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EFFECT OF CARNOSINE ON HEALING OF LUNG WOUNDS

M. I. Perel'man, Z. Kh. Kornilova, V. S. Paukova, A. K. Boikov, A. A. Priimak, T. V. Bulargina, S. E. Severin, and G. N. Tikhonova

UDC 616.24-001.44-085.324:547.784.2]-036.8-07:616.24-003.9

Key words: lungs; regeneration; stimulation; carnosine.

Carnosine dipeptide (β-alanine, α-histidine) was discovered in 1900 in the composition of "Liebig's extract," obtained from bovine muscle [7]. This preparation is known to accelerate the healing of surgical wounds, bedsores, and erosions of the cervix uteri, and to stimulate adrenocortical function [2, 9, 10]. For many years the biological role of carnosine was unexplained, but then it was found to have the property of increasing the contractility of fatigued muscles [1, 3, 4]. Severin and Yu-Shu-Yui discovered its membranotropic effect [5]. The use of carnosine was shown to prolong considerably the keeping time of mitochondria isolated from the pectoral muscles of pigeons, while maintaining coupling between respiration and phosphorylation. The beneficial effect of carnosine on processes of Ca^{++} transport into vesicles of the sarcoplasmic reticulum and of Na^+ and K^+ transport through the plasma membrane, dependent on ATP hydrolysis. These data are evidence of the beneficial action of carnosine on the structure of membranes which remain capable of effecting active transport of H^+, Ca^{++}, Na^+, and K^+ ions without leakage [6]. The study of the role of the components of carnosine has shown that β-alanine activates nucleic acid and collagen synthesis, and α-histidine is a reserve for histamine synthesis [8, 10]. The antioxidant properties of carnosine and its ability to regulate the development of inflammatory and immune reactions open up new prospects for its therapeutic use in pulmonology.

The aim of this investigation was to study the effect of carnosine on the healing of experimental lung wounds.

EXPERIMENTAL METHOD

Experiments were carried out on 90 male guinea pigs weighing 280-300 g. A penetrating incised wound of the lung served as the experimental model. Under local anesthesia with 0.5% procaine solution a linear skin incision 1.5 cm long was made on the right side, posteriorly, along the sixth intercostal space, and this was followed by division of the subcutaneous cellular tissue, the spinal muscles, and the intercostal muscles. A penetrating incised wound 8-10 mm deep was then inflicted with a special scalpel. To select the optimal dose of carnosine six series of experiments were carried out in which 10, 15, 20, 25, 30, or 45 mg of the preparation was introduced into the lung wound. Each dose was dissolved in 1 ml of physiological saline, and 0.6 ml of the solution was introduced into the lung wound during the operation and 0.4 ml was injected into muscles and skin at three or four points. Physiological saline was injected in the control. The macroscopic picture of the zone of injury to the lung, muscles, and skin was assessed 3 and 7 days after the operation. The absence of a lung tissue defect and

I. M. Sechenov First Moscow Medical Institute. Moscow Research Institute of Traumatology, Ministry of Health of the RSFSR. M. V. Lomonosov Moscow State University. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 108, No. 9, pp. 352-356, September, 1989. Original article submitted October 15, 1988.

