BIOGERONTOLOGY

Effect of Peptide Bioregulator on Healing of Excision Wounds in Old Animals

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 12, pp. 678-682, December, 2009
Original article submitted July 15, 2009

Aging is associated with reduction of protein synthesis in cells, which leads to deceleration of proliferative processes in various tissues. Recovery of damaged skin sites was stimulated with a peptide bioregulator chondrolux. This agent is based on an extract from calf cartilage and bone tissue. Its effect on healing of excision wounds was studied in old rabbits. Morphological analysis of the wound surface during various periods of healing was carried out by electron microscopy. The results indicate that chondrolux application to the wound surface stimulated and optimized the reparative process. Active development of granulation tissue was observed as early as on day 14 after wound infliction (*vs.* days 21-28 in control animals). Acceleration of wound healing was paralleled by an increase in functional activities of fibroblast organelles.

Key Words: *peptide bioregulator; chondrolux; reparation; wound surface; fibroblasts*

Body tissues are capable of restoring their morphological structure after injury. This process can be complete or partial depending on its conditions. Reparation of a damaged tissue site is characterized primarily by activation of cell proliferation in sizes adjacent to the focus of injury [6,10]. This is true for the main functional structures of tissue and for extracellular matrix and regional blood supply system [11]. However, these characteristics of the reparative process leave aside functional aspect of cell-cell interactions and dynamic changes in the humoral medium of the matrix [8]. On the other hand, these cells involved in reparation seem to be active producers of biological stimulants and inhibitors of physiological processes in various functional systems of the body [3,7,12,13].

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It was shown that reparation processes in tissues of old animals are very slow [1,5,9]. Chondrolux, a peptide bioregulator created on the basis of an extract from calf cartilage and bone tissues, markedly stimulated metabolic processes in the bone tissue of rats with experimental postmenopausal osteoporosis [2] and in the cartilage tissue of rats with experimental posttraumatic osteoarthrosis. Studies on organotypical culture demonstrated that chondrolux stimulated cell proliferation in the cartilage and bone, as well as in the skin [4]. We studied the efficiency of chondrolux used for stimulation of reparative processes in skin tissue of old animals.

MATERIALS AND METHODS

The study was carried out on 5-year-old outbred female rabbits (n=10; 4.5-5.0 kg). A linear incision of the skin (2 cm long) on the back was made. The rabbits were randomly divided into 2 groups (5 animals

0007-4888/09/1486-0915

each). Controls received no treatment, the healing process running a natural course in them. Experimental animals received applications of chondrolux (peptide bioregulator) to the wound surface from the moment of injury until complete healing.

The time course of wound healing was evaluated on days 7, 14, 21, and 28 after creation of the injury by the morphological parameters (electron microscopy). Biopsy specimens from the wound bottom and marginal regions and cicatricial tissue were collected for electron microscopy. The sections were prepared routinely, contrasted in blends with 4.5% uranyl acetate (10 min), washed thoroughly in distilled water, and poststained with lead citrate (10 min) by modified Reynolds' method with NaOH for absorption of CO_2, with subsequent washing. The preparations were examined under a JEM-100S electron microscope (JEOL) in the transmission mode.

Morphometry of fibroblast ultrastructure was carried out on saved electronograms using Videotest-Morphology 5.0 software; 10 electron microscopic images of fibroblasts from excision wound were analyzed for each period of healing (with and without chondrolux). Volume density of mitochondria, rough endoplasmic reticulum, ribosomes, lysosomes, and micropinocytotic vesicles (MPV) was evaluated in percent of the total volume of the cytoplasm.

The data were statistically processed using Statistica 5.0 software (Statsoft).

RESULTS

The cell composition of the excision wound on day 7 was presented mainly by young fibroblasts. Areas with very compact arrangement of fibroblasts and low content of extracellular substance alternated with areas enriched with the main substance surrounding individual (primarily spindle-shaped) cells.

Young fibroblasts were spindle-shaped cells with short or long processes connected to each other in a geared manner. Young fibroblasts during this period had cytoplasmic signs of degeneration: they had little mitochondria and ribosomes, the pattern of hypertrophic Golgi complex was scanty, the lysosomes and vacuoles were numerous, the content of damaged rough (RER) and smooth endoplasmic reticulum (SER) was moderate, RER cisterns were dilated and sometimes contained fine granular material.

The counts of damages mitochondria varied (Fig. 1, *a*), cells with numerous lipid droplets and myelin figures were often seen, the integrity of plasmalemma was violated. Exocytosis of granules into the extracellular space was seen.

