Studies on the Effect of Concanavalin A on the Metabolism of Low Density Lipoproteins in Skin Fibroblasts

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Concanavalin A bound to the cell surface decreased the binding and internalization of low density lipoproteins (LDL) to human skin fibroblasts. However, concanavalin A failed to inhibit the degradation of preinternalized LDL. Concanavalin A inhibited the activation of acyl-CoA: cholesterol acyltransferase by LDL. These results strongly suggest that concanavalin A inhibits the metabolism of LDL in human skin fibroblasts by affecting the receptor binding and internalization of LDL.

Key Words: Chloroquine, Coated pit, Preinternalized LDL

INTRODUCTION

The importance of LDL in the transport of cholesterol in the body has been studied in detail by Goldstein and Brown. The metabolic pathway known as “the LDL pathway” involves the high affinity binding of LDL to the specific surface receptors. Harmony et al. have shown that concanavalin A (Con A), a lectin isolated from jack beans, interacts with LDL. Con A can also interact with a variety of cells including fibroblasts and macrophages resulting in alteration of structure and function. Goldstein et al. have reported that Con A stimulates the binding of LDL at high concentrations to human skin fibroblasts, but the bound LDL was not metabolized by the cells. This was presumed to be due to the binding of LDL to the cell-bound Con A, and the prevention of the interaction with their receptors or the blocking of fusion of endocytotic vesicles with lysosomes. In this communication, we provide some evidence to show that Con A interferes with internalization of LDL in human skin fibroblasts.

MATERIALS AND METHODS

Sodium $^{125}$I-iodide was obtained from Amersham Corporation. $^{14}$C-oleic acid and $^{3}$H-cholesterol oleate were purchased from New England Nuclear. Con A and sodium oleate were purchased from Sigma Chemical Company. All tissue culture supplies were purchased from Grand Island Biological Company.

Normal human plasma was obtained from the Blood Bank, and phenyl methyl sulfonyl fluoride (0.5 mM), sodium azide (0.01%), EDTA (1 mM) and gentamycin (0.1 mg/ml) were added. LDL was isolated by sequential ultracentrifugation, LDL was washed twice by centrifugation and dialyzed extensively. Lipoprotein deficient serum was prepared by the method described by Radding and Steinberg. Iodination of LDL was carried out using iodine monochloride. Human skin fibroblasts derived from normal subjects (GM43) were obtained from the Human Genetic Mutant Cell Repository and were maintained in Dulbecco’s Modified Eagle Medium (DMEM) supplemented with...
penicillin, streptomycin and 10% fetal calf serum. Other experimental details are described in the respective legends for the figure and the tables. The values presented are means of triplicate determination.

RESULTS AND DISCUSSION

The results shown in Table 1 indicate that Con A decreases cell-bound LDL during 4 h incubation. It is possible that Con A interacts with the receptors of lipoproteins and inhibits the binding of LDL. It is also possible that Con A bound to the cell surface is not able to bind LDL to a great extent. Goldstein et al. showed that Con A stimulated the binding of LDL at high concentration of LDL, but Con A decreased the LDL binding at low concentration of LDL.

Con A inhibited the degradation of LDL by more than 90%. However, the extent of inhibition of binding and internalization was less than 50%. Since the extent of removal of Con A by methyl-α-D-mannopyranoside is not known, it is possible that the amount of LDL measured as internalized LDL in the presence of Con A includes some LDL associated with cell-bound Con A which cannot be displaced by methyl-α-D-mannopyranoside. The net amount of LDL catabolized during the 4 h incubation was 1921 ng/mg cell protein after incubation of the cells with Con A. The activation of cholesterol esterification by LDL in fibroblasts was also inhibited in a dose dependent manner by Con A (Fig. 1). From these data, two possible

Table 1. Effect of concanavalin A on LDL metabolism in human skin fibroblasts

| LDL bound LDL internalized LDL degraded (ng/mg of protein) |
|------------------|------------------|------------------|
| Control          | 127              | 556              | 1238             |
| Con A            | 69               | 312              | 93               |

Human skin fibroblasts were incubated with medium containing lipoprotein deficient serum for 48 h. The cells were then treated with 100 μg/ml of concanavalin A for 1 h at 37° C. After washing the cells with phosphate buffered saline three times, fresh medium was added and the cells were incubated with [125I]-LDL (5 μg/ml, 210 cpm/ng) for 4 h at 37° C. After this incubation the medium was collected and degraded LDL was measured by the method described by Goldstein et al. The cells were washed and the surface bound LDL was released using buffer containing heparin (2 mg/ml) and methyl-α-D-mannopyranoside (0.5M) successively. The cells were dissolved in 0.2N NaOH to determine the internalized LDL.

Table 2. Effects of concanavalin A and chloroquine on the degradation of preinternalized [125I]-LDL

<table>
<thead>
<tr>
<th>LDL degraded</th>
<th>LDL remaining</th>
<th>% degraded in the cell</th>
<th>(ng/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>330</td>
<td>98</td>
<td>77</td>
</tr>
<tr>
<td>Con A</td>
<td>275</td>
<td>191</td>
<td>70</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>176</td>
<td>257</td>
<td>41</td>
</tr>
</tbody>
</table>

The fibroblasts were incubated with medium containing lipoprotein deficient serum for 48 h. After changing to a fresh medium, [125I]-LDL (5 μg/ml, 210 cpm/ng) was added and the cells were incubated for 2 h at 37° C. At the end of this period, the cells were washed using buffer containing heparin (2 mg/ml). 435 ng of LDL was internalized per mg cell protein during this period. The cells were incubated with 100 μg/ml of concanavalin A or 25 nmol/ml of chloroquine for 4 h at 37° C. After this incubation, the degraded LDL and remaining LDL in the cells were determined.
mechanisms of action of Con A in LDL metabolism may be proposed: one is the inhibition of receptor binding and internalization of LDL and another is the inhibition of proteolytic degradation of LDL in the lysosomes. In order to clarify whether Con A can inhibit the degradation of LDL, we examined the effect of Con A on the degradation of preinternalized LDL in human skin fibroblasts. The data shown in Table 2 indicate that Con A does not have any effect on the degradation of preinternalized LDL. Chloroquine inhibited the proteolytic degradation of preinternalized LDL by about 50%. These results indicate that Con A affects the catabolism of LDL in human skin fibroblasts by inhibiting the receptor binding and internalization but not the degradation in the lysosomes.

It has been reported that Con A inhibits cap formation in lymphocytes by immobilizing the surface receptors\(^7\). Our results strongly suggest that Con A alters the uptake of LDL in human skin fibroblasts by a similar mechanism since receptor-bound LDL must migrate to the coated pit regions on the cell membrane before they can be internalized\(^8\).

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REFERENCES