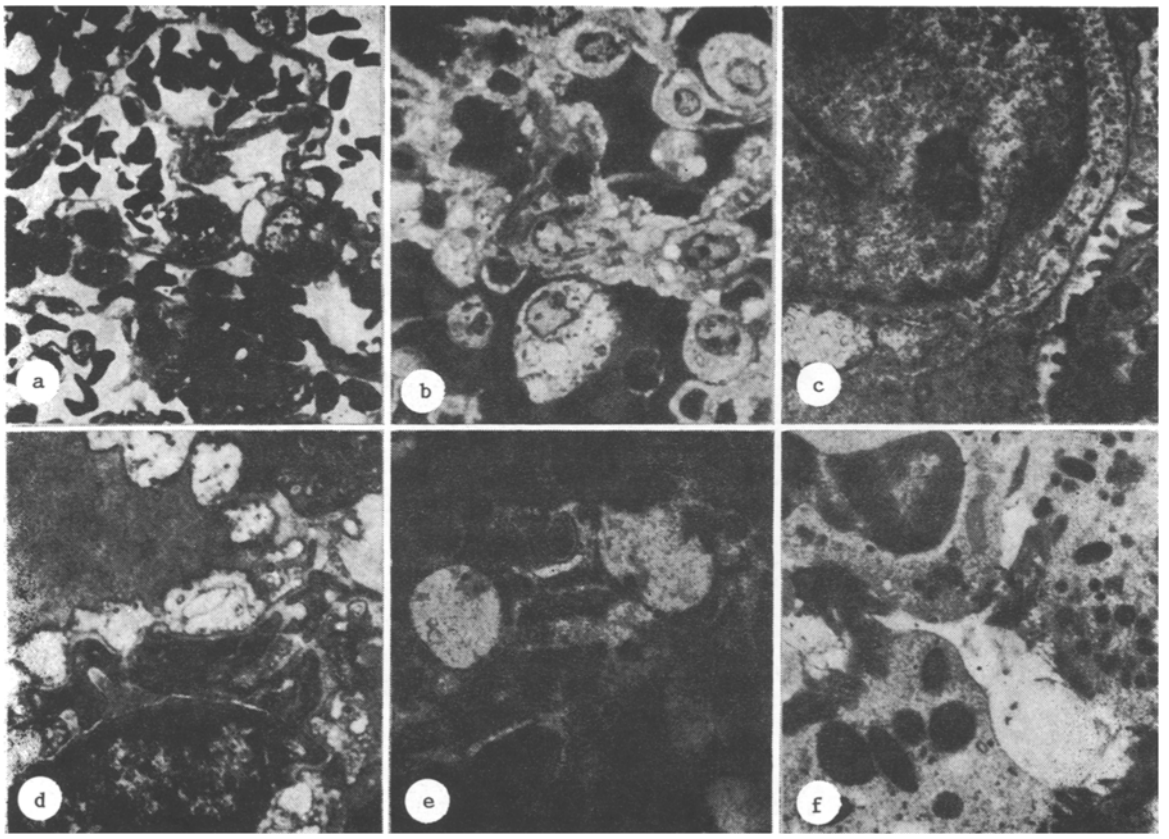

Fig. 1. Morphology of lung 1 day after injury. a) Control; atelectasis, increase in volume of all cells of lung parenchyma. 1000×; c) experiment; increase in number of ribosomes and tubules of endoplasmic reticulum in cytoplasm of Pn-II, marked indentation of outlines of nucleus and margination of chromatin in it. 8000×; d) control; control; balloon degeneration in cytoplasmic processes of Pn-I. 8000×; e) experiment; partial preservation of osmiophilic material in laminae of Pn-II. 8000×; f) experiment; phagocytosis of fibrin in alveolar space by granulocytes. 6000×.

of pleural adhesions, and the possibility of removing the skin sutures on the 3rd day after injection of 20-25 mg carnosine (60-75 mg/kg) enabled a dose of 20 mg to be settled upon in the subsequent experiments. In the main experiment, during the operation 0.6 ml of 2% carnosine solution (12 mg) was injected into the lung, 0.2 ml (4 mg) was injected intramuscularly, and 0.2 ml (4 mg) into the skin wound. In the control, instead of carnosine, 0.6, 0.2, and 0.2 ml of physiological saline respectively was injected. To evaluate the course of the repair processes in the injured lung, clinical observations were made on the 1st, 3rd, and 7th days after injury; histological methods were used, namely staining with hematoxylin and eosin and by Van Gieson's method, and semithin sections were stained with azure II-eosin. Material for electron microscopy was processed by the usual method and examined in the "Tesla BS-550" electron microscope.

EXPERIMENTAL RESULTS

On the 1st day after injury, edema and hemorrhages were less marked in the skin and lungs of the experimental animals than in the control. The lung around the wound was more inflated. On histologic examination necrobiotic and necrotic changes with hemorrhages into the alveolar space and alveolar septa, atelectasis and edema of the lung parenchyma, and stasis in the capillaries were the predominant changes in the zone of injury of the lungs in both groups of animals (Fig. 1a). However, the changes described above were less marked in the experimental group, and in semithin sections a considerable increase in area of all cells of the lung parenchyma was observed at the erges of the wound (Fig. 1b). Electron-microscopically, in the experiments with administration of carnosine, a marked increase in the number of ribosomes, mitochondria, and cisterns of the endoplasmic reticulum, more definite margination of the chromatin in the nucleus and indentation of its outlines were discovered in the type I and II pneumocytes (Pn-I,II), evidence of stimulation of intracellular

