# PREVENTION OF POSTISCHEMIC MYOCARDIAL DAMAGE BY MEANS OF LIPOSOMES

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UDC 616.127-005.4-085-039.71

KEY WORDS: ischemia; reperfusion; lipid peroxidation

Problems concerning ischemic and reperfusion tissue damage and its prevention occupy an important place in modern biology and medicine. Experimental studies have shown that ischemia and reperfusion cause a cascade of intracellular changes, of which the most important are considered to be processes of free-radical oxidation [1, 9]. Their potentiation leads to a decrease in the total content of membrane phospholipids and to a significant change in their qualitative composition, and this leads to a disturbance of the barrier function of the membranes and to overloading of the intracellular structures with $Ca^{2+}$ [13]. In myocardial ischemia these disturbances are manifested as inhibition of the pumping and contractile function of the heart. Resumption of perfusion of the ischemic myocardium, an effective way of restoring the viability of cells, at the same time aggravates all membrane lesions if the duration of ischemia is considerable [11]. Under these circumstances a "no reflow" phenomenon develops, and this enhances the existing depression of the pumping and contractile function of the heart.

Unfortunately none of the preparations traditionally used to treat severe forms of IHD has sufficient ability to prevent destructive processes in cells. The writers showed previously that lecithin liposomes can inhibit LPO, maintain activity of enzymes of antioxidant protection during hypoxia of varied genesis, and also preserve a sufficiently high level of the principal high-energy compounds in the cells [6, 12]. It is also known that liposomes can interact with damaged cells, by fitting the necessary phospholipids into their membranes [8].

The aim of this investigation was to study the possibility of using liposomes to prevent experimental reperfusion damage to the myocardium.

## EXPERIMENTAL METHOD

Experiments were carried out on 10 adult mongrel dogs of both sexes weighing 25.8 ± 1.6 kg, anesthetized with chloralose and urethane (0.05 and 0.5 g/kg respectively). The preparatory operations and catheterization of the vessels were carried out as described previously [5]. The blood pressure in the left ventricle and its first derivative, the systemic arterial pressure (SAP) and pressure in the pulmonary artery (PAP) were recorded by means of No. 746 strain-gauge transducers (Sweden), the coronary blood flow (CBF) was recorded by electromagnetic flowmetry, and the cardiac output (CO) by transthoracic tetrapolar rheoplethysmography [10]. The parameters were recorded synchronously on an eight-channel Mingograf-82 polycardiograph. The total peripheral resistance (TPR), the resistance in the pulmonary vessels (PVR), Veragut's contractility index (VI) [14], the relaxation index (RI) [4], and the rigidity of the left ventricular myocardium (VR) [7] also were calculated. Myocardial ischemia was induced by limiting perfusion of one branch of the left coronary artery by 80% for 1 h, at the end of which time the limitation was abolished. All the animals were divided into two groups: 1) control, 2) experimental group of animals, which, at the end of 1 h immediately before removal of the limitation to perfusion, were given an intravenous injection of lecithin liposomes in a dose of 50 mg/kg. The method of obtaining the liposomes was described previously [6].

A. V. Palladin Institute of Biochemistry, Academy of Sciences of the Ukraine. A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukraine, Kiev. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 113, No. 6, pp. 590-593, June, 1992. Original article submitted November 20, 1991.

0007-4888/92/0006-0787$12.50 

TABLE 1. Changes in Parameters of Hemodynamics in Animals of Both Groups, during Myocardial Ischemia and Reperfusion

| Parameter | Statistical index | Initial value | End of ischemia | Group | Reperfusion, min | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 15 | 30 | 45 | 60 |
| SAP, mm Hg | *M* | 129,8 | 107,6 | 1 | 93,2 | 91,5 | 97,5 | 92,3 |
| | *m* | 2,2 | 5,3 | | 8,0 | 11,6 | 9,5 | 11,7 |
| | *p* | | <0,05 | | <0,05 | <0,05 | <0,05 | <0,05 |
| | *M* | | | 2- | 97,0 | 106,8 | 106,8 | 118,5 |
| | *m* | | | | 1,9 | 6,1 | 5,5 | 8,4 |
| | *p* | | | | <0,01 | <0,05 | <0,05 | >0,05 |
| | $p_1$ | | | | >0,05 | <0,05 | <0,05 | <0,05 |
| CO, ml/min | *M* | 2421 | 1655 | 1- | 1682 | 1684 | 1699 | 1525 |
| | *m* | 141 | 230 | | 182 | 179 | 137 | 122 |
| | *p* | | <0,05 | | <0,05 | <0,05 | <0,05 | <0,01 |
| | *M* | | | 2- | 2012 | 2029 | 1952 | 1923 |
| | *m* | | | | 168 | 201 | 147 | 238 |
| | *p* | | | | >0,05 | >0,05 | >0,05 | >0,05 |
| | $p_1$ | | | | >0,05 | <0,05 | <0,05 | <0,05 |
| rAP, mm Hg | *M* | 13,3 | 15,2 | 1- | 15,0 | 16,5 | 17,6 | 23,8 |
| | *m* | 0,8 | 1,8 | | 1,6 | 1,5 | 1,7 | 2,8 |
| | *p* | | <0,05 | | <0,05 | <0,05 | <0,05 | <0,01 |
| | *M* | | | 2- | 10,6 | 10,5 | 10,2 | 10,1 |
| | *m* | | | | 0,9 | 1,3 | 1,2 | 1,4 |
| | *p* | | | | >0,05 | >0,05 | >0,05 | >0,05 |
| | $p_1$ | | | | <0,05 | <0,01 | <0,01 | <0,001 |
| PVR, $mN \cdot sec \cdot m^{-7}$ | *M* | 483 | 903 | 1- | 755 | 897 | 992 | 1588 |
| | *m* | 61 | 91 | | 64 | 83 | 97 | 182 |
| | *p* | | <0,01 | | <0,01 | <0,01 | <0,01 | <0,001 |
| | *M* | | | 2- | 472 | 454 | 471 | 481 |
| | *m* | | | | 37 | 51 | 48 | 51 |
| | *p* | | | | >0,05 | >0,05 | >0,05 | >0,05 |
| | $p_1$ | | | | <0,01 | <0,01 | <0,01 | <0,001 |

