Acception of Cholesterol from Cells in Men of the Russian Population Correlates with Concentration of Pre-β1 High-Density Lipoproteins

A. P. Serdyuk, K. Lasselin*, G. Castro*, O. A. Litinskaya, G. Frushar*, and V. A. Metel'skaya

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We analyzed subfraction composition of HDL and cholesterol-acceptor properties of the plasma in Russian men with high and low HDL cholesterol. HDL were subfractionated by two-dimensional electrophoresis in agarose-polyacrylamide gel. The content of pre-β1 HDL increased in individuals with high concentration of HDL cholesterol and strictly correlated with acception of cellular cholesterol in both groups.

Key Words: *high-density lipoproteins; cholesterol; reverse cholesterol transport; apoproteins*

Previous studies revealed a negative correlation between cholesterol (CH) content in plasma high-density lipoproteins (HDL) and morbidity and mortality from coronary heart disease (CHD) in Western countries [2,9]. However, these relationships were not found in Russian population [1,8]. At the same time, epidemiological studies showed that HDL CH content in Russian men is higher than in American men [8]. Moreover, mortality from CHD in Russia is highest over the world. The causes of this discrepancy remain unknown.

The major function of HDL is reverse transport of CH (*i.e.* transport of CH from peripheral tissues into the liver followed by its excretion from the body). This is an important mechanism underlying regulation of plasma level of HDL CH [11,12]. HDL particles vary in size, density, protein and lipid composition, and functional activity [3,10]. Our previous studies showed that HDL particles interacting with cells contain only apoprotein AI (apo-AI) and displays pre-β-mobility during electrophoretic separation [5]. Pre-β1-migrating apo-AI-containing HDL particles act as a major acceptor of cellular CH [5]. The efficiency of the initial stages in reverse CH transport depends on physicochemical characteristics and plasma level of these particles. In patients with dyslipidemias the ratio of pre-β HDL in the total pool of HDL varies. Factors regulating the total content of pre-β-migrating HDL, the concentration of pre-β HDL subfractions (pre-β1 HDL and pre-β2 HDL), and their functional activity remain unknown.

Here we studied whether impaired CH-binding capacity of pre-β-migrating HDL contributes to less pronounced negative correlation between the concentration of plasma HDL CH and CHD morbidity and mortality in Russian men. The method of two-dimensional electrophoresis was used to assay subfractions of HDL in 2 groups of Russian men with high and low content of HDL CH. We evaluated the ability of human plasma samples to accept CH from rat hepatoma Fu5AH cells.

MATERIALS AND METHODS

We examined men (40-59 years) living in Moscow and not having clinical signs of CHD and liver diseases. Plasma HDL CH content in patients of groups

Department of Metabolic Disorders, State Research Center for Preventive Medicine, Russian Ministry of Health, Moscow; *Department for Studies of Lipoproteins and Atherosclerosis, Pasteur Institute, Lille, France. ***Address for correspondence:*** vicamet@orc.ru. Serdyuk A. P.

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1 (n=30) and 2 (n=30) was less than 40 mg/dl (low level) and more than 50 mg/dl (high level), respectively. The blood from cubital vein was taken from fasting individuals in the morning. The contents of total CH, triglycerides, and HDL CH were measured enzymatically after precipitation of apo-B-containing lipoproteins with sodium phosphotungstate in the presence of magnesium chloride. The measurements were performed on an Airone 200 automatic analyzer using Human diagnostic kits. Plasma apo-AI concentration was estimated by immunonephelometry on a Behring automatic analyzer with kits and standards. Lecithin-cholesterol acyltransferase (LCAT) activity was measured radiometrically. The content and ratio of pre-β- and α-migrating HDL were determined by the amount of apo-AI in subfractions using two-dimensional electrophoresis in agarose-polyacrylamide gel [5]. Step I separation was performed using 0.75% agarose gel in 50 mM barbiturate buffer at 7°C (200 V) for 2 h. Step II separation was performed in 2-15% gradient polyacrylamide gel at 120 V for 16 h. Proteins were transferred from the gel on a nitrocellulose membrane (blotting). The membrane was treated with polyclonal antibodies against human apo-AI labeled with peroxidase. Subfractions containing apo-AI were identified by chemiluminescence (ECL, Amersham Pharmacia Biotech). Acception of CH was assayed using rat hepatoma Fu5AH cells [6]. The efficiency of acception was determined by incubation of 5% plasma samples with cells labeled with [^{3}H]-CH at 37°C for 4 h. We estimated the amount of radioactive cellular CH released into the medium. The data are expressed as means and errors. Intergroup differences were evaluated by unpaired Student's test. The differences were significant at p<0.05.

