.

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