Protective Effect of Phosphatidylcholine Liposomes in Cats with Hemorrhagic Shock

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Experiments on cats indicated that the use of phosphatidylcholine liposomes in hemorrhagic shock may reduce the intensity of free-radical processes in the liver, stabilize the phospholipid bilayer of plasma membranes in hepatocytes, decrease the severity of pathomorphological changes in the target organs, and raise systemic arterial pressure with its stabilization at a subnormal level. The use of phosphatidylcholine liposomes in cats with hemorrhagic shock resulted in a considerable prolongation of their survival.

Key Words: *hemorrhagic shock; phosphatidylcholine; liposomes*

A major pathogenic factor in all forms of shock is hypoperfusion of organs and tissues leading to cell starvation and inadequate removal of metabolic products [8], with consequent disturbance of cellular functions accompanied by damage to cell membranes. Structural changes in the lipid bilayer of these as a result of intensified lipid peroxidation (LPO) are regarded as one of the causes of cell damage [2,3,14]. Our previous studies showed that hemorrhagic shock is associated with drastically reduced phosphatidylcholine (PC) levels in the plasma membranes of hepatocytes [5]. Given that PC exhibits antioxidant properties [1,4], an attempt was made in the present study to diminish the activation of free-radical processes in the liver by enriching hepatocyte plasma membranes with PC administered in the form of liposomes. PC liposomes were chosen because the liver is known to be the main target organ for liposomes [6], whose lipids become incorporated into cell membranes [11]. In addition, we explored how PC liposomes might influence the time course of arterial pressure in cats with hemorrhagic shock and their mortality and evaluated the morphological status of organs in these animals.

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MATERIALS AND METHODS

The study was conducted on 48 cats of both sexes (body weight 3±0.5 kg) under Nembutal anesthesia (40 mg/kg intraperitoneally). Shortly (30 min) before they were bled, the cats received an intravenous heparin injection (2000 units/kg) to prevent thrombus formation. Liposomes were prepared as previously described [9] from dry soybean PC powder (Serva) (0.5 mg powder per ml of distilled water) and injected intravenously in a dose of 1 ml/kg.

The study consisted of three parts. In part I, liposomes were tested for their effects on LPO levels in the liver and the phospholipid composition of hepatocyte plasma membranes in cats with hemorrhagic shock. These cats were divided into three groups. Group 1 consisted of intact cats injected with heparin in the indicated dose (controls). Group 2 comprised cats in which hemorrhagic shock was produced over 90 min: arterial pressure was reduced to 40 mm Hg for 30 min and then maintained at this level for 60 min. In group 3, hemorrhagic shock was produced as above but liposomes were administered 30 min after the

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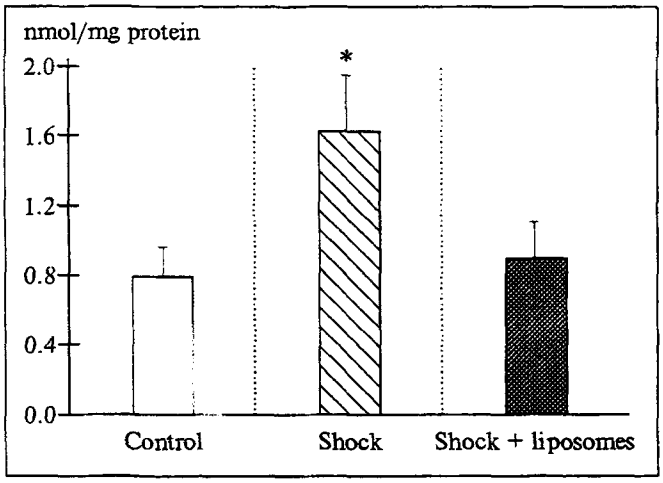

Fig. 1. Effect of liposomes on the level of TBA-reactive products in the livers of cats with hemorrhagic shock. Each of the three groups consisted of 5 cats. *$p<0.01$ in comparison with the control group.

start of bloodletting. Two hours after heparin injection, cats were killed and the liver was removed after perfusion with a cooled 1 mM sodium bicarbonate solution. The level of 2-thiobarbituric acid (TBA)-reactive products in the liver was measured as described [10]. After isolation of hepatocyte plasma membranes [7] and extraction of their total lipids [12], phospholipids were fractionated by thin-layer chromatography on Silufol plates in the solvent system chloroform:methanol:acetic acid:water (25:15:4:2 by volume) [13]. The chromatograms were subjected to densitometry in a Chromoscan-201 apparatus (Joyce-Loebl). The densitograms were read using a semiautomatic image analyzer (Leitz-A.S.M.).