The cell nuclei had intricate shape and often contained inclusions. In addition, there were autophagic vacuoles and often disordered filaments (~10 nm in diameter) sometimes forming loose bundle-like structures under the plasma membrane.

Microtubules about 20 nm in diameter were often seen. Numerous damaged blood capillaries closely connected to cells were seen in the areas with compactly lying cells. The vessels in the bottom of the wound deserve special attention. Capillaries with hypertrophic swollen endothelial cells and several layers of pericytes divided by fine collagen filaments predominated (Fig. 1, *b*).

Macrophages with signs of poor functional activity appeared in the bottom of the wound. These cells had polymorphic nuclei, their karyoplasm contained nuclear inclusions (accumulations of fine fibrillar structures forming balls bordered with a clarified zone). These cells had few pseudopodia and phagocytosed vacuoles, solitary round granules of low electron density, and small mitochondria.

Lymph capillaries were detected in the wound areas adjacent to the epidermis. These capillaries formed loops and their lumens were dilated. Structural changes in the cytoplasm of lymphatic endotheliocytes indicated reduced energy, synthetic, and transporting functions of these cells. The organoids were swollen, open interendothelial contacts emerged. The electron density of the interstitium was lower than that of the lymph capillary lumen, which presumably reflected tissue edema and disorders in the lymph discharge.

Numerous histioid young fibroblasts were seen in the bottom of the wound in experimental rabbits on day 7 (Fig. 1, *c*). These cells had processes and exhibited higher functional activity in comparison with the control.

Well-developed RER and numerous ribosomes were seen in the cytoplasm. The mitochondria were scanty, and just few cells had numerous lysosomes. Filaments and pinocytotic vesicles were sometimes seen under the plasma membrane in the peripheral compartments of the cytoplasm.

Fibroblasts closely contacted with each other or were scattered by groups of several cells in a network of connective tissue fibrils. Fibroblast nuclei were mainly elongated, sometimes of irregular shape because of depressions. Mast cells were also present in the bottom of the wound.

The forming capillaries were lined with large endotheliocytes with large round nuclei and invaginations. Endotheliocyte cytoplasm contained the Golgi complex of different degree of development, mitochondria, pinocytotic vesicles, fine processes of the plasma membrane, sometimes filaments (Fig. 1, *d*).

On day 21, ultrastructural signs of formation of granulation tissue with fibroblasts with more pro-

