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Effect of the inhibitors of different steps of cholesterol biosynthesis pathway on high density lipoprotein binding to isolated human enterocytes

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Summary

The effect of cholesterol synthesis inhibitors on high-density lipoprotein (HDL_3) binding to isolated human small intestine epithelial cells was studied. Lovastatin (2 nM), ketoconazole (1 μM), miconazole (2 μM), compound U-18666A (1 μg/ml), 25-hydroxycholesterol (1 μM) and taurocholic acid (2 mM) inhibited [^{14}C]acetate incorporation into cholesterol; lovastatin and ketoconazole also inhibited [^{14}C]oleic acid incorporation into cholesteryl esters. All inhibitors down-regulate HDL_3 binding to enterocytes; good correlation was observed between the effects of the compounds on cholesterol synthesis and HDL_3 binding. These data allow one to assume that newly synthesized cholesterol rather than cholesterol biosynthesis pathway intermediates is a regulator of HDL receptors.

Introduction

High-density lipoprotein (HDL) binding sites have been found on many cell types (Bierman and Oram, 1987); these binding sites are subject to physiological regulation by intracellular free cholesterol content or demand for it (for references, see Sviridov et al., 1990a). It was recently demonstrated in this laboratory that lovastatin, a competitive inhibitor of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA reductase, down-regulates HDL receptors on human small intestine epithelial cells (enterocytes) (Sviridov et al., 1990a) and rat adrenocortical cells (Sviridov et al., 1991), indicating that the pool of newly synthesized cholesterol could be a genuine regulator of HDL receptor. However, HMG-CoA reductase catalyses one of the first steps of cholesterol biosynthesis and, besides cholesterol itself, many intermediates of the cholesterol biosynthesis pathway could be good candidates for regulating the HDL receptor. To test this possibility, we studied the effect of six inhibitors of cholesterol biosynthesis on ^{125}I-HDL_3 binding to isolated human enterocytes. The inhibitors differed in their mechanisms of action and affected different

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Abbreviations: ACAT, acyl-CoA: cholesterol acyltransferase; HDL, high-density lipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; MEM, minimum essential medium.

steps of the cholesterol biosynthesis pathway. It was demonstrated that, irrespective of how these compounds inhibit cholesterol synthesis, they down-regulate HDL receptor proportionally to their effect on the rate of cholesterol synthesis.

Materials and Methods

A segment of middle jejunum was taken at autopsy within 1 h after death. Enterocytes were isolated and maintained as previously described (Sviridov et al., 1987). High-density lipoprotein (HDL_3) ($d = 1.125–1.216$) was isolated from blood plasma of healthy donors by sequential preparative ultracentrifugation (Lindgren, 1975) and iodinated using the iodine monochloride method (Bilheimer et al., 1972). Cholesterol synthesis and esterification were determined as previously described (Sviridov et al., 1990a). Cell protein content and lipoprotein concentration were measured according to Bradford (1976).

The binding assay has been described previously (Sviridov et al., 1987, 1990a). Briefly, the incubation mixture contained 0.5×10^6 cells, 50 μl lipoprotein-deficient serum, the indicated concentration of an inhibitor and minimum essential medium (MEM, Flow, Ayrshire, U.K.) in a total volume of 0.5 ml. The mixture was incubated in the wells of 24-well plates for 1 h at 37°C in a humidified 5% CO_2, 95% air atmosphere with shaking at 60 rpm. After incubation ^{125}I-HDL_3 was added to a final concentration of 10 μg/ml and the plates were incubated for a further 2 h under the same conditions. To determine non-specific binding, 100 μg of unlabeled HDL_3 was added to parallel incubations. After incubation the mixture was transferred into plastic tubes and cells were washed three times with 10 ml of MEM containing 1 mg/ml bovine serum albumin (Sigma, St. Louis, MO) and once with Dulbecco's phosphate-buffered solution without Ca^{2+} and Mg^{2+} (Flow) by centrifugation at $500 \times g$ for 5 min at 4°C. ^{125}I-HDL_3 binding was determined as cell-associated trypsin-sensitive radioactivity as previously described (Sviridov et al., 1987, 1990a).