In parts II and III of the study, hemorrhagic shock was produced over 120 min: arterial pressure was maintained at 40 mm Hg for 90 min, after which the communication between the vascular bed and the reservoir used for bloodletting was interrupted. In part II, liposomes were tested for their effects on the temporal profile of arterial pressure and on mortality of the cats. These were divided into two groups. One group consisted of cats with hemorrhagic shock and the other of animals with hemorrhagic shock but injected with liposomes 120 min after the start of bleeding. Part III was devoted to histological examination of the organs taken from three groups of cats with hemorrhagic shock: untreated cats that had died from the shock (group 1), liposome-treated cats injected with a lethal dose of Nembutal 70 min after liposome administration, i.e., after an interval equal to the mean survival time of untreated animals; and liposome-treated cats killed with Nembutal 8 h after liposome administration, i.e., after an interval equal to the mean survival time of treated animals. Material for histological examination was collected after complete systemic dissection by Shor's method. Histological specimens of lung, liver, spleen, kidney, and heart were prepared (frozen sections stained with hematoxylin-eosin by Goldman's method and with picrofuchsin-fuchsilene after Hart). The results were treated statistically using Student's t test.

RESULTS

Ninety minutes after the start of bleeding, the level of TBA-active products in the liver of cats with hemorrhagic shock exceeded 2-fold the control value ($p<0.01$) (Fig. 1), while the PC level in their hepatocyte plasma membranes had decreased 3.2-fold ($p<0.01$) in direct proportion to the increase in LPO ($r=0.79$) (Fig. 2). The phosphatidylinositol level in these membranes also depended on that of LPO: 90 min after the start of bleeding, it had risen significantly to exceed the control value 6.1-fold ($p<0.01$) and the increase correlated directly with that in LPO ($r=0.72$).

The level of TBA-reactive products in the liver of cats treated with PC liposomes 30 min after the start of bleeding was 1.8 times lower than in the

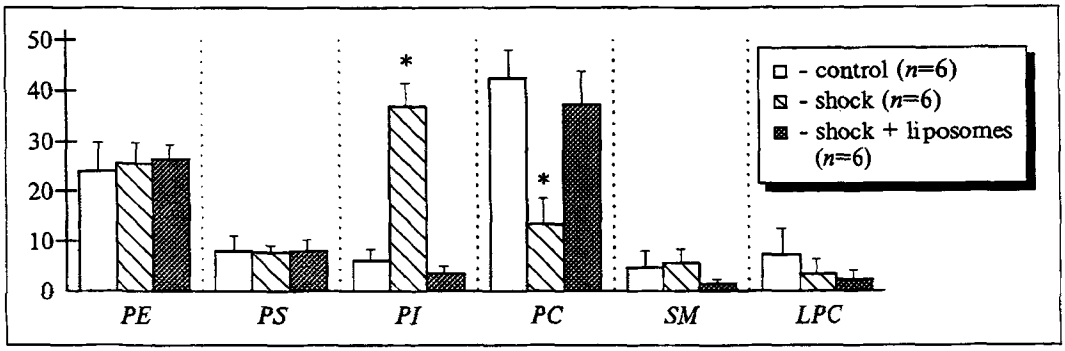

Fig. 2. Effect of liposomes on the percentage composition of phospholipids in hepatocyte plasma membranes of cats with hemorrhagic shock. *PE* = phosphatidylethanolamine; *PS* = phosphatidylserine; *PI* = phosphatidylinositol; *PC* = phosphatidylcholine; *SM* = sphyngomyelin; *LPC* = lysophosphatidylcholine. *$p<0.01$ in comparison with the control cats.

liver of untreated animals with hemorrhagic shock ($p<0.01$) and was only slightly above the control level (Fig. 1), while the PC level in the hepatocyte plasma membranes of these cats increased to approach the control value (Fig. 2), and the increase correlated with LPO depression in the liver ($r=0.67$). Phosphatidylinositol was also close to the control value, and the decrease in its level correlated directly with that in LPO ($r=0.75$).

In cats injected with liposomes 120 min after the start of bleeding, arterial blood pressure rose, on average, to 70 mm Hg over 20-30 min (Fig. 3) to remain unchanged throughout the 4-h observation period. The mean survival time of these cats was 8 h. In contrast, the untreated hypotensive cats showed a progressive fall in arterial pressure, and their mean survival time was only 70 min.

