Influence of Perftoran on Structural and Metabolic Disturbances in the Liver during Experimental Atherosclerosis

G. F. Leskova, A. B. Michunskaya, L. P. Kobozeva, and E. D. Klimenko

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 136, No. 10, pp. 386-389, October, 2003
Original article submitted July 7, 2003

We evaluated whether perftoran can be used for the correction of structural and metabolic changes in the liver during alimentary atherosclerosis. Perftoran in a dose of 0.3 g/kg normalized the contents of phosphatidylserine, phosphatidic acid, and phosphatidylcholine in the plasma membranes of liver cells in rabbits feeding an atherogenic diet for 2 months. Architectonics of hepatic trabeculae returned to normal, the degree of granular and vacuolar degeneration in hepatocytes decreased, and phagocytic activity of macrophages increased. Individual peculiarities of the effect of perftoran on structural and metabolic changes in the liver were revealed.

Key Words: *perftoran; phospholipids; plasmalemma; liver; atherosclerosis*

Dyslipidemia increases the risk of cardiovascular diseases. The study of the pathogenesis of this condition and the development of methods for the correction lipid metabolism are of considerable importance. The liver plays an important role in lipid metabolism. We studied the pathogenesis of liver cell dysfunction in rabbits with alimentary atherosclerosis. A large body of evidence indicates that membrane phospholipids (PL) are involved in the regulation of cell activity. Here we studied PL composition in liver cell membranes and evaluated whether perftoran, a perfluorocarbon acting as a highly efficient sorbent with gas-transporting, hepatoprotective, and membrane-stabilizing properties [1] can be used for the correction of structural and metabolic changes in the liver after long-term atherogenic diet (ATD).

MATERIALS AND METHODS

Experiments were performed on 14 male rabbits weighing 3.0±0.2 kg and kept in a vivarium under standard conditions. Control animals fed a standard diet and received grated carrot in a daily amount of 100 g for 2 months. Two experimental groups received carrot with cholesterol in a dose of 0.3 g/kg. Group 1 animals received only ATD. Group 2 animals fed ATD and were treated with perftoran in a dose of 4 ml/kg. Perftoran was administered 8 times over the 2nd month of ATD at 3-day intervals. Samples were taken after 2-month ATD under Nembutal anesthesia. Plasma membranes of liver cells were isolated [4]. Total lipids were extracted by the method of Folch. PL were fractionated by thin-layer chromatography on Silufol UV-254 plates using a system of solvents containing chloroform, methanol, and 7 N ammonia (12.4:4.6:1.0 v/v) [2]. Chromatograms were subjected to densitometry on a Chromoscan-201 device (Joyce-Loebl). Densitograms were analyzed on a Leitz-A.S.M. semiautomatic image scanner. PL content was expressed in percents of the total peak area in densitograms. A morphological study of the liver was performed on histological sections (7-10 μ). The sections were fixed with 10% acid formalin, embedded in paraffin, and stained with azure-2-eosin (Romanovsky technique)

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow

0007-4888/03/1364-0340$25.00

and by the method of Van-Gieson. In addition to this, we examined frozen slices stained by the method of Goldman. The severity of atherosclerosis was determined by the index of atherosclerotic damage (IAD) in the aorta proposed by Avtandilov. The results were analyzed by Student's *t* test.

RESULTS

In rabbits feeding ATD for 2 months aortic IAD was 10% (Table 1). Examination of the liver revealed various stages of disorganization in hepatic trabeculae, eosinophilia, perivascular and periductal microfocal mononuclear lymphocytic infiltrates, and tissue edema. Terminal regions of portal and hepatic veins and sinusoidal capillaries were enlarged and filled with hyperlipidemic plasma and erythrocyte aggregates. Signs of granular, fatty, and vacuolar degeneration were observed in hepatocytes. The composition of PL in the plasma membranes of liver cells underwent considerable changes. The contents of phosphatidic acid and phosphatidylserine increased by 3.0 ($p<0.01$) and 2.8 times ($p<0.05$), respectively. The amount of phosphatidylcholine decreased by 26.7% ($p<0.05$).

Administration of perftoran to rabbits feeding ATD normalized the contents of PL in the plasma membranes of liver cells. In animals receiving perftoran the amount of phosphatidylserine, phosphatidic acid, and phosphatidylcholine did not differ from the control. The study of structural characteristics of the liver revealed recovery of architectonics in hepatic trabeculae, decrease in the severity of granular and fatty degeneration in hepatocytes, and increase in the count of binuclear cells. We observed pronounced phagocytic reaction of macrophages. The degree of mononuclear infiltration in the liver tissue decreased.

