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EFFECT OF RECENT ALCOHOL INTAKE ON ACCEPTOR PROPERTIES OF HIGH-DENSITY LIPOPROTEINS AND THEIR INTERACTION WITH LIVER CELLS

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Alcohol is one of the factors that raises the blood serum high-density lipoprotein (HDL) level. Epidemiological studies conducted in various countries have demonstrated positive correlation between alcohol consumption and the cholesterol (ChS) and HDL levels [11]. The causes of the rise of the HDL level after alcohol intake (alcohol hyperalphacholesterolemia — hyper-AChS) have not been finally elucidated, although they clearly depend on the dose of alcohol taken and the duration of its administration [14]. One of the most probable mechanisms is an inducing effect of alcohol on synthesis of the principal apoproteins of HDL and, correspondingly, of nascent HDL, in the liver [6]. Another pathway of HDL accumulation is increased synthesis of very low-density lipoproteins (VLDL) and an increase in the rate of their catabolism, leading to the formation of mature HDL [14]. A third possibility is slowing of catabolism of HDL as a result of disturbance of their interaction with liver cells, but this has received the least study. Another point for discussion is the problem of the validity of alcohol hyper-AChS from the point of view of the ability of HDL to accept ChS from cells of peripheral tissues and, in particular, blood vessel walls.

We know that small and ChS-deprived particles of the HDL_3 subclass (acceptance of ChS) interact with peripheral cells, and large and ChS-rich particles of HDL_2 (transfer of ChS into the liver) with liver cells [5]. The writers previously demonstrated that hepatoma HepG2 cells and ChS-loaded fibroblasts can be used as models of liver cells and peripheral cells, respectively,

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TABLE 1. Changes in Subfractional HDL Spectrum during Incubation of Test Sera with Fibroblasts ($X \pm m$)

Group of subjects tested	HDL fractions (in % of control)				
	HDL_{2b}	HDL_{2a}	HDL_{3a}	HDL_{3b}	HDL_{3c}
Normolipidemia					
abstainers (n=18)	25,4±8,6+*	23,7±6,1+	−12,5±3,2+	−17,8±5,1+	−24,8±3,8+
after overindulgence in alcohol (n=17)	5,4±2,6+*	9,5±2,6+	−8,0±2,9+	−2,7+±5,9	12,9±11,6
AChS:					
abstainers (n=11)	12,5±2,6+	4,6±0,7+	−4,8±2,2+	−9,0±2,3+	−17,2±10.4
after overindulgence in alcohol (n=8)	10,4±2,1+	1,1±3,6	−7,6±3,0+	−6,7±5,7	−3,3±11,6

Legend. Here and in Table 2: +) significance of difference from control (by Wilcoxon's paired test); *) between corresponding groups of abstainers and overindulging in alcohol (by Student's test).

TABLE 2. Changes in Subfractional HDL Spectrum during Incubation of Test Sera with Hepatoma HepG2 Cells ($M \pm m$)

Group of subjects tested	Subfraction HDL_{2b}	HDL fractions (in % of control)			
		HDL_{2a}	HDL_{3a}	HDL_{3b}	HDL_{3c}
Normolipidemia					
abstainers	−10,0±2,9**	0,4±7,8	13,6±5,6*	7,9±12,2	−15,4±12,2
after overindulgence in alcohol					
a) n=8	−15,7±3,9*	1,1±4,9	9,9±3,1*	3,7±6,9	2,8±4,9
b) n=9	20,8±8,4**	4,7±4,2	2,3±2,7	−16,3±9,4*	−28,4±8,8*
AChS:					
abstainers	1,7±6,2	13,0±4,6*	−1,4±4,8	−21, ±5,4*	−35,0±3,3**
after overindulgence in alcohol	4,1±1,4*	12,1±3,9*	−18,5±11,1*	−21,5±12,5	0,2±19,1*

in order to study the dynamics of the subfractional spectrum of HDL during transport of ChS between HDL and cells in dyslipidemias [2]. The dynamics of the subfractional spectrum of HDL in normolipidemic blood sera from healthy abstainers and of persons after acute overindulgence in alcohol has now been studied by incubation of the sera with fibroblasts and with hepatoma HepG2 with the aim of determining the causes of the raised ChS of HDL level under the influence of alcohol.

EXPERIMENTAL METHOD

Blood was taken in the fasting state from the cubital vein of men aged between 25 and 53 years, admitted to a medical sobering-up unit after acute overindulgence in alcohol, and with evidence of alcohol dependence (25 persons), and also men of the control group of corresponding age (29 persons), not taking alcohol systematically or during 2 weeks before investigation. Total ChS, triglycerides (TG), and ChS of LDL in the test sera were determined quantitatively on a "Technicon AAP" automatic analyzer (USA).

