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Use of Phosphatidylcholine Liposomes for Correction of Mitochondrial Phospholipid Composition in the Medulla Oblongata and Frontal Lobes in Hemorrhagic Shock

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Phosphatidylcholine liposomes normalize phosphatidylcholine and lysophospholipid levels in mitochondrial membranes of the medulla oblongata of cats with hemorrhagic shock. In the frontal lobes of brain hemispheres of liposome-treated cats, phospholipid levels in the mitochondria and their membranes are close to the norm, with the exception of phosphatidylserine and lysophosphatidylserine: their contents in the inner mitochondrial membranes remain below the control. It is concluded that phosphatidylcholine liposomes exert protect brain mitochondrial membranes in hemorrhagic shock.

Key Words: *phospholipids; mitochondria; brain; hemorrhagic shock; liposomes*

Hemorrhagic shock involving long-lasting pronounced hypotension is accompanied by impaired cerebral circulation and brain hypoperfusion and hypoxia. Of particular significance in such situations is the damage sustained by mitochondrial membranes that are the principal site of energy generation. Impaired metabolism of membrane phospholipids is associated with altered energy generation in the mitochondria [1,13]. It was shown [4,5] that phosphatidylcholine (PCh) liposomes, which inhibit lipid peroxidation [2], exert an appreciable membrane-protecting effect in hepatocytes of cats subjected to hemorrhagic shock. The purpose of the present study was to explore the possibility of using PCh liposomes for correction of the phospholipid composition of mitochondrial membranes in two different parts of the brain in hemorrhagic shock.

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MATERIALS AND METHODS

The study was performed on 17 cats (body weight 3.0±0.5 kg) under Nembutal anesthesia (40 mg/kg intraperitoneally). Hemorrhagic shock was produced by the method of Wiggers—Fine [17]. In order to prevent blood coagulation in catheters the cats were

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injected with 2000 U/kg heparin. Thirty minutes after the injection, they were bled until blood pressure dropped to 40 mm Hg; it was maintained at this level for 60 min. Intact cats given same dose of heparin served as controls.

Liposomes were prepared from dry soybean PCh powder (Serva) (0.5 mg/ml distilled water) as described previously [13] and injected intravenously in a dose of 1 ml/kg body weight 30 min after the start of bleeding. The cats were sacrificed 120 min after the start of anesthesia. Mitochondria were isolated from the medulla oblongata and the frontal lobes of cerebral hemispheres by the method [12] in a medium containing 0.32 M sucrose, 10 mM Tris-HCl (pH 7.4), and 1 mM EDTA [9]. The outer and the inner mitochondrial membranes were isolated as described [11]. After extraction of total lipids from the mitochondria and their membranes [14], phospholipids were fractionated by thin-layer chromatography on Silufol UV-254 plates (Cavalier) in a chloroform: methanol:7 N ammonia mixture (12.4:4.6:10 v/v) [3]. Chromatograms were processed in a Chromoscan-201 (Joyce-Loebl) densitometer and semiautomatic image analyzer (Zeitz-A.S.M.). Statistical analysis was performed using Student's t test.

RESULTS

In cats subjected to hemorrhagic shock, the PCh level in bulbar mitochondrial membranes was 2.2 times lower than in the control cats given heparin ($p<0.05$-0.01) (Table 1). On the other hand, these membranes accumulated large amounts of lysophospholipids (Table 1); thus, the level of lysophosphatidylcholine (LPCh) rose 5.2-fold in the outer membranes and 3.0-fold in the inner membranes ($p<0.05$), while that of lysophosphatidylserine (LPS) rose 4- and 2-fold, respectively ($p<0.05$-0.01); a 2.2-fold rise of lysophosphatidylethanolamine (LPE) was observed in the inner membranes ($p<0.05$). Generally, alterations of phospholipid levels in whole bulbar mitochondria were similar to those in their outer and inner membranes, although less pronounced: the PCh level decreased by 46.5% ($p<0.05$), while the increase in the lysophospholipid content was due to the rise in LPE and LPS (2.3 and 4.6 times, respectively; $p<0.02$).