RESULTS

The patients with low and high content of HDL CH were of comparable age (53.0±1.0 and 51.0±0.9 years, respectively). We did not reveal significant differences in the total content of plasma CH in patients of these groups (Table 1). The amount of HDL CH was a major criterion for selection of patients. In group 2 patients HDL CH content was higher, while plasma triglyceride level was lower than in group 1 patients. The total content of apo-AI in group 2 patients was 32% higher than in group 1 patients. Activity of LCAT playing a key role in reverse transport of CH considerably decreased in Russian men with high HDL CH content. However, in these patients the ability to accept CH from hepatoma Fu5AH cells was higher than in group 1 patients.

Two-dimensional electrophoresis of apo-AI-containing HDL revealed considerable intergroup differences in the composition of HDL subfractions (Table 2). The study of the relative concentration of apo-AI showed that in group 2 patients the content of pre-β and pre-β1 subfractions was higher, while the amount of subfractions pre-β2 and α was lower than in group 1 patients. In group 2 patients the absolute concentrations of apo-AI in subfractions pre-β, pre-β1, and α surpassed those in group 1 patients by 52, 62, and 24%, respectively. However, the absolute concentration of subfraction pre-β2 in group 2 patients was 42% lower than in group 1 patients. The observed increase in the content of pre-β and pre-β1 HDL and decreased LCAT activity in group 2 patients are consistent with the fact that pre-β HDL are the substrate for LCAT, and the amount of pre-β HDL decreases with increasing LCAT activity [7]. However, our findings are contradictory to the data obtained on Argentinean po-

TABLE 1. Plasma Lipid and Apo-AI Content, LCAT Activity, and Binding of Cellular CH in Patients with High and Low Levels of HDL CH (M±m)

Parameter	Group 1, low level of HDL CH	Group 2, high level of HDL CH
Total CH, mg/dl	218.0±6.1	226.0±6.7
Triglycerides, mg/dl	159.0±5.5	101.0±9.5*
HDL CH, mg/dl	35.8±0.8	58.2±1.6*
Apo-AI, mg/dl	101.0±2.4	133.0±3.2*
LCAT activity, nmol/ml/h	11.55±1.16	7.32±1.02**
Binding of cellular CH, %	31.04±0.71	36.59±1.16*

Note. Here and in Table 2: *p<0.01 and **p<0.05 compared to group 1.

TABLE 2. Concentration of Apo-AI-Containing HDL Subfractions in Patients with High and Low Levels of HDL CH (M±m)

Apo-AI subfraction	Group 1, low level of HDL CH	Group 2, high level of HDL CH
Pre-β, % apo-AI	30.42±0.76	34.59±1.47**
α, % apo-AI	69.37±0.71	65.39±1.46**
Pre-β1, % apo-AI	27.62±0.86	33.28±1.50*
Pre-β2, % apo-AI	2.80±0.23	1.31±0.16*
Pre-β, mg apo-AI/dl plasma	30.69±0.91	46.77±2.61*
α, mg apo-AI/dl plasma	69.94±1.90	86.59±2.20*
Pre-β1, mg apo-AI/dl plasma	27.82±0.88	45.09±2.64*
Pre-β2, mg apo-AI/dl plasma	2.88±0.27	1.68±0.20*

pulation where LCAT activity decreases in male individuals with low HDL CH content [4].

Our experiments showed that pre-β particles effectively accept cellular CH during incubation of plasma samples from patients of both groups (Table 1). Intergroup differences were revealed after correlation analysis of the absolute and relative concentrations of pre-β1 HDL and binding of cellular CH. The absolute and relative concentrations of pre-β1 correlated with binding of cellular CH in group 2 patients (r=0.522, p<0.01; and r=0.633, p<0.01, respectively). In group 1 patients only the absolute content of pre-β1 correlated with CH release from cells (r=0.372, p<0.05). We found that in group 2 patients the concentrations of plasma HDL CH and apo-AI correlated with binding of cellular CH (r=0.500, p<0.01; and r=0.606, p<0.01, respectively). In group 1 patients binding of cellular CH correlated only with apo-AI content (r=0.536, p<0.01).

Thus, our experiments demonstrated unimpaired CH-acceptor capacity of pre-β1 HDL in Russian men. In patients with low level of HDL CH binding of cellular CH decreased and less significantly correlated with the content of subfraction pre-β1 (compared to patients with high level of HDL CH). No correlation was found between the content of pre-β HDL and the risk of atherosclerosis [7]. However, high concentration of pre-β HDL in Russian people promotes the initial stage in reverse CH transport (binding of CH excess from cells). At the same time, decreased LCAT activity in patients with high level of HDL CH probably indicates impairment of subsequent stages in reverse CH transport, e.g. CH esterification and transport of CH esters to apo-B-containing lipoproteins and into the liver. Further investigations are required for evaluation of the efficiency of the late stages in reverse CH transport in Russian people.

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