Effect of Phosphatidylcholine Liposome on Regeneration of Surgical Wound in Guinea Pig Lung

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Phosphatidylcholine liposomes in a dose of 25 mg/kg displayed wound-healing properties. They increased the count of alveolar macrophages, lymphocytes, and microvessels in damaged regions and, therefore, attenuated emphysematous changes in the lungs.

Key Words: *phosphatidylcholine; liposomes; lungs; surgical wound; regeneration*

Various forms of phospholipids (e.g., liposomes) are used as medicinal preparations [5]. The pharmacological effect of so-called "empty" liposomes was investigated in experimental and clinical studies. Experiments on animals with gunshot wounds showed that liposomes containing phosphatidylcholine (PCL) and cholesterol produce wound-healing and antiinflammatory effects [10]. Phospholipid synthesis in the surfactant of mouse lungs was accelerated on days 4-7 after inhalation of liposomes [8]. Liposomes promoted recovery of lung tissues in irradiated mice [12].

Clinical observations on volunteers showed the harmlessness of empty liposome inhalations [11]. This treatment produces positive effects during bronchial asthma in adults [4] and respiratory insufficiency in newborns [9].

Here we studied the effects of PCL in various doses on healing of surgical skin and lung wounds in guinea pigs.

MATERIALS AND METHODS

Experiments were performed on 10-month-old guinea pigs weighing 300-350 g and treated with PCL in concentrations of 1.3, 7.5, and 13 mg/ml. Phosphatidylcholine was obtained from the Biolek company (Ukraine). Liposomes were prepared as described previously [2].

We used the model of lung surgical wound. Narcotized animals underwent partial segmental resection of the left lung under aseptic conditions. Anterior pleurotomy was performed in the 4th intercostal space after surgical incision of the skin and muscles. A wedge-shaped fragment (10-12 mm in height and 5-6 mm in width) was excised from the anterior segment of the upper pulmonary lobe. Lung wound was closed with 3 stitches, and 0.6 ml PCL were introduced into the damaged region. PCL in a dose of 0.4 ml were administered during skin and muscle suturing.

Group 1, 2, and 3 animals were treated with 1 ml PCL in concentrations of 1.3 (5 mg/kg), 7.5 (25 mg/kg), and 13 mg/ml (40 mg/kg), respectively. Control animals (n=48) received 1 ml physiological saline.

The dynamics of skin wound healing was studied by planimetry [3]. Guinea pigs (6-7 animals of each group) were euthanized under hexenal anesthesia 1, 7, and 14 days after surgery. Skin and lung samples from the wound area (granulation zone) were assayed histologically by staining with hematoxylin and eosin. The cell composition of granulation tissue in damaged lungs was studied under a microscope equipped with a rectangular grid. The number and percentage of damaged cells, alveolar macrophages (AM), lymphocytes, and fibroblasts were calculated in 20 visual fields.

The ratio between lung components was estimated by measuring areas of alveoli, stroma, vessels, bronchi, and lymphatic system under a microscope

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TABLE 1. Effects of PCL on Area of Skin Wound and Cell Ratio in Lung Wound of Guinea Pigs on Days 1 and 7 after Surgery

Parameter	Control	PCL, mg/kg		
		5	25	40
Wound area, mm^2	42.6±2.2/33.5±1.5	39.2±2.5/32.9±1.4	31.0±1.8/18.6±0.9	28.2±1.5/19.0±0.6
Cell ratio, %				
damaged cells	26/9	24/10	16/—	14/—
AM	53/21	50/21	61/31	64/30
lymphocytes	12/10	13/11	13/16	14/17
neutrophils	6/6	8/4	3/—	3/—
fibroblasts	3/55	5/54	7/53	5/53

equipped with an Avtandilov 25-point grid [1]. The results were analyzed by Student's *t* test.