In the frontal lobes of cats with hemorrhagic shock, the phosphatidylinositol (PI) level decreased by 30% ($p<0.05$) in whole mitochondria and by 42.7% in the outer membranes ($p<0.02$), while the phosphatidylethanolamine (PE) level decreased by 32% in the inner membranes ($p<0.02$) (Table 2). Lysophospholipids were accumulated in the frontal lobe mitochondria as well as in the medulla oblongata

TABLE 1. Phospholipid Levels (%) in Bulbar Mitochondria and Their Membranes from Cats with Hemorrhagic Shock ($M\pm m$)

Group	Phosphatidic acid	Cardiolipin	Phosphatidylethanolamine	Phosphatidylcholine	Phosphatidylinositol	Sphingomyelin	Lysophosphatidylethanolamine	Phosphatidylserine	Lysophosphatidylcholine	Lysophosphatidylserine
Mitochondria										
Control (6)	5.1±1.1	5.7±1.0	27.6±3.5	32.9±4.1	17.2±1.8	3.6±0.7	2.1±0.5	3.2±0.6	1.5±0.3	0.7±0.2
Shock (4)	9.4±3.5	8.1±1.8	34.8±7.8	17.6±5.1*	11.2±3.4	4.0±1.4	4.8±0.8*	4.9±1.2	6.1±2.8	3.2±0.9*
Liposomes (4)	4.3±1.6	3.2±0.7	19.4±1.1	43.8±0.8**	21.0±3.0	3.4±1.4	1.6±0.4**	1.7±0.4**	0.6±0.2	0.8±0.2**
Outer membranes										
Control (5)	7.4±2.1	—	28.8±3.3	38.6±4.1	11.4±1.8	5.8±1.0	2.4±0.8	3.8±0.4	2.1±0.6	1.3±0.3
Shock (5)	7.5±2.6	—	26.9±2.6	17.4±1.2*	13.1±2.1	6.6±1.8	6.4±1.8	6.4±1.1	11.0±3.1*	5.2±1.4*
Liposomes (4)	5.1±0.6	—	34.4±2.8	25.0±2.8**	18.3±2.0	5.6±1.0	2.6±0.4	5.0±1.1	2.6±0.8**	1.8±0.4
Inner membranes										
Control (4)	5.0±1.2	3.0±0.5	33.8±4.4	26.1±4.8	13.5±3.0	3.0±0.8	3.0±0.6	7.5±2.1	2.4±0.6	2.2±0.4
Shock (4)	13.1±2.4*	4.9±1.3	28.6±1.4	11.3±1.1*	11.6±2.0	5.3±1.1	6.6±1.2*	6.1±1.2	7.2±1.8*	4.6±0.7*
Liposomes (4)	2.7±0.4**	1.7±0.3**	32.7±4.2	36.5±3.5**	14.6±2.1	2.8±0.2	1.9±0.6**	3.7±1.0	1.7±0.3**	1.8±0.4**

Note. Here and in Table 2: *significantly different from the control value (heparin-treated intact cats), **significantly different from the value in cats with hemorrhagic shock not treated with liposomes. The number of animals is given in parentheses.

TABLE 2. Phospholipid Levels (%) in Frontal Lobe Mitochondria and Their Membranes from Cats with Hemorrhagic Shock (M±m)

Group	Phosphatidic acid	Cardiolipin	Phosphatidylethanolamine	Phosphatidylcholine	Phosphatidylinositol	Sphingomyelin	Lysophosphatidylethanolamine	Phosphatidylserine	Lysophosphatidylcholine	Lysophosphatidylserine
Mitochondria										
Control (4)	5.4±2.5	4.6±1.2	30.0±3.1	28.6±3.0	17.0±1.4	2.5±0.3	2.3±0.9	5.3±1.5	2.1±0.3	0.8±0.2
Shock (4)	12.2±3.8	5.0±0.4	33.8±4.4	21.0±2.7	11.9±1.4*	5.2±1.4	2.7±1.5	3.7±1.1	3.2±0.8	1.9±1.1
Liposomes (4)	2.5±1.6	1.6±0.4	37.5±3.4	29.9±3.2	19.0±1.7**	3.4±1.7	1.5±0.4	2.0±0.6	1.4±0.7	1.5±0.6
Outer membranes										
Control (4)	6.1±1.7	—	28.8±4.3	28.0±5.2	21.3±2.3	6.2±2.4	2.2±0.5	3.4±0.6	1.7±0.4	2.0±0.1
Shock (6)	9.2±0.9	—	31.2±0.6	25.4±1.8	12.2±1.8*	6.6±1.6	4.1±0.5*	4.9±1.3	5.6±0.7*	2.8±0.4
Liposomes (4)	2.0±0.4**	—	33.7±3.7	35.2±2.0**	19.1±1.0**	3.1±0.4	2.8±0.7	2.3±0.4	1.8±0.6**	0.6±0.2*
Inner membranes										
Control (4)	7.5±1.4	6.6±1.4	37.2±2.1	25.4±0.6	8.5±1.2	2.8±0.4	3.4±0.4	5.0±0.1	2.2±0.4	1.4±0.2
Shock (4)	5.4±2.3	4.4±1.4	25.3±2.9*	19.6±3.4	10.8±2.6	2.6±1.0	4.6±1.8	6.9±2.6	13.7±3.8*	5.9±2.4
Liposomes (5)	4.2±0.7	3.2±0.6	40.6±3.7**	26.0±1.9	12.5±1.2	4.0±0.6	2.7±0.5	3.0±0.2**	2.0±0.6**	1.9±0.8