Lovastatin (MSD, Rahway, NJ), ketoconazole (Sigma), miconazole (Sigma), U-18666A (a kind gift of Upjohn Co, Kalamazoo, MI), and compound 58-035 (Sandoz, Switzerland) were prepared as a 100 × stock solutions in dimethylsulfoxide. Taurocholic acid (sodium salt, Sigma) and mevalonate (lactone, Sigma) were prepared as 100 × stock solutions in MEM, and 25-hydroxycholesterol (Sigma) as a 100 μM solution in ethanol.

Results and Discussion

The effect of six inhibitors of cholesterol biosynthesis on cholesterol synthesis and esterification and on ^{125}I-HDL_3 binding to isolated human enterocytes was studied. The inhibitors were lovastatin, an inhibitor of HMG-CoA reductase (Sviridov et al., 1990b), ketoconazole, an inhibitor of lanosterol 14-methyl demethylase and squalene-2,3-oxide cyclase (Gupta et al., 1986; Favata et al., 1987), miconazole, an inhibitor of lanosterol 14-methyl demethylase (Favata et al., 1987), U-18666A, an inhibitor of squalene-2,3-oxide cyclase (Boogaard et al., 1987), 25-hydroxycholesterol, which has multiple sites of action (Carlson and Kottke, 1989), and taurocholic acid, a specific inhibitor of cholesterol synthesis in enterocytes with an unidentified mechanism of action (Sviridov et al., 1986). Optimal concentrations of the compounds were taken from the papers cited above.

All compounds effectively inhibited [^{14}C]-acetate incorporation into cholesterol by isolated human enterocytes (Table 1); the most effective compounds were lovastatin and ketoconazole, and the least effective was U-18666A.

It has recently been indicated that lovastatin, in addition to inhibition of cholesterol synthesis, can also inhibit cholesterol esterification (Kam et al., 1990). Since inhibition of cholesterol esterification affects cell free cholesterol content and up-regulates HDL binding to enterocytes (Sviridov et al., 1990a), we examined the effect of all compounds on [^{14}C]oleic acid incorporation into cholesteryl esters. Lovastatin and ketoconazole inhibited cholesterol esterification by about 60%, i.e., they were even more effective than the reference ACAT inhibitor, compound 58-035 (Table

TABLE 1

Effect of cholesterol synthesis inhibitors on biosynthesis of cholesterol by isolated human enterocytes

Additions	[^{14}C]Acetate incorporation into cholesterol	
	dpm/mg cell protein [a]	% of control
None	2813 ± 89	100
Lovastatin (2 nM)	389 ± 239	14 [b]
Ketoconazole (1 μM)	428 ± 125	15 [b]
Miconazole (2 μM)	639 ± 255	23 [b]
U-18666A (1 μg/ml)	1834 ± 300	65 [b]
25-Hydroxycholesterol (1 μM)	1011 ± 400	36 [b]
Taurocholate (2 mM)	911 ± 405	32 [b]

[a] Mean ± SE of triplicate determinations.
[b] $p < 0.01$ (vs control).

2). Other compounds did not affect cholesterol esterification. Mevalonic acid stimulated cholesteryl esters synthesis (Table 2).