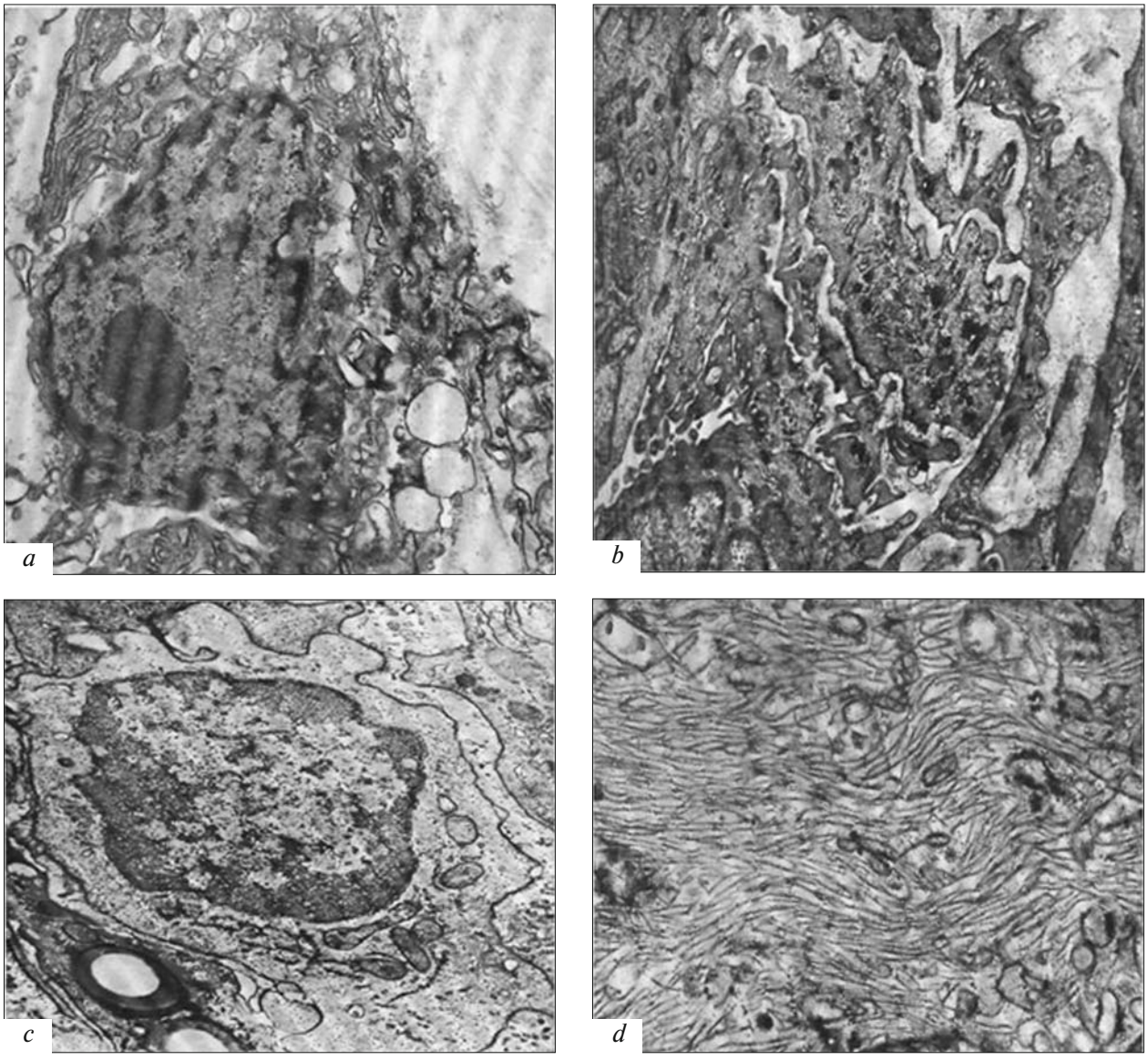

Fig. 1. Excision wound, day 7. *a*) control. Damaged RER lesions in fibroblasts, peculiar membrane structures appearing in its dilated cisterns. Pinocytotic vesicles, dilated mitochondria, autophagic vacuoles in the cytoplasm, ×16,500; *b*) control. Capillaries on the bottom of the wound. The vascular lumen is limited by swollen endotheliocytes, adhering to each other by the edges and connected to each other. Several layers of pericytes surrounded by the basal membrane nearby, ×13,200; *c*) chondrolux treatment. Well-developed RER and numerous ribosomes in the cytoplasm of young histioid fibroblasts. Filaments and pinocytotic nuclei under plasma membrane, ×10,500; *d*) chondrolux treatment. Forming capillaries lined with large endotheliocytes with large round nuclei and invaginations, ×6500.

nounced functional activity and histiocytes developed in the wounds of control animals. Bundles of nonspecific (usually fine) filaments chaotically scattered in the cytoplasm formed in the fibroblasts and histiocytes. Cells with filaments located parallel to the long axis of the cell were rare (Fig. 2, *a*).

Numerous capillaries were formed from large endothelial cells, exhibiting functional activity. The nuclei in these cells were large, elongated, with invaginations. The cytoplasm contained numerous organelles, RER was well developed, the Golgi complex was clearly seen, there were many mitochondria and pinocytotic vesicles. Fine processes of the plasma membrane, sometimes filaments were seen.

Some macrophages were characterized by high functional activity, which was seen from cytoplasmic processes (pseudopodia) and invagination of the cytoplasm. The macrophage cytoplasm, in addition to the lysosomes, mitochondria, and a small amount of RER, contained phagocytic vacuoles and siderosomes.

On day 21, well-formed granulation tissue in the wounds of experimental rabbits was presented by thin-walled blood vessels located in connective tissue stroma with spindle and stellate fibroblasts, lympho-

