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# Malondialdehyde-modified HDL leads to accumulation of cholesterol in rat liver endothelial cells

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In order to study in vivo the effect of modified high density lipoprotein (HDL) on the movement of free cholesterol to liver cells and bile, we injected i.v. into rats, native or malondialdehyde modified HDL labelled with [$^{14}$C]cholesterol. Bile analysis indicated that the contribution of labelled cholesterol to bile acid secretion was diminished in the group receiving MDA-modified HDL when compared to control group. On the other hand, the liver analysis revealed higher radioactivity in the treated group. A separation of liver cells into parenchymal, endothelial, and kupffer at 90 min after the injection of MDA-modified HDL or native HDL indicated that the endothelial cell uptake of labelled free cholesterol from MDA-modified HDL was 2.6-fold higher than for native HDL. It is suggested that liver endothelial cells may be involved in the protection against atherogenoic oxidized lipoprotein. However, with regard to our finding, the uptake of cholesterol from modified HDL was detrimental to bile acid secretion. 

Epidemiological studies have consistently shown that high-density lipoprotein (HDL) concentrations are inversely correlated with the incidence of coronary heart disease (1,2). The protective function of HDL may be explained by their major role in reverse cholesterol transport. They facilate the efflux of cholesterol from peripheral tissues and promote its delivery to the liver (3). Evidence has been increasing that

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oxidation of low-density lipoproteins (LDL) may play an important role of the pathogenesis of the atherosclerosis process (4,5). While considerable amount a work has been carried out on the functional properties of LDL, only very few reports have dealt with those of HDL. However, oxidative modification can occur in HDL as in LDL (5). The resulting alterations in the HDL physicochemical properties may affect its biological functions (6-11). Recently, we have documented that the uptake of oxidized HDL is increased in the liver (12). In these in vivo experiments, the hepatic accumulation of cholesterol was not accompanied by increased secretion of bile acids, which is the ultime pathway for the elimination of excess cholesterol. As the liver consists of different cell types (parenchymal, endothelial, Kupffer and fat-storing cells), it is possible that the nonparenchymal liver cells were far more active in the uptake of modified HDL than the hepatocytes, which are responsible for the conversion of cholesterol into bile acids. The purpose of the current investigation was, therefore, to examine the effectiveness of MDA-modified HDL in altering the cholesterol distribution into the different types of liver cells.

## MATERIAL AND METHODS

### Animals

Male Sprague-Dawley rats were obtained from Charles River Breeding Farms (St Constant, Québec, Canada) and allowed 3 to 4 days of acclimatization in a room maintained at 22 $^{o}$ C with a 12- hr light cycle, before experimentation.

### Preparation of lipoproteins

High-density lipoproteins (HDL) were prepared by sequential ultracentrifugation, as previously reported (12,13). After exhaustive dialysis against 0.05M phosphate-buffer, pH 7.4, containing 154 mM NaCl and 1mM EDTA, lipoproteins were stored in the darkness under nitrogen at 4$^{o}$C. HDL were used within two weeks of isolation.

### Chemical modification of HDL

HDL was modified as well described previously (12). Control incubations were performed omitting MDA solution. Reaction was stopped by dialysis against PBS

(0.05M) pH 7.4, containing 154 mM NaCl and 1mM EDTA. The modification of HDL was monitoring by Agarose gel electrophoresis (Beckman Pargon LIPO GEL) and sodium dodecyl sulfate polyacylamide gel electrophoresis.

**Labelling of HDL**

MDA-modified and control HDL were radiolabelled with [$^{14}$C]-cholesterol according to the procedure of Terpstra (14). The HDL fraction was reisolated by sequential ultracentrifugation. Most of the radioactivity (99%) was recovered in the free form of HDL-cholesterol.

**Bile sampling**

After anesthesia of overnight fasted rat with an intraperitoneal injection of pentobarbital (48 mg/kg body wt), the main bile duct was cannulated with a PE-10 catheter and bile was collected for 30 min. Then, labelled HDL was injected into jugular vein catheter and 6X1h bile samples were collected, as previously described (12).