All cholesterol synthesis inhibitors decreased specific ^{125}I-HDL$_3$ binding to enterocytes (Table 3). Non-specific binding was not affected by any of the compounds. The most effective compounds were lovastatin, 25-hydroxycholesterol and ketoconazole, while U-18666A was the least. Addition of mevalonic acid (15 mM) reversed the effect of lovastatin on ^{125}I-HDL$_3$ binding, but had no influence in combination with other compounds (not shown); mevalonic acid itself stimulated ^{125}I-HDL$_3$ binding (Table 3). Good correlation was observed between the effects of the compounds on cholesterol synthesis and ^{125}I-HDL$_3$ binding to enterocytes ($r = 0.73$; $p < 0.01$) (Fig. 1). Simultaneous inhibition of cholesterol synthesis and

TABLE 2

Effect of cholesterol synthesis inhibitors on cholesterol esterification by isolated human enterocytes

Additions	[^{14}C]Oleate incorporation into cholesteryl esters	
	dpm/mg cell protein [a]	% of control
None	5766 ± 13	100
Lovastatin (2 nM)	1944 ± 512	34 [b]
Ketoconazole (1 μM)	2300 ± 481	40 [b]
Miconazole (2 μM)	4053 ± 1017	70
U-18666A (1 μg/ml)	5763 ± 642	100
25-Hydroxycholesterol (1 μM)	4053 ± 154	70
Taurocholate (2 mM)	4474 ± 787	78
Mevalonate (15 mM)	8191 ± 477	142 [b]
58-035 (5 μg/ml)	3124 ± 477	54 [b]

[a] Mean ± SE of quadriplicate determinations.
[b] $p < 0.01$ (vs control).

TABLE 3

Effect of cholesterol synthesis inhibitors on ^{125}I-HDL$_3$ binding to isolated human enterocytes

Additions	^{125}I-HDL$_3$ binding [a]	
	ng/mg cell protein [b]	% of control
Expt I		
None	158 ± 35	100
Lovastatin (2 nM)	75 ± 3	47 [d]
Ketoconazole (1 μM)	96 ± 9	61 [d]
Miconazole (2 μM)	105 ± 8	66 [d]
U-18666A (1 μg/ml)	134 ± 10	85 [c]
Expt II		
None	195 ± 25	100
Lovastatin (2 nM)	73 ± 14	37 [d]
25-Hydroxycholesterol (1 μM)	75 ± 17	38 [d]
Taurocholate (2 mM)	148 ± 15	76 [c]
Mevalonate (15 mM)	224 ± 24	146 [d]

[a] Specific binding (i.e., total minus non-specific binding, determined in the presence of a 20-fold excess of unlabeled HDL$_3$) is presented.
[b] Mean ± SE of quadriplicate or triplicate determinations.
[c] $p < 0.05$; [d] $p < 0.01$ (vs control).

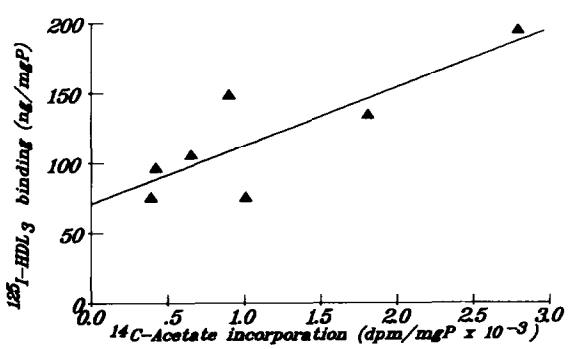

Fig. 1. Correlation between cholesterol synthesis inhibitor-induced changes in cholesterol synthesis and ^{125}I-HDL$_3$ binding to human enterocytes. Data were obtained from the experiments described in Tables 1 and 3.

esterification by lovastatin and ketoconazole resulted in down-regulation of ^{125}I-HDL$_3$ binding to enterocytes.

Although the tested compounds inhibit different steps of the cholesterol biosynthesis pathway, including those before and after formation of known oxysterol regulators of HMG-CoA reductase and low-density lipoprotein receptor in intestinal cells (Gupta et al., 1986, 1990), their influence on HDL binding was proportional only to the effect on cholesterol synthesis. This allows one to assume that newly synthesized cholesterol itself rather than intermediates of cholesterol biosynthesis is a regulator of HDL receptor.

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