RESULTS

Physiological saline (control) and 5 mg/kg PCL produced similar effects on the area of skin wound on days 1 and 7 after surgery. Therefore, PCL in a dose of 5 mg/kg were inefficient. PCL in a dose of 25 mg/kg decreased the area of skin wound. On day 1 after surgery, 25 mg/kg PCL attenuated edema and hyperemia and decreased the area of skin wound. This effect was more pronounced on day 7 after surgery (Table 1). This decrease in the area of skin wound indicated that PCL in a dose of 25 mg/kg possessed considerable wound-healing properties. Increasing the dose of PCL to 40 mg/kg did not enhance the wound-healing effect. Therefore, the optimal dose of PCL was 25 mg/kg.

One year after surgery, we revealed scar tissues in control animals and guinea pigs treated with 5 mg/kg PCL. Emphysematous alveoli were found in the adjacent tissue. In group 2 guinea pigs, 25 mg/kg PCL induced cicatrization of lung wounds similar to that in control animals. However, the number of emphysematous alveoli in group 2 animals was much lower than in the control, which was probably related to an increase in the amount of microvessels (Fig. 1). Thus, intrapulmonary administration of PCL had no effect on the type of scar tissue, but normalized the architectonics of adjacent lung tissue.

On days 1 and 7 after surgery, the ratio between cells in granulation tissue of lung wound after treatment with 5 mg/kg PCL did not differ from the control, which confirmed the inefficiency of liposomes in low doses (Table 1).

On day 1 after surgery, there were many damaged cells, AM, and lymphocytes in all groups of animals. Fibroblast activity in damaged lung decreased. These results are consistent with published data that AM intensively phagocytize damaged cells and initiate removal of necrotized tissues at the first stage of wound healing.

On days 1 and 7 after surgery, the number of AM in group 2 and 3 animals was much higher than in the control (Table 1), which probably contributed to a considerable decrease in the content of damaged cells and neutrophils. PCL in doses of 25 and 40 mg/kg had no effect on fibroblast count. The number of lympho-

TABLE 2. Effects of PCL on the Specific Area (%) of Lung Tissue Components in Guinea Pigs before and after Surgery (*M*±*m*)

Lung tissue components	Intact lung (*n*=10)	Operated lung			
		without PCL		PLC, 25 mg/kg	
		14 days (*n*=24)	1 year (*n*=12)	14 days (*n*=13)	1 year (*n*=12)
Alveoli	44±2	25±3	35±2	36±2*	39±2**
Stroma	48.0±4.3	68±4	58±4	55±2*	55±2**
Bronchi	3.0±0.5	2.4±0.3	2.0±0.3	2.3±0.1**	2.1±0.1**
Vessels	2.4±0.3	1.8±0.2	2.8±0.3	3.5±0.2**	1.5±0.2
Lymphoid tissue	2.3±0.5	1.8±0.5	2.2±0.3	3.2±0.2**	2.4±0.1

Note. *$p<0.01$ and **$p<0.05$ compared to unoperated lung.

