Differences in processing of cholesterol between rat liver endothelial cells and rat peritoneal macrophages

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Isolated rat liver endothelial cells and rat peritoneal macrophages were incubated with [^{3}H]cholesteryl oleate labeled acetylated LDL in the presence of [^{14}C]oleate in order to compare the fate of cholesteryl esters introduced into the cells by a so-called atherogenic lipoprotein. It was found that 5-times more [^{3}H]cholesteryl oleate became associated with the endothelial cells per mg cell protein than with the macrophages. Hydrolysis of the cholesteryl esters proceeded in both cell types at a similar rate. In macrophages, however, the cholesterol formed is re-esterified at a 5-fold higher rate than in the liver endothelial cells. It is concluded that the cholesteryl esters introduced into the liver endothelial cells by acetylated LDL are rapidly hydrolysed and only to a very limited extent reconverted to cholesteryl esters, which contrasts the fate of this substrate with peritoneal macrophages. This property may allow the endothelial liver cells to function as a proper protection system against circulating atherogenic lipoproteins.

Acetyl-LDL *Acyl-CoA:cholesterol acyltransferase* *Rat liver endothelial cell* *Rat peritoneal macrophage* *Cholesterol processing* *Re-esterification*

1. INTRODUCTION

It is generally accepted that a significant fraction of the foam cells found in atherosclerotic lesions are derived from cells of the mononuclear phagocyte system and that a major portion of the cholesterol deposited in the atheroma originates from LDL [1–3]. Paradoxically, in vitro incubation of macrophages with LDL fails to result in massive cholesterol deposition in these cells. However, incubation of the cells in the presence of a modified form of LDL (acetylated LDL or biologically modified LDL) can lead to cholesteryl ester accumulation of as much as 300 μg/mg cell protein [4]. The cholesterol is deposited as cholesteryl esters in the cytoplasm. The intracellular processes leading to the cholesteryl ester deposition can be studied by incubating the macrophages with acetylated LDL, labeled with [^{3}H]cholesteryl oleate, in the presence of [^{14}C]oleate or [^{14}C]stearate-albumin. After uptake of the modified LDL by its specific receptor (the scavenger receptor) the cholesteryl ester is delivered to the lysosome and the ester bond is hydrolyzed by acid cholesteryl esterase. The liberated cholesterol passes the lysosomal membrane and is either excreted from the cell or re-esterified with the aid of the microsomal fatty acyl CoA:cholesterol acyltransferase (ACAT). For formation of cholesteryl esters exogenous fatty acid (either oleate or stearate) is obligatory [4]. We reported earlier that iodine-labelled, acetylated or biologically modified LDL, when injected into rats, is quantitatively taken up by the liver [5,6] and that the liver endothelial cell is mostly responsible for this active uptake. In vitro studies indicated that the high uptake by liver endothelial cells is caused by a relatively high concentration of

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scavenger receptors on this cell type [5]. We suggested that the liver endothelial cell could form a protection system against the atherogenic action of modified LDL at other sites in the body. A prerequisite for such a protection function is however that the uptake of modified LDL will not lead to pathological deposition at the protection site.

To compare the metabolism of scavenger receptor-mediated uptake at the pathological and supposed protection site, we incubated both peritoneal macrophages and endothelial cells under comparable conditions and determined the cellular processing of cholesterol.

2. MATERIALS AND METHODS

Throughout this study 12-week-old male Wistar rats (TNO, Zeist) were used, with free access to food and water. Rat liver endothelial cells were isolated by centrifugal elutriation after a collagenase perfusion as described [5]. Peritoneal macrophages were obtained by peritoneal lavage and purified by flotation on a 17.5% (w/v) metrizamide density cushion essentially according to [7].

Low density lipoprotein (LDL) was isolated from the blood of healthy volunteers as described by Redgrave et al. [8]. After density gradient ultracentrifugation the LDL ($1.024 < d < 1.055$ g/ml) and lipoprotein-deficient serum (LPDS $d > 1.21$ g/ml) were collected. Cholesteryl oleate was incorporated into LDL as described by Blomhoff et al. [9], 25 μCi [1,2,6,7-(n)-[^{3}H]cholesteryl oleate (82.7 Ci/mmol) was dissolved in 100 μl acetone. 1 ml LPDS was added and the acetone evaporated under a stream of N_2. After 15 min the tube was transferred to a water bath at 37°C and shaken for 10 min. LDL (1 ml, 2.5 mg protein/ml) was added and the mixture incubated for 4 h at 37°C under an atmosphere of N_2. The [^{3}H]cholesteryl oleate labeled LDL was reisolated according to Redgrave et al. [8]. The radiolabeled LDL was acetylated according to Basu et al. [10].

Rat liver endothelial cells and peritoneal macrophages were incubated in Ham's F-10 medium containing 25 μg lipoprotein and [^{14}C]oleate-albumin complex (molar ratio 5:1, final concentration FFA, 0.12 mM). At the indicated times 1-ml samples were taken and centrifuged at 600 × g. The cells were washed twice with 50 mM Tris-HCl (pH 7.4), 0.15 M NaCl, and 2 mg/ml BSA and once with 50 mM Tris-HCl, 0.15 M NaCl alone. From the final cell pellet and the first supernatant the lipids were extracted according to Bligh and Dyer [11]. The extracts were subjected to thin-layer chromatography using heptane-diethyl ether-acetic acid (60:40:1) as solvent. After visualisation of the spots by I_2 vapour the spots were scraped from the plates and counted in a Packard Tricarb scintillation counter, programmed to correct for quenching.

