Volume 227, number 2, 179–182 FEB 05511 January 1988

The effect of inhibition of cholesterol esterification on the fate of cholesterol derived from HDL in rat hepatocyte monolayers

William J. Sampson, Kathleen M. Botham+, Brian Jackson and Keith E. Suckling

Department of Cellular Pharmacology, Smith Kline & French Research Ltd, The Frythe, Welwyn AL6 9AR and
+*Department of Veterinary Basic Sciences, Royal Veterinary College, Royal College Street, London NW1 0TU, England*

Received 23 November 1987

Rat HDL_2 is known to stimulate bile acid synthesis in rat hepatocyte monolayers. The intracellular fate of the cholesterol derived from the HDL_2 was studied using the inhibitor of cholesterol esterification, Sandoz compound 58-035. Rat HDL_2 added to rat hepatocyte monolayers caused a stimulation of cholesterol esterification of 32%. This stimulation could be inhibited by 58-035. A small significant increase in bile acid synthesis was also observed in cells in the presence of HDL_2, confirming our earlier observations. 58-035 prevented this increase. These observations imply that cholesterol entering the cell from HDL_2 is first esterified and can only enter the substrate pool for bile acid synthesis after subsequent intracellular hydrolysis.

Bile acid; HDL; Acyl-CoA:cholesterol acyltransferase; Lipoprotein; Cholesterol metabolism; (Rat hepatocyte)

1. INTRODUCTION

Lipoproteins are taken up by cells by a variety of mechanisms. Of these the best characterised is receptor-mediated endocytosis by the LDL pathway [1]. In some tissues non-receptor mediated uptake also contributes, and mechanisms in which the apoprotein of HDL is taken up at a slower rate than its lipid core have also been described [2]. In all these cases some of the metabolic consequences of uptake of cholesterol are known but it is not clear whether cholesterol entering a cell by one of these routes is constrained to a particular cellular pathway or whether it can immediately equilibrate with the cellular metabolically active pool [3,4]. In some tissues, such as the bovine adrenal cortex, it has been possible to demonstrate the route followed by cholesterol derived from LDL within the cell under different metabolic conditions [5,6]. In other tissues, such as the liver, there are indications that there may be compartmentation of cholesterol metabolism [3,4] but there has been no direct evidence of this.

Free cholesterol derived from lipoproteins in the liver may have a number of fates. This free cholesterol may be secreted into the plasma as a component of lipoproteins or, with phospholipid and bile acids, into bile. Alternatively it may be used as a substrate for bile acid synthesis or be esterified by the enzyme acyl-CoA:cholesterol acyltransferase (ACAT) [4]. We demonstrated a few years ago that a rat lipoprotein fraction, HDL_2 can stimulate the production of bile acids in monolayers of rat hepatocytes [7]. Here we show that this stimulation can be completely blocked by the inhibition of cholesterol esterification in the cells by the Sandoz compound 58-035.

Correspondence address: K.E. Suckling, Department of Cellular Pharmacology, Smith Kline & French Research Ltd, The Frythe, Welwyn, AL6 9AR England

This work was presented in abstract form at the Smith Kline & French Research Symposium on Hyperlipidaemia and Atherosclerosis, Cambridge, December 1987

2. MATERIALS AND METHODS

Rat hepatocyte monolayers were prepared by collagenase perfusion of livers obtained from rats that had been maintained on

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

a diet containing 4% cholestyramine to induce bile acid synthesis. The culture conditions and the measurement of bile acids by radioimmunoassay were carried out as described [8]. The ACAT inhibitor, Sandoz compound 58-035, provided by Dr J. Heider (Sandoz Research Institute, E. Hanover, NJ), was added to cell monolayers dissolved in dimethyl sulphoxide (200 μg per 2 ml dish in 20 μl dimethyl sulphoxide [9]), and cholesterol esterification was measured by determining the incorporation of [^{3}H]oleic acid into cholesteryl esters [9].

Hepatocyte monolayers were prepared independently from 4 rats. The cells were pretreated with 58-035 or control solvent (dimethyl sulphoxide) as described [9]. Mevinolin (obtained from Mr A. Alberts, Merck Sharp & Dohme, Rahway, NJ) was added (5 μM) to remove the effect of endogenous cholesterol synthesis, making the cells more dependent on added lipoprotein. Incubations were carried out for 5 h and bile acids estimated in both cells and medium as described in [7]. In some of the incubations [^{3}H]oleic acid was added to estimate cholesterol esterification during the final 2 h of incubation [9]. HDL_2 was added to incubations at a concentration of 500 μg per dish, about that corresponding to the plasma concentration in rats [7,10]. The lipoprotein fraction was prepared by standard centrifugation techniques from rat plasma [11], the fraction of density 1.085–1.210 being used for the experiments. This fraction contained 251 μg protein, 160 μg cholesteryl ester and 20 μg free cholesterol in 100 μl of the preparation that was added to cells. The composition of this fraction is close to that reported in [12]. Analysis of this fraction by SDS-gel electrophoresis showed that apo-A-I was the main apolipoprotein present. There were also significant amounts of apo-E and apo-A-IV and trace amounts of apo-C-II and apo-D. No apo-B was present.