mitochondria: LPCh rose 3.3 times in the outer membranes ($p<0.01$) and 6.2 times in the inner ($p< 0.02$), while LPE rose 1.9 times ($p<0.05$) compared with the control.

In cats treated with PCh liposomes, the PCh level in bulbar mitochondria was 2.5 times higher than in untreated cats with hemorrhagic shock ($p< 0.01$) and 33.1% higher than in the control ($p<0.05$). In the outer membranes of these mitochondria, the PCh level was 43.7% higher than in untreated cats ($p<0.05$) and 35.2% lower than in control cats ($p< 0.05$). In the inner mitochondrial membranes of liposome-treated cats, the level of this phospholipid was completely restored, while the level of phosphatidic acid decreased to the normal value. Furthermore, the LPE and LPS contents in bulbar mitochondria of liposome-treated cats were lower (3- and 4-fold, $p<0.02$ and $p<0.05$, respectively) than in untreated animals. The lysophospholipid content in the membranes of bulbar mitochondria from liposome-treated cats was close to those recorded in the control group.

In the mitochondria from frontal lobes of liposome-treated cats, PI rose to the normal level (being 59.7% higher than in the untreated group; $p<0.02$), which was accompanied by PI normalization in the outer membranes. The PE level in the inner membranes was 60.5% higher than untreated cats ($p< 0.02$). The phosphatidylserine levels in the mitochondria and their membranes were much lower than in untreated and control (by 40%, $p<0.001$) groups, suggesting an enhanced decarboxylation of this phospholipid with subsequent formation of phosphatidylethanolamine. Treatment with PCh liposomes also lowered mitochondrial levels of lysophospholipids in the frontal lobes. Normalization of LPCh was observed both in the inner and the outer membranes of the frontal lobe mitochondria. In the outer membranes, the LPE level was close to the control, while that of LPS was 3.3-fold lower ($p<0.001$).

These results indicate that changes in the mitochondrial phospholipid composition in the medulla oblongata of cats with hemorrhagic shock are more pronounced than in the frontal lobe, primarily due to a greater decrease in the mitochondrial membrane PCh level. This supports the concept that choline-containing neuronal phospholipids are utilized for the synthesis of acetylcholine when the demand for its precursor is increased [8], which results in damage to cellular membranes, impaired cell function, and cell death [7]. PCh liposomes administered to cats with hemorrhagic shock raised PCh levels in the bulbar mitochondria and completely restored them in the inner membranes. This finding indicates that PCh liposomes partially compensate the increased demand for the acetylcholine precursor in hem-

orrhagic shock. The decrease to near-control values of phosphatidic acid in the inner membranes of these mitochondria can be interpreted as a consequence of diminished PCh breakdown.

Restoration of the PI content in the frontal lobes is an important beneficial effect produced by PCh liposomes in hemorrhagic shock. PI metabolites play an important role in brain functioning [10], participating in the regulation of neurochemical processes associated with neurotransmission [6,16]. During hemorrhagic shock, PI of the outer mitochondrial membranes in cortical neurons is probably used to replenish the intracellular stores of second messengers, and PCh liposomes are likely to reduce the demand for the major PI metabolites. Concerning the role of PE in the regulation of membrane permeability for Ca^{2+} [18], the rise to near-control values of this phospholipid in the inner mitochondrial membranes of liposome-treated cats with hemorrhagic shock may promote the restitution of intracellular ionic homeostasis. A considerable contribution to this process is undoubtedly made by the concurrent normalization of mitochondrial membrane lysophospholipids in view of their important role in the regulation of transmembrane ion transport [15].

The use of PCh liposomes in cats with hemorrhagic shock prevents the phospholipid metabolism disorders in brain mitochondrial membranes, thus promoting normalization of metabolic processes in its neurons.

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