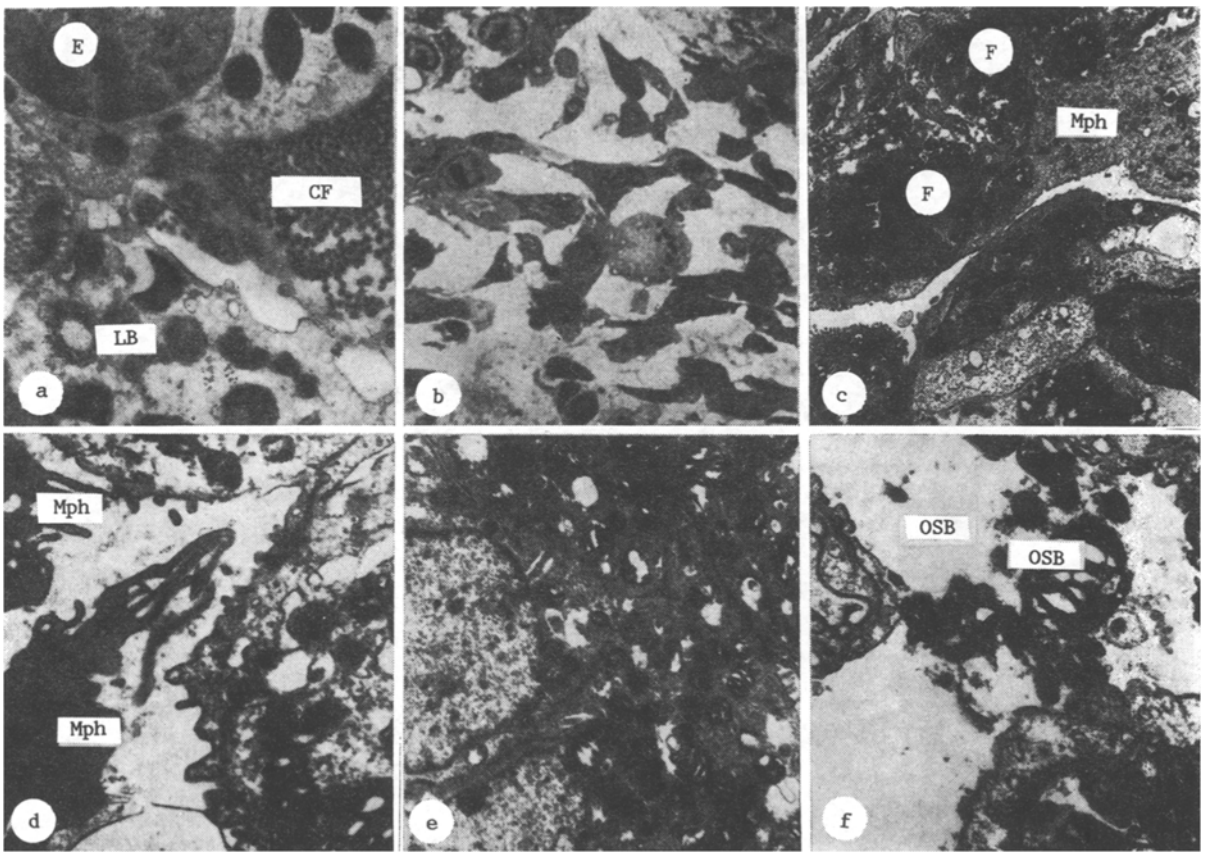

Fig. 2. Regenerative processes in lung wound treated with carnosine, 3rd-4th day of experiment. a) Increase in number of eosinophils (E), labrocytes (LB), and collagen fibers (CF) in central space. 8000×; b) many fibroblasts in wound edges. 1000×; c) Phagocytosis of fibrin (F) by macrophages (Mph). 2000×; d) Increase in number of active Mph in alveolar lumen. 8000×; e) Increase in number of Pn-II and of normal osmiophilic bodies (OSB) in their cytoplasm. 4000×; f) Outflow of OSB and their contents into alveolar space.