Mass cholesterol and cholesteryl ester were determined by an enzymatic colorometric method, using cholesteryl esterase, cholesterol oxidase and peroxidase (CHOD-PAP kit, Boehringer Mannheim, cat.no.310328). Protein was determined according to Lowry et al. [12] using BSA as a standard.

Type I collagenase and BSA (fraction V) were from Sigma, St. Louis, MO; metrizamide from Nyegaard, Oslo; Ham's F-10 from Gibco-Europe, Hoofddorp, The Netherlands; [^{3}H]cholesteryl oleate from New England Nuclear, Dreieich; and TLC silica gel 60 plates from Merck, Darmstadt.

3. RESULTS AND DISCUSSION

Isolated rat liver endothelial cells and rat peritoneal macrophages were incubated with [^{3}H]cholesteryl oleate labeled acetyl-LDL (25 μg apolipoprotein/ml) in the presence of [^{14}C]oleate-albumin. Fig.1A shows the amount of ^{3}H radioactivity recovered as cholesteryl ester as a function of the incubation time, expressed as ng cholesteryl oleate associated per mg cell protein. Both in liver endothelial cells and in macrophages, acetyl-LDL becomes rapidly associated with the cells, although the extent of association with endothelial cells is 4–5-times higher than with macrophages. After the initial cell association, the cholesteryl esters are hydrolysed and free [^{3}H]cholesterol accumulates in the cells (fig.1B). The same relative cellular enrichment as found for cholesteryl esters is also reflected in the amount of cholesterol. This indicates that in both cell types hydrolysis of the cholesteryl ester bond proceeds with approximately the same efficiency. In fig.1C the amount of exogenous fatty acids recovered inside the cells as cholesteryl ester is shown as a function of incubation time. In contrast with the results of fig.1A and

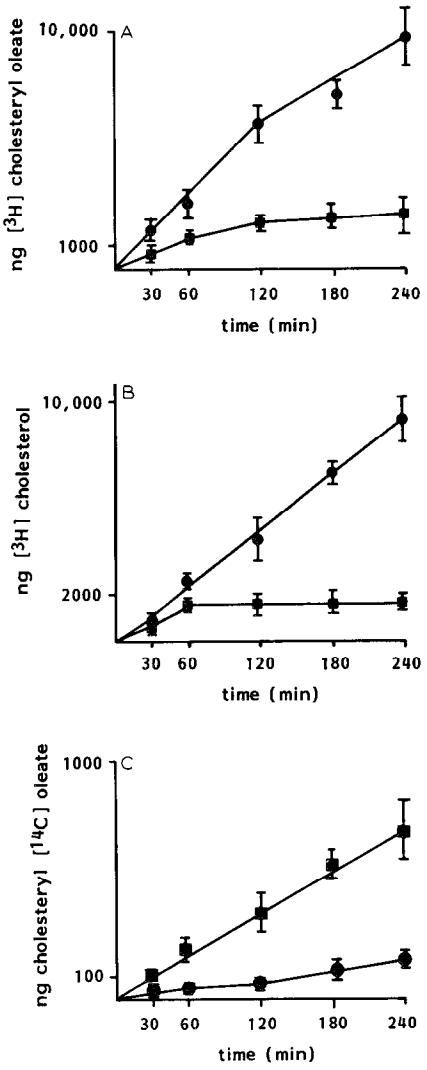

Fig.1. Time course of in vitro association and metabolism of cholesteryl esters from acetyl-LDL with rat liver endothelial cells (●) and peritoneal macrophages (■). Cells were incubated with 25 μg apoprotein/ml of [^{3}H]cholesteryl oleate labeled acetyl-LDL and [^{14}C]oleate-albumin complex, final concentration 0.12 mM. (A) Cell association of [^{3}H]cholesterol oleate. (B) Formation of free [^{3}H]cholesterol. (C) Formation of cholesteryl [^{14}C]-oleate. Values are expressed as ng/mg cell protein ± SE (n = 4).

B, it is obvious that the macrophages contain 5-times more newly formed cholesteryl ester than the endothelial cells. Because the amount of radiolabeled oleate inside the macrophages and endothelial cells is comparable (approx. 500 ng [^{14}C]oleate/mg cell protein) and the amount of free cholesterol formed inside endothelial cells is 5-times higher than in macrophages, it can be calculated that a cholesterol molecule liberated from a cholesteryl ester in macrophages has an approx. 25-times higher chance of being re-esterified and stored as cholesteryl ester than in the endothelial cells.

It has been suggested [13] that acyl-CoA:cholesterol acyltransferase, the enzyme responsible for cholesteryl esterification in the cytosol, is activated by an increase in cholesterol concentration inside the cell. To investigate such a mechanism the amount of cholesterol in endothelial cells and macrophages was determined (table 1). Indeed, it was found that the amount of cholesterol was twice as much in macrophages as in endothelial cells. Therefore this higher amount of cholesterol could explain the observed difference in re-esterification, although other explanations are not excluded.

In conclusion, the present data show that functional differences exist in the handling of cholesterol either internalized by peritoneal macrophages (coupled to pathological accumulation) or by liver endothelial cells (protection system). The relatively inefficient conversion of the cholesterol inside the endothelial cells to cholesteryl esters might allow direct further transport from endothelial to parenchymal cells as suggested earlier [5] and recently indicated [9]. Such a mechanism would allow an optimal catabolic route of the atherogenic particles which are internalized by liver endothelial cells without further local pathological implications.

Table 1

Cholesterol content of isolated cells

	Cholesterol
Endothelial cells	12 ± 1
Macrophages	22 ± 4

Cholesterol was determined by a colorimetric method (see section 2). Values are expressed as μg/mg cell protein ± SE (n = 4)

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