Changes in the Lipid-Transporting System in Rats with Experimental Peritonitis

S. S. Osochuk

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 8, pp. 169-171, August, 2002
Original article submitted March 7, 2002

In experiments on outbred albino rats the spectrum of plasma lipids and composition of HDL were studied during the early period of experimental peritonitis. The content of HDL cholesterol, activity of lecithin-cholesterol acyltransferase, and intensity of VLDL lipolysis increased. We also revealed accumulation of dihomo-γ-linolenic, a marker of essential fatty acid deficiency, in HDL cholesterol esters.

Key Words: *high-density lipoproteins; phospholipids; cholesterol; fatty acids*

Peritonitis is a severe complication of some acute diseases and abdominal surgery. The mortality rate of patients with generalized purulent peritonitis reaches 70-100% [2]. Therefore, studies of the pathogenesis and etiology of peritonitis are of considerable importance.

For a long time, high-density lipoproteins (HDL) were considered only from the viewpoint of their antiatherogenic activity. Recent studies showed that HDL are polyfunctional particles. For example, HDL are involved in the regulation of early stages of inflammation [7]. The data on changes in the HDL composition during inflammatory diseases are contradictory.

Previous experiments demonstrated that rats are highly resistant to the development of inflammatory processes. Here we studied the spectrum of plasma lipids and composition of HDL in outbred albino rats with experimental peritonitis.

MATERIALS AND METHODS

Peritonitis was modeled by single intraperitoneal injection of *E. coli* (strain O-26, 4×10^9 microbial cells) to 15 male outbred albino rats weighing 180-200 g. The control group included 12 intact rats. The animals were decapitated 4 h postinjection. The blood was collected into tubes with citrate. HDL were isolated from the plasma by chemical precipitation of apo B-containing lipoproteins with heparin in the presence of manganese ions [4]. Cholesterol content was measured using Analiz-Kh kits (Belarussian State University). The analysis is based on the Liberman—Burhard reaction. The lipid fraction of HDL was extracted with a chloroform-methanol mixture (2:1). The amount of total phospholipids in HDL was determined after their mineralization by the content of inorganic phosphate in the reaction with ammonium molybdate in the presence of ascorbic acid. Phospholipids separated by two-dimensional thin-layer chromatography [1] were mineralized in chloric acid at 220-240°C. The percentage was estimated by the yield of inorganic phosphate [6]. To evaluate the spectrum of fatty acids in HDL cholesterol esters the lipid extract was separated by thin-layer chromatography in a hexane-diethyl ether-glacial acetic acid system (73, 25, and 2 ml respectively) (Fig. 1). The fractions of cholesterol esters were scraped into vials and methylated with 0.75 N H_2SO_4 in methanol at 65°C for 24 h. Methyl esters were extracted with hexane. The extract was evaporated under nitrogen flow, immediately dissolved in acetone, and assayed on a Tsvet 500M gas chromatograph using a 2-m Reoplex 400 column (gas flow rate 30 ml/min) and a flame ionization detector. Fatty acid methyl esters (Sigma-Aldrich) were used. The ratio between detected fatty acids was evaluated by peak areas and expressed in percents. Activity of lecithin-cho-

Central Research Laboratory, Department of Biochemistry, Vitebsk State Medical University

0007-4888/02/1342-0145$27.00

lesterol acyltransferase (LCAT) was measured by the rate of ^{14}C-cholesterol incorporation into plasma cholesterol esters using Immunotech kits. Enzyme activity was expressed in mmol cholesterol/liter plasma/h. The results were analyzed using Excel and Statistica 5.0 softwares.

RESULTS

The contents of plasma total cholesterol (p=0.023) and HDL cholesterol (p=0.021) increased, while the concentration of VLDL cholesterol decreased (p=0.015, Table 1) 4 h after administration of *E. coli*. The decrease in VLDL cholesterol concentration was probably associated with activation of lipoprotein lipase and conversion of VLDL into HDL. Intensification of VLDL lipolysis is probably a result of increased energy consumption during peritonitis.

Thus, in the early period of peritonitis changes in the spectrum of blood lipids were associated with activation of lipolytic conversion of VLDL into HDL. The content of lysophosphatide in HDL significantly increased (p=0.00002), while the content of sphingomyelins (p=0.006), phosphatidylcholines (p=0.006), and phosphatidylethanolamines decreased (p=0.038). HDL protein content remained unchanged. The concentration of total phospholipids decreased, which led to an increase in the protein/phospholipid ratio (p=0.002). LCAT activation can be explained by decreased content of sphingomyelins, specific inhibitors of this enzyme [5]. LCAT activation is accompanied by a decrease in HDL phosphatidylcholine content and accumulation of lysophosphatides.