synthesis (Fig. 1c). Edema of the cells in this group was less marked and was manifested as an increase in vesiculation of the islets of Pn-I and endotheliocytes, and swelling of the mitochondria. Edema of the cells in the control group was more clearly defined and signs of balloon degeneration were observed in the cytoplasmic processes of Pn-I (Fig. 1d). When carnosine was used, Pn-II preserved remnants of osmiophilic laminae in the osmiophilic bodies, whereas in the control the osmiophilic bodies consisted of vacuoles, some of which contained only amorphous osmiophilic material (Fig. 1e). An important finding was the appearance of a larger number of neutrophilic granulocytes (polymorphs) and macrophages (Mph), which evidently accelerates the process of removal of tissue breakdown products from the wound (Fig. 1f).

On the 3rd-4th day the macroscopic changes in the zone of injury in both groups of animals were less marked than on the previous occasion. When carnosine was used, healing of the skin incision was discovered in the region of the wound, a whitish area 2-3 mm in diameter was present on the pleura, sometimes with slight in drawing, and areas of atelectasis in the lung were preserved. In animals of the control group edema and hyperemia were noted in the region of the skin incision, fibrinous deposits were present on the pleura and hemorrhages into it, fluid was present in the pleural cavity, and areas of atelectasis and hemorrhages in the lung were extensive.

On histologic and electron-microscopic investigation the number of Pn-II, Mph, labrocytes, and eosinophils in the wound edges was increased in both groups compared with their number at the previous time of the experiment (Fig. 2a). Hypertrophy and hyperplasia of the organelles still remained in all cells of the lung parenchyma. When carnosine was used, the formation of young granulation tissue was more advanced in the region of injury, and many fibroblasts appeared (Fig. 2b). Edema of the lung parenchyma was slight in degree compared with the control, and most of the fibrin had undergone phagocytosis by Mph (Fig. 2c). Many active Mph with long cytoplasmic outgrowths and numerous lysosomes could be seen (Fig. 2d). At the edges of the tissue defect concentrations of Pn-II appeared; they contained many osmiophilic bodies, in