3. RESULTS AND DISCUSSION

The addition of HDL_2 to the cells in culture caused an increase in the synthesis of cholesteryl ester of 32% (table 1). Esterification could be inhibited by the addition of 58-035, as we had observed previously [9]. This increase, which is significant ($p < 0.01$), provides a scale over which the changes due to HDL_2 can be compared. The data in table 1 suggest that the cholesterol present in the HDL_2 was able to enter the cell and increase the supply of substrate to the pool for esterification, which is probably not saturated under normal conditions [4]. In other experiments we have shown that HDL_2 labelled with [^{14}C]cholesterol in either the unesterified or esterified form can enter rat hepatocytes in monolayer culture and provide substrate for bile acid synthesis (Ford, R.P., unpublished). In related work in vivo and in the perfused liver Stone et al. [13] have shown that cholesteryl ester-rich rat HDL can stimulate hepatic ACAT activity by 45%, which is a comparable increase to that found in our experiments

Table 1

Esterification of [^{3}H]oleate by rat hepatocyte monolayers in the presence of 58-035 and rat HDL_2

Additions	pmol cholesteryl ester formed
1. Control (5 μM mevinolin)	237 ± 13
2. 1 + 58-035	52 ± 6
3. 1 + HDL_2	312 ± 18
4. 2 + 58-035	69 ± 10

Cells (4 independent preparations) were incubated in the presence of additions for 5 h. During the last 2 h of this period potassium [^{3}H]oleate was added. At the end of the incubations the lipids were extracted and the radioactivity in the cholesteryl esters determined after separation on silica Bond-Elut columns. Data are the means ± SD of 4 independent preparations assayed in duplicate culture dishes

in hepatocytes. However, unlike the experiments in cell culture [7,10], no increase in bile salt synthesis was observed.

We have shown previously that 58-035 does not inhibit cholesterol 7α-hydroxylase [7] or HMG-CoA reductase [5]. Other enzymes of cholesterol metabolism in certain cell types (e.g. cholesteryl ester hydrolase in bovine adrenal cortical cells) are also unaffected by 58-035 [5]. In the present experiments cholesterol synthesis in the cultured hepatocytes was inhibited by mevinolin, so any indirect effects of 58-035 on HMG-CoA reductase activity over the 5 h incubation period would not be of significance. The basal rate of synthesis and secretion of bile acids by the cells in 5 h was 2.47 ± 0.46 nmol per 2×10^6 cells. The changes caused by the addition of HDL_2 and 58-035 in each of the 4 animals studied (and the corresponding mean values) are shown in fig.1. Comparison of the absolute values from which these differences were calculated by the two-tailed paired t-test showed that the changes were significant with $p < 0.05$.

The data in fig.1 show that, in addition to causing an increase in cholesterol esterification, the added HDL_2 caused a small but significant stimulation of bile acid synthesis. This observation was reported previously from our group [7] and has been confirmed under slightly different experimental conditions [10]. Thus cholesterol derived from the added lipoprotein was available as substrate for both ACAT and cholesterol 7α-hydroxylase, the initial step in bile acid synthesis [4].

Free cholesterol is required as the substrate for

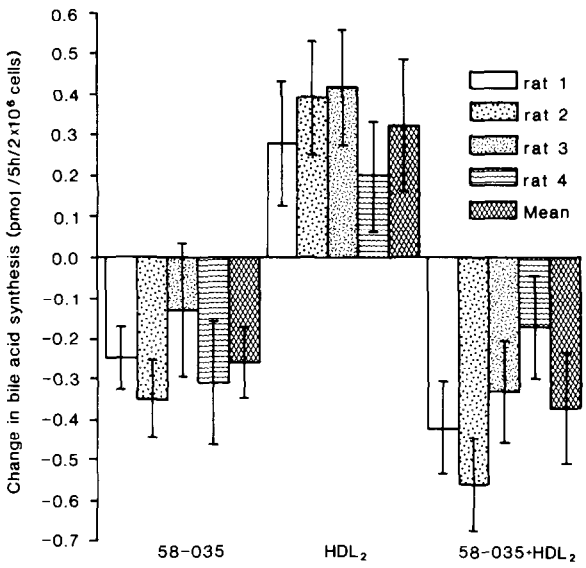

Fig.1. Change in bile acid synthesis by rat hepatocyte monolayers in the presence of mevinolin, 58-035 and rat HDL_2. Rat hepatocytes were incubated with the additions shown under the conditions described in section 2. The data shown are the changes in bile acid synthesis compared with the control value for preparations of cells from each of 4 rats. Error bars show the standard deviation of triplicate determinations of the secreted bile acids by radioimmunoassay [8].