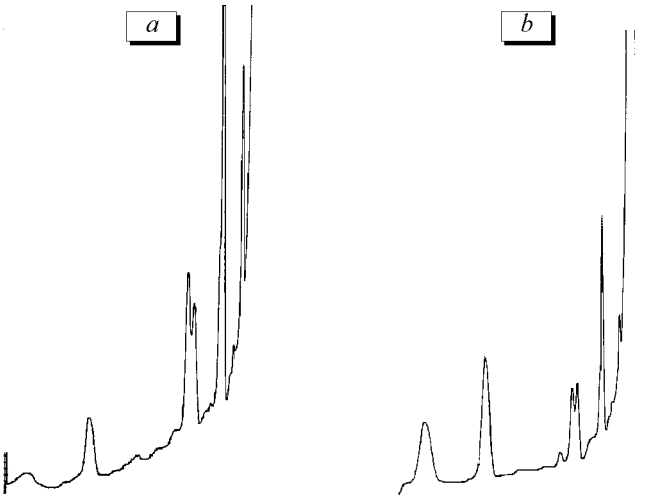

Fig. 1. Chromatogram of fatty acids of HDL cholesterol esters in intact rats (*a*) and animals with experimental peritonitis (*b*).

The content of myristic, heptadecanoic, and heptadecenoic acids in HDL cholesterol esters decreases. The content of stearic acid tended to decrease (p=0.07). We also revealed accumulation of palmitic and dihomo-γ-linolenic acids. The concentration of linoleic acid tended to increase (p=0.06, Table 2).

Accumulation of dihomo-γ-linolenic acid is a marker of essential fatty acid deficiency. Its synthesis from oleic acid is catalyzed by D^6- and D^5-desaturases [3].

TABLE 1. Plasma Lipid Spectrum ($M\pm m$, n=6-12)

Parameter	Control	Experiment
Triacylglycerides, mmol/liter	0.86±0.06	0.63±0.04*
Total cholesterol, mmol/liter	2.24±0.09	2.50±0.03*
LDL cholesterol, mmol/liter	0.39±0.07	0.55±0.03
VLDL cholesterol, mmol/liter	0.39±0.03	0.29±0.02
LCAT activity, μmol/liter/h	3.77±0.62	16.88±2.82
HDL cholesterol, mmol/liter	1.47±0.06	1.65±0.01
HDL total phospholipids, mmol/liter	3.42±0.23	2.37±0.04
Phospholipid spectrum, %		
lysophosphatides	33.12±0.87	38.66±0.34*
sphingomyelins	11.74±0.61	9.08±0.57*
phosphatidylcholines	44.22±0.81	40.03±0.56*
phosphatidylethanolamines	6.38±0.37	5.20±0.36*
glycerophosphate	5.21±0.44	7.01±0.87
Total protein, g/liter	78.18±2.35	76.75±2.80
Protein/total phospholipids	23.59±1.63	31.66±1.21*
Protein/cholesterol	56.56±3.13	45.75±2.10*
Total phospholipids/cholesterol	2.40±0.19	1.45±0.03*

Note. *Significant differences from the control.

TABLE 2. Percentage of Fatty Acids in HDL Cholesterol Esters ($M \pm m$, n=5)

Acid	Control	Experiment
Myristic	15.40±1.41	1.25±0.40*
Palmitic	50.04±3.00	66.29±3.80*
Heptadecanoic	0.82±0.17	0.15±0.03*
Heptadecenoic	0.73±0.11	0.19±0.03*
Stearic	5.60±0.85	3.27±0.48**
Oleic	8.29±0.61	2.35±0.18*
Linoleic	0.66±0.16	1.47±0.28**
Dihomo-γ-linolenic	0.020±0.00^{+}	24.6±4.3*
Arachidonic	17.35±2.30	—

Note. Differences compared to the control: *significant, **tendency to significance. ^{+}n=2.

These changes probably attest to increased requirement for essential fatty acids and enhanced production of their ω-9-substituents. This is confirmed by a decrease in the ratio of oleic acid (precursor of ω-9-fatty acids) and increase in the content of linoleic acid (product of D^6-desaturase).

Our results indicate that the early period of experimental peritonitis is characterized by an increase in blood HDL cholesterol level, which is probably associated with intensive lipolysis of VLDL. Activation of LCAT is probably related to accumulation of its preferential substrate HDL and low content of enzyme inhibitor sphingomyelin. The synthesis of ω-9-fatty acids is intensified during this period.

REFERENCES

1. M. Keits, *Technics of Lipidology* [in Russian], Moscow (1975).
2. A. N. Kosinets, *Prevention and Therapy of Purulent and Inflammatory Complications after Urgent Surgeries on Abdominal Organs (Clinical and Experimental Observations)*, Abstract of Doct. Med. Sci. Dissertation, Vitebsk (1994).
3. R. Marri, D. Grenner, P. Meies, and V. Roduel, *Human Biochemistry* [in Russian], Moscow (1993).
4. *Modern Methods for Studying High-Density Lipoproteins (Methodical Recommendations)*, Ed. N. V. Perova, [in Russian] Moscow (1983).
5. A. Jonas, *Biochim. Biophys. Acta*, **1529**, 245-256 (2000).
6. V. E. Vaskowsky, E. Y. Kostetsky, I. M. Vasendin, *J. Chromatogr.*, **114**, No. 1, 129-141 (1975).
7. K. Vosbek, P. Tobias, H. Mueller, *et al.*, *J. Leukoc. Biol.*, **47**, No. 2, 97-104 (1990).