**In vivo uptake of modified HDL in the different liver cell types**

At 90 min after injection of labelled HDL, the vena porta was canulated, and liver perfusion was performed at 8$^{o}$C according to the procedure of Nagelkerke et al (15). The low temperature allows cell isolation without active metabolism of cholesterol. The obtained cell suspension was filtered through 250 µM nylon filter, and hepatocytes were collected after 5 min of centrifugation at 50 g. These hepatocyte cells were resuspended in 20 ml of DMEM medium, counted with a hemocytometer and cell viability was estimated by the exclusion of 0.25 % Trypan blue. The combined supernatants were centrifuged for 10 min at 300 g, and the pellet (mainly non-parenchymal cells) was stored on ice. The material remaining on the nylon gauze filter was suspended in Hank's buffer containing 0.01% DNAse and 0.25% Pronase (BDH, Montréal, Canada), and incubated for 20 min at 8$^{o}$C. The suspension was placed on a 75 µM nylon filter, and the filtrate centrifuged at 300 g for 10 min. The pellets (non parenchymal cells) were combined and suspended in 5 ml of buffer, mixed with 7 ml

Gey's buffer solution (GBS), without NaCl and containing 30 % (w/v) Metrizamide (Nyegaard Oslo Norway). One ml of buffer was layered on top of the mixture and the tubes were spun for 15 min at 1400 g . Non parenchymal cells found in the top layer of Metrizamide gradient were washed with Hank's buffer at 300 g for 5 min before being further separated by centrifugal elutriation according to the procedure of Ferland et al (16). Cell suspensions were centrifugated at 300 g for 5 min. The radioactivity present in isolated cells was measured in a Beckman liquid scintillation spectrophotometer. Protein content was quantified by the method of Lowry (17) with bovine serum albumin as standard.

## RESULTS AND DISCUSSION

Modification of lipoproteins by MDA is frequently employed to assess their in vitro chemical and physiological behavior (18-21). To our knowledge, this is the first in vivo investigation aimed at defining the effect of HDL modification on liver cellular cholesterol distribution.

Initial studies were carried out to determine the hepatic handling of [$^{14}$C]-cholesterol vehiculated by HDL fractions. As shown in Table 1 following the injection of radioactive HDL, the contribution of labelled cholesterol to biliary bile acids was smaller with MDA-modified HDL compared to native HDL. On the other hand, higher liver uptake of [$^{14}$C]-cholesterol was noted with MDA-modified HDL (Table 1). At this stage, it was still unclear whether MDA-modified HDL was present at lower level in

Table 1

**Amount of recovered [$^{14}$C] radioactivity in the liver and bile**

| Injected lipoprotein | Liver weight (g) | Liver radioactivity (total dpm) | Biliary [$^{14}$C] distribution | |
|---|---|---|---|---|
| | | | Cholesterol (dpm/6h) | Bile acids (dpm/6h) |
| Native HDL | 7.99±0.45 | 140,387±7,798 | 11,969±2,408 | 35,327±2,582 |
| MDA-modified HDL | 7.87±0.62 | 161,865±11,610 | 11,973±2,446 | 24,923±3,280* |

Rats were injected with native and MDA-modified HDL-[$^{14}$C] cholesterol as described in Methods. Lipids from the liver and bile were extracted using chloroform:methanol (2:1, v/v). Lipid extracts were dried under $N_2$ and assayed by liquid scintillation spectrometry. Each value represents the mean±SEM of 5 rats/group.
* $p<0.025$.

parenchymal cells, which may explain its reduced availibility for biliary secretion. Therefore, the different liver cell types were isolated after the injection of labelled native and MDA-modified HDL, and the in vivo uptake of [$^{14}$C]-cholesterol was determined in specific cell fractions. It should be noted that redistribution and degradation during technical procedures were avoided by using a low temperature as described by Nagelkerke et al (15). Figure 1 shows the distribution of [$^{14}$C]-cholesterol (% of injected dose x $10^3$/mg protein) into parenchymal, Kupffer and endothelial cells. Although a similar pattern of radioactivity distribution characterized liver cell types of both groups of rats, higher percent of injected dose was associated with endothelial cells belonging to animals administered modified HDL. The