cholesterol 7α-hydroxylase [14]. Therefore cholesterol entering the cell as cholesteryl ester must be hydrolysed at some stage before it enters the substrate pool for synthesis of bile acids. Free cholesterol entering the cell could in principle penetrate either directly to the substrate pool for bile acid synthesis or be esterified by ACAT. If the route followed in the cell by either free cholesterol or cholesteryl ester from the lipoprotein leads compulsorily through esterification by ACAT, this should be detectable by inhibition of this activity by 58-035. Fig.1 shows that the increase in bile acid synthesis due to the addition of HDL_2 is completely blocked by the inhibition of ACAT (table 1). Under the present conditions, therefore, the immediate fate of the cholesterol taken up by the cells from HDL_2 must be esterification.

This observation is in contrast to our recent experiments in which the fate of excess cholesterol newly synthesised from endogenous mevalonate was studied [9]. Here cholesterol esterification was stimulated about 2-fold by the addition of mevalonic acid and bile acid synthesis was also slightly stimulated. However, in this case 58-035 caused a significant increase in the secretion of bile acids, implying that excess cholesterol could be diverted to bile acid synthesis from the ACAT substrate pool.

It is clearly important in experiments with hepatocyte monolayers to attempt to correlate them with the in vivo situation. In the present experiments HDL_2, a specific homologous lipoprotein fraction which carries a substantial amount of cholesterol in the rat, can deliver cholesterol to the hepatocyte. This cholesterol follows a defined intracellular pathway, through ACAT to form the ester, followed by hydrolysis before it can become a substrate for bile acid synthesis. The limiting step in this system is probably the uptake of the lipoprotein by the cultured cells. This experimental design represents a close approximation in a hepatocyte monolayer culture to the situation in vivo. In our earlier experiments [9] the intracellular transport and regulatory mechanisms were probably saturated by the excess cholesterol synthesised from the added mevalonate. Uptake mechanisms were by-passed.

It is possible in cell culture experiments to devise conditions that will channel cholesterol from different sources in any desired way. Such control can be carried out with bovine adrenal cortical cells, which can use cholesterol from any of several sources as a substrate for synthesis of cortisol [4,6]. Studies such as those reported here show what pathways are possible for movement of cholesterol in the cultured cell. Other techniques would be required to demonstrate the operation of these pathways in an intact animal.

REFERENCES

[1] Brown, M.S. and Goldstein, J.W. (1985) J. Clin. Invest. 72, 743–747.
[2] Pittman, R.C., Knecht, T.P., Rosenbaum, M.S. and Taylor, C.A. (1987) J. Biol. Chem. 262, 2443–2450.
[3] Stange, E.F. (1987) Biochem. Soc. Trans. 15, 189–192.
[4] Suckling, K.E. and Stange, E.F. (1985) J. Lipid Res. 26, 647–671.
[5] Jamal, Z., Suffolk, R.A., Boyd, G.S. and Suckling, K.E. (1985) Biochim. Biophys. Acta 834, 230–237.
[6] Ochoa, B. and Suckling, K.E. (1987) Biochim. Biophys. Acta 918, 159–167.

[7] Ford, R.P., Botham, K.M., Suckling, K.E. and Boyd, G.S. (1985) FEBS Lett. 179, 177–180.
[8] Ford, R.P., Botham, K.M., Suckling, K.E. and Boyd, G.S. (1985) Biochim. Biophys. Acta 836, 185–191.
[9] Sampson, W.J., Suffolk, R.A., Bowers, P., Houghton, J.D., Botham, K.M. and Suckling, K.E. (1987) Biochim. Biophys. Acta 920, 1–8.
[10] Mackinnon, A.M., Drevon, C.A., Sand, T.M. and Davis, R.A. (1987) J. Lipid Res. 28, 847–855.
[11] Oschry, Y. and Eisenberg, S. (1982) J. Lipid Res. 23, 1099–1106.
[12] Jansen, H., Schoonderwoerd, G.C. and Dallinga-Thie, G.M. (1983) Biochim. Biophys. Acta 754, 271–278.
[13] Stone, B.G., Schreiber, D., Alleman, L.D. and Ho, C.-Y. (1987) J. Lipid Res. 28, 162–172.
[14] Myant, N.B. and Mitropoulos, K.A. (1977) J. Lipid Res. 18, 135–153.