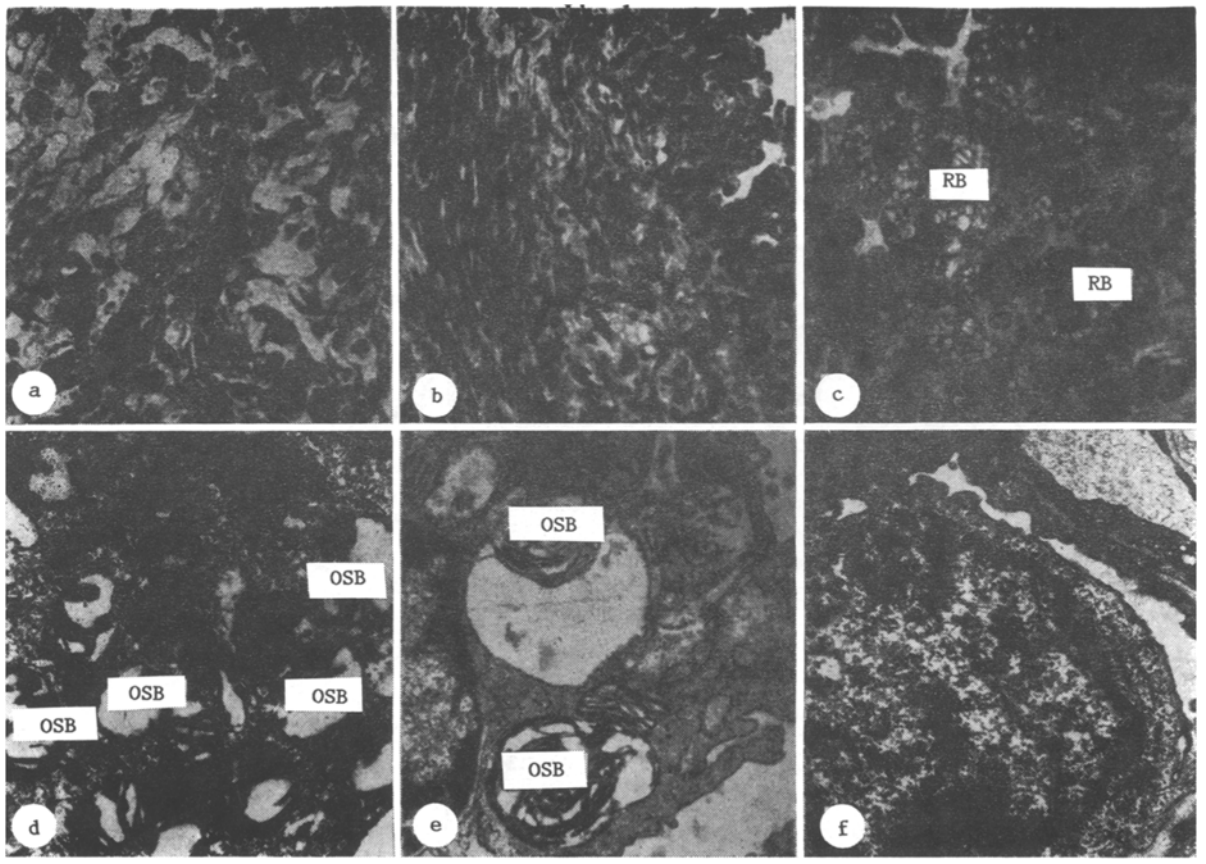

Fig. 3. On 7th-8th day after lung injury. a) Control; young granulation tissue in zone of wounding. Hematoxylin-eosin. 1000×; b) Experiment, formation of loose, amorphous connective tissue in zone of injury. Hematoxylin-eosin, 1000×; c) experiment, proliferation of Pn-II, which form regenerating buds (RB). 1000×; d) Experiment; intensive restoration of OSB in Pn-II. 8000×; e) Control; state of OSB in Pn-II. 8000×; f) Experiment, restoration of air-blood barrier, activation of pinocytosis in capillary endothelium and outgrowths of Pn-II. 8000×.

which the osmiophilic laminae were relatively orderly and occupied 1/2-3/4 of the volume of the osmiophilic body (Fig. 2e). An increase was observed in the outflow of contents of the osmiophilic bodies into the alveolar space (Fig. 2f). In the control group of animals, osmiophilic material occupied only 1/10 of the volume of the osmiophilic body.

On the 7th-8th day after injury, no changes in the pleura and in the lung tissue could be observed visually in animals of the experimental group, whereas in the control group these changes corresponded to the injuries observed on the 3rd-4th day of the experiment with carnosine. Histologically, granulation tissue continued to form in the zone of injury in the control group. In the experimental group the defect of the lung parenchyma was filled with young connective tissue, with a fascicular arrangement of fibroblasts and with the formation of fibrous structures (Fig. 3a, b). In semithin sections, in the edges of the tissue defect in animals of the same group concentrations of Pn-II appeared, in the form of buds including 4-20 cells, which, as our investigations showed, is the initial stage of formation of new alveoli (Fig. 3c). No such formations could be seen in animals of the control group. When carnosine was used, the lung tissue next to the granulations was more inflated, the Pn-II were larger than in the control and less vacuolated, and they contained osmiophilic bodies, mainly filled with laminae, which were rapidly excreted into the lumen of the alveoli (Fig. 3d). In the control, intensification of synthetic processes also was observed in Pn-II, but they contained fewer restored osmiophilic bodies (Fig. 3e). In both groups of animals the number of Mph in the alveolar space was reduced. The air-blood barrier was somewhat thickened and pinocytosis was activated in the capillary endothelium (Fig. 3f). Sometimes stasis of blood could be observed in the capillaries of animals of the control group. In both groups swollen Pn-I could be seen, projecting into the lumen of the alveoli. Margination of the chromatin in their nuclei was intensified, and the number of ribosomes and polysomes in their cytoplasm was increased. Polymorphs were still present in the parenchyma of the lungs at the periphery of the wound.

Carnosine accelerated repair processes in the injured lung almost twofold compared with the control, by activating proliferation of fibroblasts, connective tissue formation, and cellular and intracellular regeneration of the lung. The formation of osmiophilic bodies and osmiophilic laminae contained in them took place more intensively in Pn-II. On the 7th-8th day after injury, alveoli were formed in the wound edges from concentrations of Pn-II, and this may have contributed to the increased outflow of osmiophilic bodies and their material from Pn-II and to surfactant formation.

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