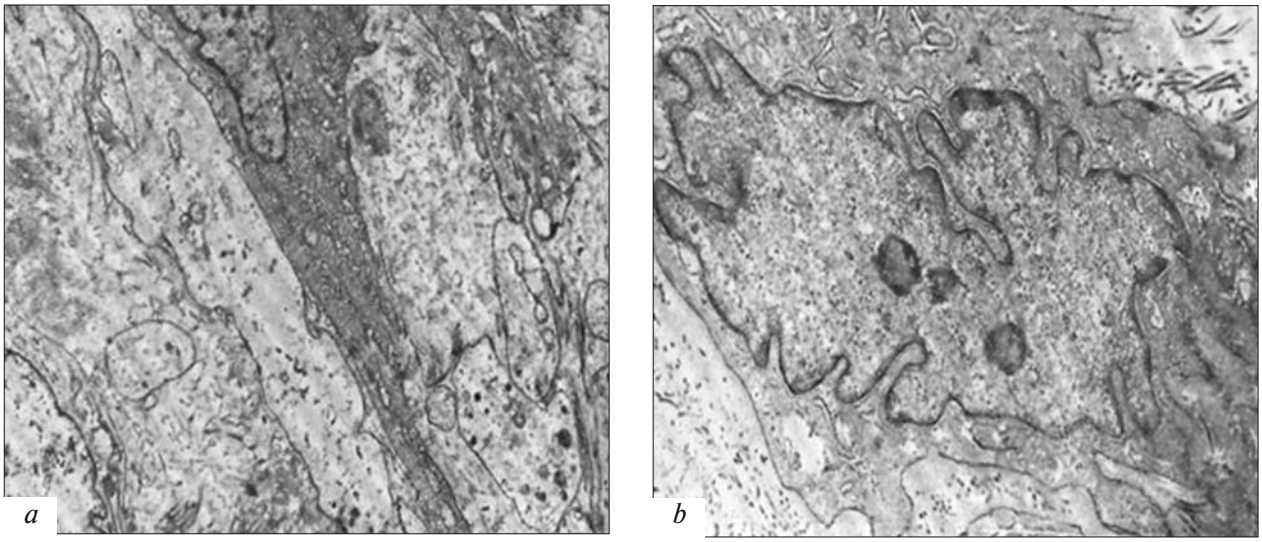

Fig. 2. Excision wound, day 21. *a*) control. In some histiocytes the filaments are parallel to the long axis of the cells. Histiocyte nuclei have large nucleoli. The cytoplasm contains numerous moderately dilated RER cisterns filled with finely granular contents. The mitochondria are oval with clear matrix, ×13,800; *b*) chondrolux therapy. Microfibroblasts have large nuclei with lacerated contour. Numerous filaments are seen in the cytoplasm, ×6200.

cytes, plasma and mast cells, and macrophages. The fibroblasts were presented by the "classical" type and contained dilated RER cisterns and numerous cytoplasmic filaments (Fig. 2, *b*). The nuclear membrane of a "classical" fibroblast formed structures (loops and pouches); the nucleus containing condensed heterochromatin. There were also histioid fibroblasts with hypertrophic Golgi complex, numerous vesicles, including those under the cell membrane, sometimes filaments, solitary lysosomes, and lipid droplets.

The myofibroblasts already present by this term had large nuclei with lacerated contour, the cytoplasm contained numerous microfilaments 7-8 nm in diameter, somewhere arranged parallel to each other (Fig. 2, *b*).

Morphometric analysis of fibroblast ultrastructure (Table 1) on day 28 showed a significant increase of volume density of hypertrophic mitochondria after chondrolux treatment. This fact reflects more intense energy metabolic processes in fibroblasts, promoting the regeneration processes.

The increase in the volume density of RER along with changed ultrastructure of its cisterns packed with fine granular contents indicates more intense intracellular transporting processes after chondrolux treatment.

Hyperplasia of ribosomes, fixed to the RER membranes and free, reflects primarily the fact that intensification of the transporting processes is associated with protein producing function of cells.

The increase of MPV volume density reflects the active secretory function of fibroblasts. Particularly high concentration of MPV at the cellular basal membrane indicates active secretion of signal proteins into

TABLE 1. Results of Morphometric Analysis of Excision Wound Fibroblasts on Day 28 (*M±m*)

Parameters (Vv)	Control group	Experimental group (chondrolux)
Mitochondria	5.2±0.2	9.0±0.3*
RER	9.2±0.1	13.4±0.1*
Adhered ribosomes	11.2±0.3	15.1±0.2*
Free polysomal ribosomes	14.3±2.4	20.8±2.5*
Lysosomes	13.6±2.2	15.4±2.2
Luminal MPV	2.2±0.05	2.4±0.08
Cytoplasmic MPV	13.6±1.16	20.1±1.45*
Basal MPV	20.2±2.2	29.3±2.4*

Note. Vv: volume density of structures (% of cytoplasm volume); *$p<0.05$ compared to the corresponding parameter in the control group.

the cell-to-cell space by the fibroblasts, which, in turn, stimulates the formation of collagen fibers and formation of a cicatrix.

Hence, electron microscopy showed that chondrolux (peptide bioregulator) is characterized by pronounced reparative effect and stimulates and optimizes healing of excision wounds in old animals. Active development of granulation tissue was observed as early as on day 14 after damage (*vs.* days 21-28 in the control). Morphometric parameters of the fibroblast organelle status in animals treated with chondrolux reflected significant intensification of their functional activity on day 28 of healing in comparison with the control.

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