EFFECT OF OXIDIZED DERIVATIVES OF CHOLESTEROL ON UPTAKE AND DEGRADATION OF VERY LOW-DENSITY β-LIPOPROTEINS BY HUMAN AND RABBIT HEPATOCYTES

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Commercial cholesterol stored for a long time and also cholesterol subjected to heat treatment contain up to 5% of oxidized cholesterol derivatives [5, 6]. This can be clearly seen on a chromatogram obtained by HPLC (Fig. 1). It has been shown [31 that autooxidation products of cholesterol can suppress the synthesis of cell receptors responsible for binding and uptake of lipoproteins. To create a model of this process we chose a culture of hepatocytes, for the liver plays a central role in lipid and lipoprotein metabolism, including their synthesis and secretion, uptake, and degradation, and also their excretion into the bile.

EXPERIMENTAL METHOD

Old commercial cholesterol was fractionated by HPLC on a reversed phase column (Altex, Ultrasphere-ODS, 250 × 4.6 nm, particle size 5 μ), using an isocratic method with elution system of 5% H_2O/95% methanol. The principal oxidized derivatives of cholesterol were 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, cholestane-3β,5α,6β-triol (Fig. 1). Human liver was obtained from persons dying between the ages of 30 and 45 years (from head injuries), in the course of 30 min after death. Human and rabbit hepatocytes were isolated by a modified collagenase perfusion method [2]. The cells were seeded in culture dishes with a density of $2 \cdot 10^5/cm^2$ in Eagle's minimal medium containing 10% fetal calf serum, 100 μg/ml kanamycin, and essential amino acids (1 mM), and cultured at 37°C in an atmosphere of 95% air and 5% CO_2. Very low-density β-lipoproteins (β-VLDL) were isolated from blood plasma by gradient ultracentrifugation. The protein concentration in the lipoproteins was determined by Lowry's method [4]. The lipoproteins were labeled with ^{125}I with the aid of IC1 [1]. To determine binding of the ^{125}I-β-VLDL with hepatocytes the cells were destroyed in 0.1 N NaOH. Specific binding of ^{125}I-labeled lipoproteins by hepatocytes was calculated by subtracting from values of total binding, those values obtained in the presence of an excess of unlabeled lipoproteins. Degradation of lipoproteins was determined by measuring radioactivity in the culture medium. Values of radioactivity obtained by incubation of medium without cells were subtracted from values determined in the cell culture medium.

EXPERIMENTAL RESULTS

Of all the oxidation products of cholesterol we chose for the experiments 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, and cholestane-3β,5α,6β-triol because they were present in the largest amounts in long-stored commercial cholesterol. Their effect on degradation of β-VLDL by rabbit hepatocytes and on binding and degradation by human hepatocytes was studied. The cholesterol derivatives were added to the hepatocyte culture in a concentration of 10 μg/ml.

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TABLE 1. Effect of Oxidation Products of Cholesterol on β-VLDL Degradation by Human Hepatocytes (M ± m, n = 3)

Substance	Degradation	
	ng/mg cell protein	percent of control
Control	130,0±13,0	100
Cholestanetriol	151,0±21,2	116
7-Ketocholesterol	140,0±17,5	107,6
7a- and 7β-hydroxycholesterol	139,0±6,8	107

TABLE 2. Effect of Oxidation Products of Cholesterol on β-VLDL Binding by Human Hepatocytes (M ± m, n = 3)

Substance	Binding	
	ng/mg cell protein	percent of control
Control	609,0±27,4	100
Cholestanetriol	550,0±32,3*	90,3
7-Ketocholesterol	437,0±31,6*	71,7
7a- and 7β-hydroxycholesterol	491,0±36,7*	80,6

Legend. *$p < 0.05$.

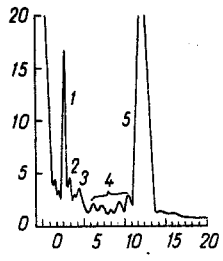

Fig. 1

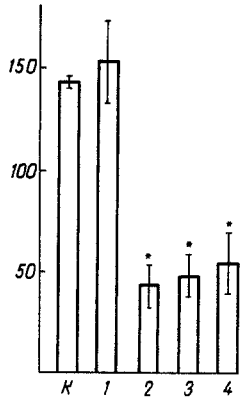

Fig. 2

Fig. 1. Chromatogram of commercial cholesterol stored for 5 years, obtained by HPLC. 1) 7α- and 7β-hydroxycholesterol; 2) 7-ketocholesterol; 3) cholestane-3β,5α,6β-triol; 4) unidentified products; 5) cholesterol. Abscissa, time (in min); ordinate, $A_{209\ nm}$ (in relative units).

Fig. 2. Effect of oxidation products of cholesterol on degradation of VLDL by rabbit hepatocytes. K) Control; 1) cholestane-3β,5α,6β-triol; 2) 7-ketocholesterol; 3) 7α- and 7β-hydroxycholesterol; 4) total fraction. Ordinate, degradation of β-VLDL (in ng/mg cell protein). *$p < 0.05$.

Oxidized products 7-ketocholesterol and 7α- and 7β-hydroxycholesterol were shown to inhibit degradation of β-VLDL in a culture of rabbit hepatocytes ($p < 0.05$). Cholestanetriol does not affect the dynamics of β-VLDL degradation compared with the control. The total fraction of oxidized derivatives also had an inhibitory action on β-VLDL degradation (Fig. 2). The results are in agreement with data in the literature on inhibition of synthesis of receptors for β-VLDL [3], leading to reduction of their binding and subsequent degradation. However, this kind of effect was not present when a culture of human hepatocytes was used. As Table 1 shows, none of the oxidized forms of cholesterol affected β-VLDL degradation in cell culture. Meanwhile, the study of binding of β-VLDL with human hepatocytes (Table 2) demonstrated a significant decrease in β-VLDL binding by

cells after the addition of oxidized derivatives of cholesterol to the culture. Under these circumstances 7-ketocholesterol gave the greatest effect, whereas 7α- and 7β-hydroxycholesterol and cholestanetriol had a weaker effect, but they still significantly reduced binding. In other words, definite specificity is revealed in their inhibitory action.

The results are evidence of definite species-specific features in the dynamics of β-VLDL degradation under the influence of oxidized derivatives of cholesterol. Inhibition of β-VLDL degradation by rabbit liver cells under the influence of oxidation products of cholesterol may lead to their reduced utilization from the blood plasma and to the development of hypercholesterolemia. In this situation, the hypercholesterolemia in rabbits may be provoked and be more marked in degree under the influence of oxidized forms of cholesterol. Species differences in β-VLDL degradation by human hepatocytes are characterized by absence of the inhibitory effect of oxidized forms of cholesterol. It can be postulated that degradation of β-VLDL in human hepatocytes under these conditions is not strictly the target of action of oxidized forms of cholesterol. The key stage at which oxidized derivatives of cholesterol exert their influence in this chain of conversions is binding of β-VLDL with receptors. The mechanism of the effect of oxidized forms of cholesterol may perhaps be realized in two ways: by inhibition of receptor synthesis and the formation of modified forms of β-VLDL, which possess weaker affinity for these receptors. Whereas the first mechanism has been sufficiently well confirmed experimentally and is not in dispute, the second requires further study of a possible effect of oxidized derivatives of cholesterol on the kinetics of binding of β-VLDL with receptors. However, both these mechanisms, as a result of reduced binding of β-VLDL with receptors, may help to reduce β-VLDL utilization and to promote the development of hypercholesterolemia.

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