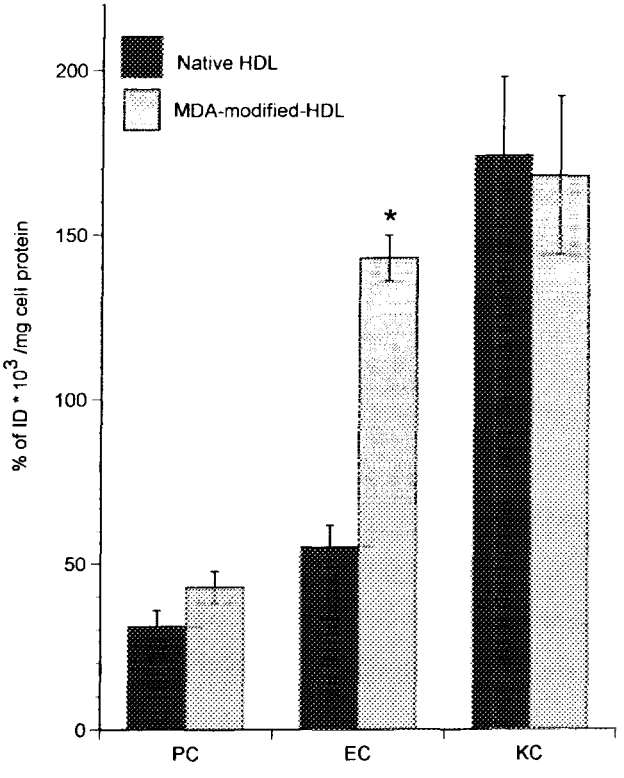


**Figure 1.** In vivo distribution of [$^{14}$C]cholesterol HDL between parenchymal (PC), endothelial (EC), and Kupffer cells (KC). Labelled HDL was injected into anesthetized rats and 90 min after injection a liver perfusion was started. The association to the isolated (at 8$^{o}$C) parenchymal, endothelial, and Kupffer cells was determined. The bars represent values ± SEM of 5 rats/group. * $p < 0.0025$.

Table 2

**Relative contribution of different liver cell types to the total liver uptake of [$^{14}$C]cholesterol HDL**

| Injected lipoprotein | Parenchymal cells (%) | Endothelial cells (%) | Kupffer cells (%) |
|---|---|---|---|
| Native HDL | 81.57±2.10 | 5.36±0.39 | 13.08±1.81 |
| MDA-modified HDL | 78.42±5.80 | 11.48±2.91* | 10.10±2.99 |

Data are given as percentages based upon the amount of radioactivity/mg cell protein and multiplied by the amount of protein that each cell type contributes to total liver protein (92.5%, 3.3%, and 2.5% for parenchymal, endothelial, and Kupffer cells, respectively). Values are means±SEM for 5 rats/group.
*$p<0.05$.

endothelial cell fraction contains 2.6 times more radioactivity with modified HDL than with native HDL. The contribution of different liver cell types to the total liver uptake of HDL [$^{14}$C]-cholesterol was also calculated in order to take into account the relative protein contribution of the different cell types to the total liver content in vivo. Again a significant ($p< 0.05$) difference was noted between endothelial cells receiving modified and native HDL (Table 2). The apparent affinity of the endothelial cells for modified HDL is similar to that described for acetylated LDL (15 ). Indeed, rat liver nonparenchymal cells were highly effective in removing oxidized LDL from the circulation by scavenger receptors (22). Likewise, scavenger receptors for malondialdehyde-modified HDL was localized in rat sinusoidal liver cells (23). Thus, it was suggested that liver non-parenchymal cells elaborate an efficient system against the atherogenic action of oxidized lipoproteins. However, with regard to our findings, the uptake of [$^{14}$C]-cholesterol from modified HDL was detrimental to bile acid secretion. Further studies are therefore needed to delineate the physiological relevance of this process. This is particularly important since HDL was found to be necessary for the transport of cholesterol from endothelial cells to liver parenchymal cells and bile in vivo (24,25).