Individual peculiarities in the influence of perforant on structural and metabolic changes in the liver were revealed. Depending on IAD of the aorta, perftoran-treated rabbits were divided into 2 groups. In group 1 animals aortic IAD was 3.5 times lower than in untreated rabbits (2-5%, $p<0.01$). Aortic IAD in group 2 animals varied from 10 to 40% and did not differ from that in untreated rabbits. Perftoran produced a strong antiatherogenic effect and normalized structural changes in the liver produced by ATD in group 1 rabbits. These animals displayed more pronounced phagocytic reaction of macrophages without mononuclear infiltration of the liver tissue and with minimum eosinophilia. These data indicate that perftoran more efficiently prevented the development of autoimmune inflammation in group 1 rabbits receiving ATD. The intergroup differences were revealed in the lipid bilayer of plasma membranes in liver cells. In group 2 rabbits with high aortic IAD phosphatidylinositol content surpassed that in group 1 and control animals by 1.9 ($p<0.05$) and 2.1 times ($p<0.05$), respectively.

Our results illustrate profound structural and metabolic changes in the liver of rabbits feeding ATD for 2 months. The study of PL composition of membranes in liver cells revealed increased content of phosphatidic acid and decrease in phosphatidylcholine concentration. These changes reflect intensification of metabolic processes regulated by phospholipase D [8]. It should be emphasized that the release of phosphatidic acid from phosphatidylcholine during activation of phospholipase D is a key transmembrane pathway of cell signaling. Phosphatidic acid formed during stimulation of cells acts as a messenger and regulates activities of kinases, phospholipases, and low-molecular-weight G proteins [7]. Phosphatidic acid stimulates superoxide generation [7], initiates Ca^{2+} mobilization from intracellular stores, and increases its intracellular concentration [11]. Published data show that phospholipase D in cell membranes is activated by receptor-dependent signals of various agonists [8]. Among these agonists, growth factors play a particular role in the pathogenesis of atherosclerosis [5,8]. Previous studies showed that the sensitivity of liver cells to growth factors increases after consumption of excess cholesterol [5].

It should be emphasized that structural changes in the PL bilayer of liver cells in rabbits feeding ATD include a considerable increase in the ratio of phosphatidylserine in plasma membranes. Phosphatidylserine plays a major role in the mechanisms of action of phosphatidylserine-dependent scavenger receptors responsible for recognition and removal of apoptotic cells, modified or oxidized lipoproteins, and membrane fragments [14]. These receptors are localized on the surface of hepatocytes, Kupffer cells, and endothelial cells of the liver [10]and their activity is important for the maintenance of homeostasis in the organism. Asymmetry of PL in the cell membrane serves as a signal for recognition by scavenger receptors and removal from the organism [14]. Phosphatidylserine is transferred from the inner to the outer surface of membranes and initiates binding of abnormal cells to scavenger receptors. It can be suggested that enrichment of plasma membranes in rabbit liver cells with phosphatidylserine after long-term of ATD is related to the increase in scavenger receptor occupancy. These changes make difficult elimination of damaged cells and modified lipoproteins from the blood, which induces inflammatory processes in the organism. It is important that phosphatidylserine is a predominant activator of protein kinase C [13]. Therefore, activity of this enzyme can increase in plasma membranes of liver cells during alimentary dyslipide-

TABLE 1. Effect of Perftoran on Phospholipid Composition of Liver Cells and Aortic IAD in Rabbits Feeding ATD (*M*±*m*, %)

Parameter	Control (*n*=3)	ATD (*n*=3)	ATD and perftoran	
			group 1 (*n*=4)	group 2 (*n*=4)
Aortic IAD	0	10.8±0.9*	3.1±0.7+	25.5±6.4o
Phospholipids				
phosphatidic acid	2.0±0.2	6.0±0.3*	4.2±1.4	4.1±0.9
phosphatidylethanolamine	31.2±2.7	27.0±1.6	22.6±2.9	24.8±1.5
phosphatidylcholine	43.5±2.2	31.9±3.2	46.0±3.8+	38.7±1.9
phosphatidylinositol	9.5±3.1	15.1±2.7	13.4±2.0	20.4±1.1*o
sphingomyelin	3.8±0.2	3.5±0.7	3.8±0.2	4.4±0.9
lysophosphatidylethanolamine	2.3±0.8	1.6±0.6	1.9±0.4	1.5±0.2
phosphatidylserine	3.9±1.0	11.0±1.9*	4.8±1.8	3.7±0.9+
lysophosphatidylcholine	1.3±0.2	2.3±0.6	1.9±0.5	1.3±0.1
lysophosphatidylserine	2.4±0.8	1.8±0.2	1.8±0.7	1.1±0.2

Note. Number of animals is shown in brackets. $p<0.05$: *compared to the control; +compared to ATD group (untreated animals); ocompared to group 1 treated animals (ATD and perftoran).

mia. Published data show that activation of protein kinase can accompany intensification of lipid peroxidation (LPO) [15]. Exhaustion of phosphatidylcholine reserves inhibiting LPO can impair antioxidant defense in liver cell membranes [15].