In the group of men with normolipidemia, those with the following plasma lipid values were selected: ChS ≤ 250 mg/dl. TG ≤ 200 mg/dl, ChS of HDL ≤ 73 mg/dl, but ≥ 35 mg/dl. In the group with hyper-AChS, subjects were chosen with normal values of total ChS and TG, but with ChS of HDL > 73 mg/dl [2, 13].

Cultures of human hepatoma HepG2 and ChS-loaded human skin fibroblasts were used. The cells were grown as described in [2]. The 2% test serum was added to the cells in growth medium for 24 h. Under identical conditions (37°C, 24 h) but without cells, another aliquot of the same serum was incubated, and served as the control [2].

Lipoproteins were concentrated from 2% serum (after incubation with or without cells) on an ultracentrifuge (105,000g, 24 h, 17°C) [10]. Gradient gel-electrophoresis of HDL was carried out in polyacrylamide gel plates with concentration gradient of 4-30%. To calibrate the particle size of HDL we used a commercial protein mixture with particle diameter from 7.1 to 17 nm (HMW Calibration Kt, from "Pharmacia," Sweden). The limits of particle diameters for each HDL subfraction were chosen in accordance with data obtained in [4]. The scanning data were processed planimetrically: the integral curve was divided by vertical lines into zones corresponding to 5 HDL subfractions. The contribution of each subfraction to the total area of the whole HDL fraction was calculated and was taken to be the relative content of this subfraction in the total HDL pool. The content of the

HDL subfractions during interaction of lipoproteins with cells was determined as the difference between the content of these subfractions in samples incubated with and without cells under otherwise identical conditions.

The significance of the results was assessed by Wilcoxon's paired test and Student's test.

EXPERIMENTAL RESULTS

Changes in the subfractional spectrum of HDL during incubation of the test sera with fibroblasts are shown in Table 1. On incubation of normolipidemic sera of abstaining men with ChS-loaded fibroblasts, significant changes took place with all HDL subfractions; moreover, the proportion (content in the total HDL pool) of small HDL_3 particles decreased, in association with a simultaneous increase in the content of HDL_2 particles. This dynamics of the subfractional HDL spectrum reflects the process of ChS acceptance from fibroblast membranes, in which small HDL_3 particles are converted into large and ChS-rich HDL_2. In the case of normolipidemic sera obtained after acute overdulgence in alcohol, the general rule found for abstainers still held good, although the degree of the increase in the HDL_2 content was less.

As a result of incubation of hyperalphacholesterolemic sera of abstaining men with fibroblasts, no fundamental differences were found from the dynamics of the HDL subfractions observed in the case of normolipidemia (Table 1). The less intensive conversion of HDL_3 into HDL_2 in hyper-AChS compared with normolipidemia can be explained on the grounds that the total pool of HDL particles in hyper-AChS is low in HDL_3 particles [3].

In experiments with incubation of hyperalphacholesterolemic sera obtained after acute overindulgence in alcohol with fibroblasts, a significant decrease was found only for the HDL_{3a} subfraction, and a significant increase only for HDL_{2b}, although the general rule regarding these changes likewise was preserved.

Incubation of the same samples of sera with hepatoma HepG2 cells showed a different dynamics of the HDL subfractions (Table 2). For instance, in abstainers with normolipidemia there was a decrease in the content of large HDL_2 particles (mainly on account of HDL_{2b}), with a corresponding increase in the content of small HDL_{3a} particles, reflecting transport of ChS from HDL into the cells. However, on incubation of normolipidemic sera obtained after acute overindulgence in alcohol with hepatoma cells, the results were in a different direction. For some sera the changes were similar to those obtained in abstainers with normolipidemia, but for others the dynamics of the subfractional spectrum was opposite in direction: a significant increase in the content of large HDL_{2b} with a significant reduction in the content of HDL_{3c}.

The results obtained for hyperalphacholesterolemic sera, from both abstainers and from those indulging to excess, were largely similar. The differences related only to the degree of changes in individual subfractions. The distinguishing feature of the dynamics of the subfractional HDL spectrum in all hyperalphacholesterolemic sera, when incubated with hepatoma cells by contrast with normolipidemic sera, was a decrease in the HDL_3 content and an increase in the HDL_2 content. The reason for this may be low activity of the protein carrier of ChS esters, responsible for selective transport of esterified ChS from HDL into hepatoma HepG2 cells [8], found both in hyper-AChS [9] and in persons systematically taking alcohol [12].

Systematic alcohol consumption thus does not change the basic tendencies of subfractional conversions of HDL during acceptance of ChS from peripheral cells. The fact that in a high proportion of persons with normolipidemia and systematically taking alcohol the dynamics of the subfractional spectrum of HDL during their interaction with hepatoma cells is analogous to that in hyper-AChS may be evidence of disturbances of HDL metabolism, preceding the development of alcohol hyper-AChS. The postalcoholic rise of the ChS of HDL level in the blood plasma can be explained by accumulation of large HDL_2 particles in the general pool of HDL particles during their interaction with liver cells, on account of the slowing of ChS transport into the liver.

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