**Legend.** p) Significance of differences compared with initial values; $p_1$) significance of differences between groups. Student's t calculated by the difference method.

## EXPERIMENTAL RESULTS

Limitation of perfusion of one branch of the left coronary artery by 80% for 1 h led to inhibition of myocardial contractility in the animals of both groups, and to depression of its pumping function (Tables 1 and 2). Myocardial ischemia was accompanied by a marked increase in PVR. Reperfusion of the ischemic myocardium in animals of the first group aggravated existing circulatory disturbances. Despite removal of the obstruction to perfusion of the left coronary artery, restoration of the blood flow in it was delayed. Not until the 30th minute did it amount to 70% of its initial values. Later it decreased, possibly due to development of the "no reflow" phenomenon (Tables 1 and 2). Values of VI and PVR continued to rise. As will be clear from Fig. 1, limitation of CBF and subsequent reperfusion cause a sharp decrease in the reaction of reactive hyperemia, evidence of impairment of the functional state of the coronary vascular endothelium [5].

In animals of the experimental group receiving liposomes by intravenous injection before restoration of the blood flow, reperfusion of the ischemic myocardium was not accompanied by such a marked change in activity of the cardiovascular system as in animals of the control group. It will be clear from Table 1 that levels of SAP and CO in animals of group 2 by the 60th minute of reperfusion were 28 and 26% higher and did not differ significantly from their initial values. Under these conditions, the contractile function of the myocardium also suffered less severely. Injection of liposomes prevented an increase in MR and the development of pulmonary hypertension. Almost complete recovery of the reaction of reactive hyperemia of the coronary vessels also took place (Fig. 1).

The results are evidence that injection of lecithin liposomes enables the contractile and pumping functions of the heart to be restored in the period of reperfusion and the hemodynamics to be maintained in a sufficiently stable condition. At the same time, the functional state of the coronary vascular endothelium improves, as is shown by restoration of reactive hyperemia. The results described above differ from those of some other studies [2, 3], possibly due to differences in the models used, the dosage of the preparation, and its mode of injection. However,

TABLE 2. Changes in Coronary Blood Flow and Myocardial Contractility in Animals of Both Groups during Ischemia and Reperfusion