Perftoran protected PL bilayer in plasma membranes of liver cells in rabbits feeding ATD. This compound completely normalized the content of PL that serve as the targets for atherogenic modification (phosphatidylserine, phosphatidic acid, and phosphatidylcholine). Perftoran produced a strong antiatherogenic effect on rabbits with aortic IAD of 2-5%. In these animals the PL composition of liver cell membranes corresponded to normal. In rabbits receiving perftoran and having high aortic IAD (10-40%) the content of phosphatidylinositol in plasma membranes of liver cells markedly surpassed the control. The influence of phosphatidylinositol-3-kinase on metabolic activity of insulin is of particular interest in this respect. The resistance to insulin is one of the risk factors for atherosclerosis. Phosphatidylinositol-3-kinase inhibitors block the effects of insulin (*e.g.*, stimulation of glucose and glycogen transport and lipid synthesis) [12] and abolish an insulin-dependent increase in viability of apoptotic macrophages [9]. Our results probably reflect inhibition of phosphatidylinositol phosphorylation in liver cell membranes of rats with aortic IAD of 10-40%, which causes considerable pathogenetic consequences. Previous studies showed that incorporation of cholesterol into cell membranes can inactivate its integral proteins [6]. Therefore, the protective mechanisms underlying removal of cholesterol excess from the organism play a role in the individual resistance to ATD.

Thus, experiments on rabbits with aortic IAD <10% and resistant to the development of atherosclerosis [3] revealed changes in the PL composition of liver cell membranes produced by variations in the contents of phosphatidylserine, phosphatidic acid, and phosphatidylcholine. Perftoran completely prevented the development of these changes, probably due to sorption of cholesterol and inhibition of its accumulation in cell membranes. In rabbits with aortic IAD corresponding to that in untreated animals predisposed to atherosclerosis [3] perftoran produced a less pronounced hepatoprotective effect. This can be explained by genetically determined decrease in compensatory properties of the organism (*e.g.*, low activity of scavenger receptors in liver cells).

Our results indicate that metabolic disturbances in liver cell membranes play a pathogenetic role during alimentary atherosclerosis. Perftoran produces a hepatoprotective effect under these conditions. It should be emphasized that perftoran induces various changes, which is related to differences in the individual resistance to atherogenic factors.

REFERENCES

1. A. M. Golubev, *Byull. Eksp. Biol. Med.*, **125**, No. 5, 484-491 (1998).
2. A. V. Kargapolov and S. V. Kartsova, *Vopr. Med. Khimii*, **21**, No. 3, 325-327 (1975).
3. L. P. Kobozeva, E. D. Klimenko, A. B. Michunskaya, and O. M. Pozdnyakov, *Byull. Eksp. Biol. Med.*, **132**, No. 10, 378-381 (2001).
4. A. V. Pospelova, *Modern Biochemical Methods* [in Russian], Moscow (1977), pp. 326-329.

5. G. Baccante, G. Mincione, and C. D. Febbo, *Atherosclerosis*, **152**, 51-57 (2000).
6. M. M. Cleason, M. S. Medow, and T. N. Tulenko, *Circ. Res*., **69**, 216-227 (1999).
7. D. English, *Cell Signal*., **8**, 341-347 (1996).
8. Y. Humean, N. Vitale, and S. Chasserot-Golaz, *Proc. Natl. Acad. Sci. USA*, **98**, 15,300-15,305 (2001).
9. K. T. Iida, H. Suzuki, H. Sone, *et al*., *Arterioscler. Thromb. Vasc. Biol*., **22**, 380-386 (2002).
10. L. Malerod, K. Juvet, T. Gjoen, *et al*., *Cell Tissue Res*., **307**, 173-180 (2002).
11. G. Pelassy, J. P. Breittmayer, and C. J. Mary, *Lipid. Mediat*., **4**, 199-209 (1991).
12. A. R. Saltiel and C. R. Kahn, *Nature*, **414**, 799-806 (2001).
13. B. Sturbois-Bakcerzak, S. J. Stone, A. Sreenivas, *et al*., *J. Biol. Chem*., **276**, 8205-8212 (2001).
14. V. Terpsta and T. J. C. van Berkel, *Blood*, **95**, 2157-2163 (2000).
15. K. Yoshida and V. Mohserin, *Life Sci*., **49**, 1359-1365 (1991).