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## REFERENCES

1. Miller, G.J., and Miller, N.E. (1975) Lancet 1,16-19.
2. Gordon, T., Castelli, W., Hjortland, M.C., Kannel, W.B., and Dawber, T.R. (1977) Am. J. Med. 62,707-714.
3. Glomset, J.A. (1968) J.Lipid Res. 9,155-163.
4. Witztum, J.L (1993) Br. Heart J. 69, S12-S18.
5. Parthasarathy, S., Barnett, J., and Fong, L.G. (1990) Biochim. Biophys. Acta 1044,275-283.
6. Nagano, Y., Arai, H., and Kita, T. (1991) Proc. Natl. Acad. Sci. USA 88,6457-6461.
7. Ghiselli, G., Giorgini, L, Gelati, M., and Musanti, R. (1992) Arteriosclerosis and Thrombosis 12, 929-935.
8. Salmon, S., Mazière, C., Auclair, M., Theron, L., Santus, R., and Mazière, J.C. (1992) Biochim. Biophys. Acta 1125, 230-235.
9. LaVille, A.E., Sola, R., Balanya, J., Turner, P.R., and Mansana, L. (1994) Atherosclerosis 105, 179-189.
10. Musanti, R., and Ghiselli, G. (1993) Arteriosclerosis and Thrombosis 13, 1334-1345.
11. Morel, D.W. (1994) Biochem. Biophys. Res. Com. 200, 408-416.
12. Guertin, F, Brunet, S, Gavino, V, and Levy, E. (1994) Biochim. Biophys. Acta 1214, 137-142.
13. Levy, E., Lepage, G., Bendayan, M., Ronco, N., Thibault, L., Galeano, N., Smith, L, and Roy, C.C. (1989) J. Lipid Res. 30, 1197-1209.
14. Terpstra, A.H.M., Nicolosi. R.J., and Herbert, P.N. (1989) J.Lipid Res. 30, 1663-1671.
15. Nagelkerke, J.F., Barto, K.P., and Van Berkel, Th.J.C. (1983) J.Biol.Chem. 212221-122227.
16. Ferland, G., Perea, A., Tuchweber, B (1990) Mech. of ageing and dev. 56, 143-154.

17. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J.Biol.Chem. 193, 265-275.

18. Fogelman, A.M., Schchter, I., Seager, J., Hokom, M., Child, J.S., and Edwards, P. A. (1980) Proc. Natl. Acad. Sci. (USA) 77, 2214-2218.

19. Haberland, M.E., Fogelman, A.M., and Edwards, P.A. (1982) Proc. Natl. Acad. Sci. USA 79, 1712-1717.

20. Haberland, M.E., Olch, C.L., Fogelman, A.M (1984) J. Biol. Chem. 259, 11305-11311.

21. Fogelman, A.M., Haberland, M.E., Seager, J., Hokom, M., and Edwards, P.A. (1981) J. Lipid Res. 22, 1131-1141.

22. Van Berkel, Th.J.C., de Rijke, Y.B., and Kruijt, J.K. (1991) J. Biol. Chem. 266, 2282-2289.

23. Murakami, M., Horiuchi, S., Takata, K., and Morino, Y. (1986) Biochem. Biophys. Res. Comm. 137, 29-35.

24. Bakkeren, H.F., Kuipers, F., Vonk, R.J., and Van Berkel, T.J.C. (1990) Biochem J. 268, 685-691.

25. Pieters, M.N., Esbach, S., Schouten, D., Brouwer, A., Knook, D.L., and Van Berkel, Th. J. C. (1994) Hepatology 19, 1459-1467.