Lung specimens from untreated cats (Fig. 4, *a*) contained areas of lysed epithelium in alveolar and bronchiolar walls; perivascular and peribronchial stromata were edematous and infiltrated with granulocytes, and erythrodiapedesis, erythrostasis, and leukostasis were prominent in capillaries and venules. Manifestations of edema were less conspicuous in the lungs of cats bled for 120 min and killed 70 min after being injected with liposomes (Fig. 4, *b*). Venular stasis and macrophages in alveolar lumens were observed, and the so-called "broad" capillaries appeared congested. Specimens from cats killed 8 h after liposome injection contained serous hemorrhagic exudates in alveolar lumens.

Liver specimens from untreated cats with hemorrhagic shock (Fig. 4, *c*) contained empty venules and sinusoids and showed clear signs of fatty and vacuolar cytoplasmic degeneration. Liver specimens from cats killed 70 min and 8 h after liposome treatment presented similar appearances: no signs of fatty or vascular degeneration were in evidence, formed elements of blood were seen in sinusoid lumens, and microvessel walls seemed less edematous (Fig. 4, *d*). However, there was some evidence suggesting dissociation of hepatic lobules, the stroma was infiltrated with lymphoid cells in places, and signs of hepatocyte disintegration were occasionally present.

In spleen specimens from untreated cats, the red pulp was of decreased size and depleted of erythrocytes and the reactive sites of lymphatic follicles were dilated. The red pulp in specimens from liposome-treated cats was considerably larger, suggesting augmented utilization function of the spleen vis-a-vis erythrocytes that underwent pathological changes. The histological appearance of specimens from cats of both groups was similar.

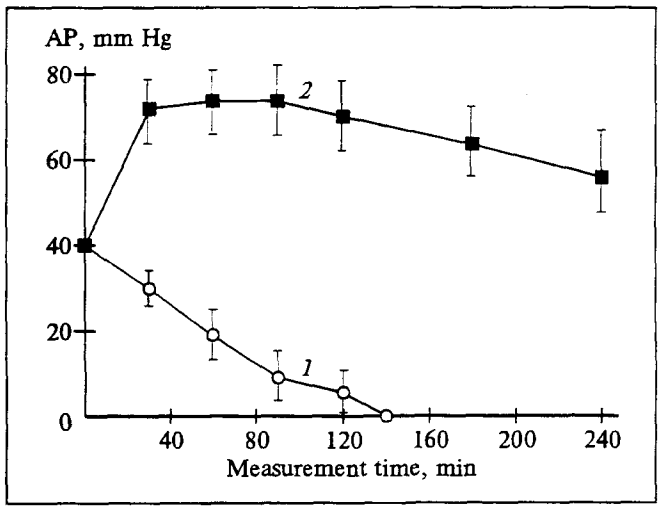

Fig. 3. Effect of liposomes on the time course of arterial pressure (AP) in cats with hemorrhagic shock. *1*) untreated cats ($n=10$); *2*) cats treated with liposomes ($n=11$).

In kidney specimens from both untreated and liposome-treated cats, gross destructive changes were observed in the glomeruli and tubular epithelium. Similarly, liposome treatment failed to improve the histological appearance of heart specimens, which contained multiple microhemorrhages and lesions in the walls of intramural arteries.

Thus, as the findings presented above indicate, the use of PC liposomes in cats early during hemorrhagic shock development largely prevents the intensification of LPO in the liver and alterations in the phospholipid profile of hepatocyte plasma membranes. These effects appear to be associated with antioxidant properties of the PC entering the liver [1,4]. An important effect from the use of PC liposomes in a late stage of hemorrhagic shock is arterial pressure elevation to a subnormal level and its prolonged maintenance at that level, indicating that the hemodynamics is partially restored, which undoubtedly results in improved perfusion of organs and tissues and thus their better supply with oxygen and nutrients. However, the liposome treatment of cats with hemorrhagic shock was found to improve the condition of only the organs that are targets for liposomes (liver, lung, and spleen) without producing any beneficial effects in other organs such as the kidney and heart.