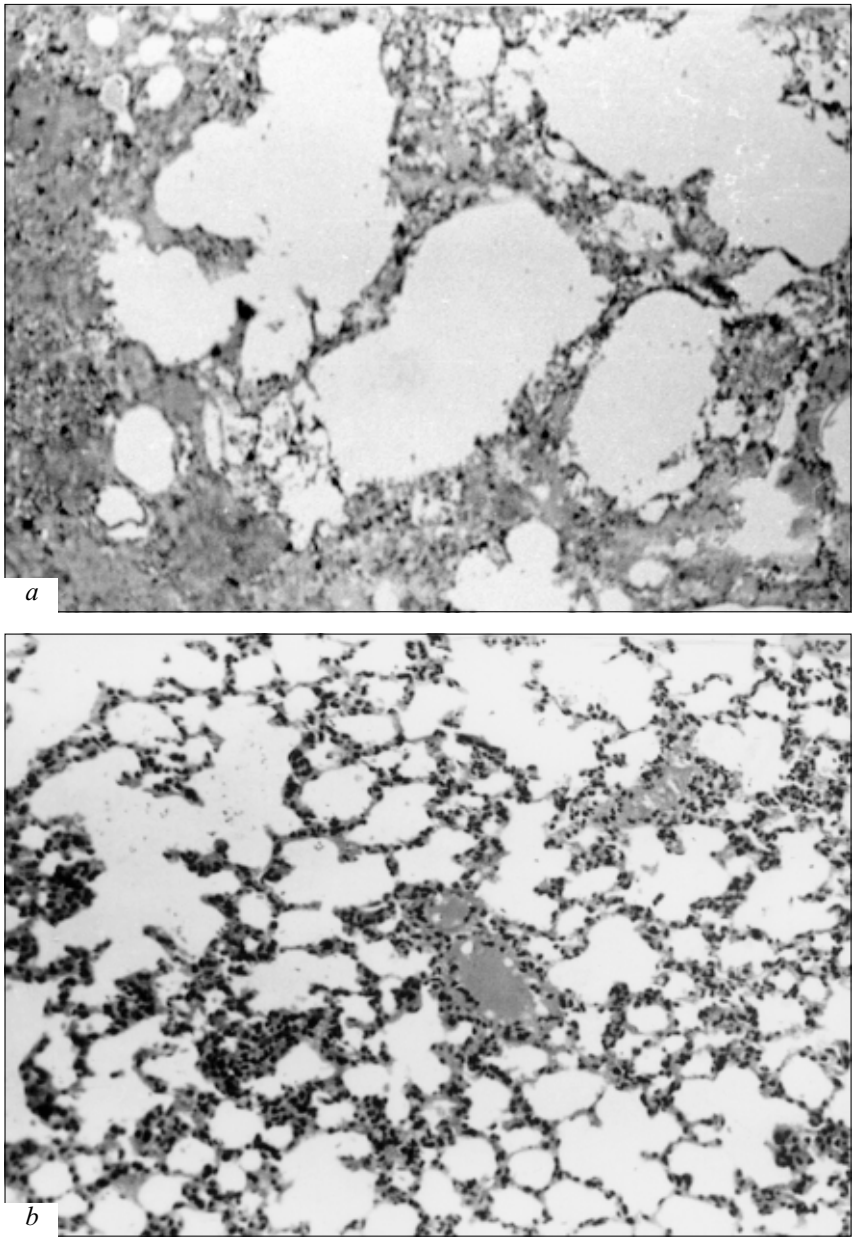

Fig. 1. Lung wound in animals not treated (*a*) and treated with phosphatidylcholine liposomes (*b*). Staining with hematoxylin and eosin, ×100.

cytes in animals treated with PCL in optimal concentrations increased on day 7 after surgery, which was probably due to the regulatory effect of lymphocytes on phagocytosis and proliferation.

On day 14 after surgery, specific area of wound stroma in control animals increased by 50% due to a decrease in specific area of alveoli, bronchi, vessels, and lymphoid tissue (Table 2). One year after surgery, the amount of vessels and lymphoid cells returned to normal. By contrast, specific area of alveoli and bronchi remained below the control, which determined the development of emphysematous changes in damaged lung regions.

On day 14 after surgery, the decrease in specific area of alveoli and bronchi was less pronounced, and the area of microvessels and lymphoid tissue increased in group 2 animals treated with 25 mg/kg PCL (Table 2).

Thus, single intrapulmonary administration of PCL promotes regeneration of the lung parenchyma in the adjacent area without emphysematous and inflammatory changes, accelerates growth of vessels, and increases the content of lymphoid cells.

It was hypothesized that pharmacological properties of PCL are related to their interaction with mononuclear phagocytes [6]. It was reported that phagocytes absorb liposomes [7,13]. Our previous studies of PCL effects on superoxide radical generation in human AM showed that liposomes induce respiratory burst in these cells [2]. The effects of liposomes on AM and lung tissue suggest that their pharmacologi-

cal properties are realized not only via absorption by phagocytes, but also via activation of processes accompanying functional changes in cells.

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