| Parameter | Statistical index | Initial value | End of ischemia | Group | Reperfusion, min | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 15 | 30 | 45 | 60 |
| CBF, ml/min | *M* | 68,4 | 12,3 | 1- | 39,1 | 48,4 | 28,3 | 28,7 |
| | *m* | 6,2 | 1,2 | | 4,7 | 4,3 | 2,3 | 3,5 |
| | *p* | | <0,001 | | <0,01 | <0,05 | <0,001 | <0,001 |
| | *M* | | | 2- | 44,4 | 48,3 | 51,2 | 53,2 |
| | *m* | | | | 4,9 | 5,0 | 5,7 | 5,1 |
| | *p* | | | | <0,01 | <0,05 | >0,05 | >0,05 |
| | $p_1$ | | | | <0,05 | <0,05 | <0,01 | <0,01 |
| VI, $sec^{-1}$ | *M* | 57,1 | 38,4 | 1- | 35,5 | 32,5 | 32,2 | 30,6 |
| | *m* | 5,4 | 3,3 | | 3,7 | 2,4 | 2,7 | 2,9 |
| | *p* | | <0,05 | | <0,01 | <0,01 | <0,01 | <0,01 |
| | *M* | | | 2- | 48,6 | 56,4 | 50,0 | 54,4 |
| | *m* | | | | 5,1 | 6,9 | 4,3 | 6,8 |
| | *p* | | | | >0,05 | >0,05 | >0,05 | >0,05 |
| | $p_1$ | | | | <0,05 | <0,01 | <0,01 | <0,01 |
| RI, conventional units | *M* | 25,0 | 25,0 | 1- | 20,7 | 22,4 | 18,9 | 18,4 |
| | *m* | 1,9 | 3,1 | | 2,5 | 2,6 | 1,9 | 1,9 |
| | *p* | | >0,05 | | >0,05 | >0,05 | <0,05 | <0,05 |
| | *M* | | | 2- | 33,2 | 31,4 | 30,8 | 30,6 |
| | *m* | | | | 3,3 | 3,0 | 3,6 | 3,9 |
| | *p* | | | | >0,05 | >0,05 | >0,05 | >0,05 |
| | $p_1$ | | | | <0,01 | <0,05 | <0,05 | <0,05 |
| MR, mm Hg/ml | *M* | 0,23 | 0,39 | 1- | 0,29 | 0,33 | 0,38 | 0,44 |
| | *m* | 0,03 | 0,06 | | 0,04 | 0,05 | 0,05 | 0,04 |
| | *p* | | <0,05 | | >0,05 | >0,05 | <0,05 | <0,01 |
| | *M* | | | 2- | 0,25 | 0,26 | 0,24 | 0,23 |
| | *m* | | | | 0,04 | 0,04 | 0,03 | 0,05 |
| | *p* | | | | >0,05 | >0,05 | >0,05 | >0,05 |
| | $p_1$ | | | | >0,05 | >0,05 | <0,05 | <0,01 |

**Legend.** p) Significance of differences compared with initial values; $p_1$) significance of differences between groups. Student's t calculated by the difference method.

evidence has been obtained of an increase in the survival rate of rats in the reperfusion period under the influence of liposomes [1].

In the discussion of the mechanism of the corrective action of lecithin liposomes during reperfusion of the ischemic myocardium, the most probable explanation, in our opinion, is that liposomes and/or their degradation products prevent any increase in free-radical oxidation during tissue hypoxia, and thus exert a protective action on the myocardium. The possibility likewise cannot be ruled out that maintenance of the level of the principal high-energy compounds, which we found in the experiments with ischemia and reperfusion of the spinal cord [12], may also play an important role. No less important is the possibility that with the aid of liposomes the functional state of the cell membranes of both cardiomyocytes and of coronary vascular endotheliocytes can be restored to normal.

In the study described above we used lecithin liposomes, which are currently undergoing clinical trials under the name of "Lipin."

Injection of lecithin liposomes thus largely prevents the development of postischemic disturbances of the cardio- and hemodynamics, and clinical application of the method may result.

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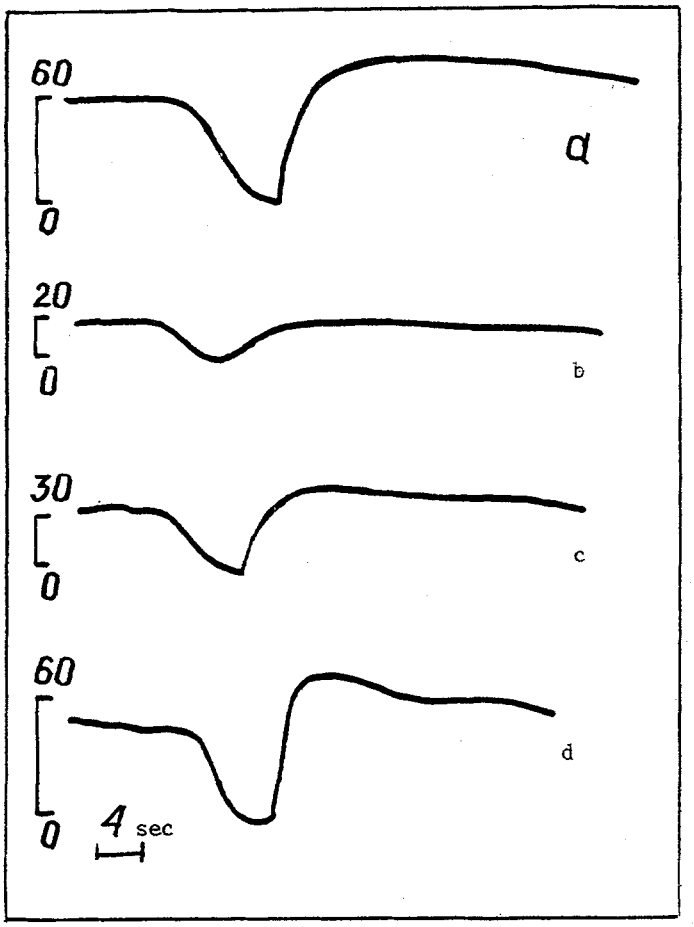


Fig. 1. Effect of ischemia and reperfusion on reactive hyperemia in left coronary artery: a) before ischemia, b) 60th minute of ischemia, c) 60th minute of reperfusion without liposomes, d) 60th minute of reperfusion with liposomes.


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