To summarize, the administration of PC liposomes to cats with hemorrhagic shock reduced the intensity of free-radical processes in the liver, stabilized the phospholipid bilayer of hepatocyte plasma membranes, mitigated histopathological changes in the target organs, and elevated systemic arterial pressure to a subnormal level, at which it remained for a relatively long period of time. As

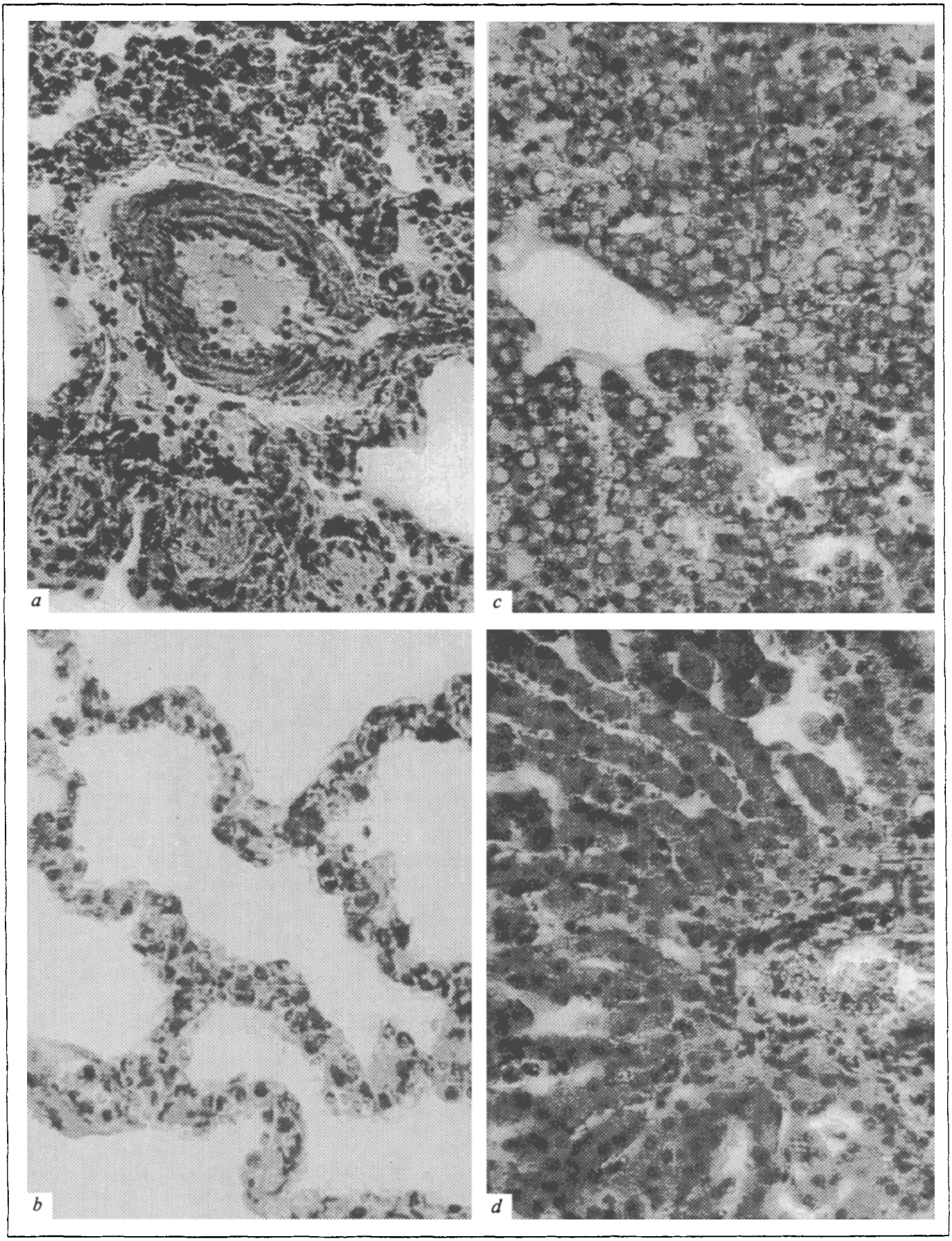
a
c
b
d

Fig. 4. Pathomorphological appearance of lung and liver specimens from an untreated (*a* and *c*) and a liposome-treated (*b* and *d*) cat with hemorrhagic shock. ×160. *a*) lung specimen showing an edematous periarterial stroma infiltrated with granulocytes, edematous alveolar walls, and erythrocyte diapedesis and leukostasis in the capillaries. *b*) lung specimen taken 70 min after inoculating the cat with liposomes: signs of generalized edema are less prominent than in *a* and erythrocyte aggregates are absent. *c*) liver specimen showing fatty and vacuolar degeneration; the central veins and sinusoids appear empty. *d*) liver specimen taken 70 min after inoculating the cat with liposomes: there is no evidence of fatty or vacuolar degeneration, and erythrocytes can be seen in the lumens of sinusoids and veins; microvessel walls are less edematous than in *c*.

a result, the liposome-treated cats survived much longer than the untreated animals.

The results of this study suggest that PC liposomes hold promise of being useful in the management of patients with shock and may therefore be considered for inclusion in multidrug therapies.

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