EFFECT OF THE OPIOID PEPTIDE DALARGIN ON REPAIR PROCESSES DURING WOUND HEALING

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Opioid receptors and their ligands determine the cytoprotective function of the body [9, 10], they participate in DNA synthesis in the developing organism [7], and they are involved in cellular events connected with proliferation and differentiation [11, 12]. High activity of endogenous neuropeptides [2] has enabled the synthesis of classes of analogs, which are very promising pharmacological preparations for the regulation of regeneration. The hexapeptide dalargin, which possesses a fairly broad spectrum of activity, is the most interesting of these substances [1, 3, 5].

The morphological basis of healing of skin wounds under the influence of dalargin was studied in this investigation.

EXPERIMENTAL METHOD

Full-thickness skin wounds in the dorsal region, 17 mm in diameter, were inflicted on 200 male Wistar rats, initially weighing 200-220 g, under pentobarbital anesthesia (35 mg/kg, intraperitoneally). Immediately after the operation an ointment base (lanolin—petrolatum, 5:1) was applied to the wounds of the animals of group 1 for 6 days. In group 2 (experiment) dalargin in the ointment base in a concentration of 10 μg/g ointment, was applied. Rats of group 3 (control) received physiological saline by intraperitoneal injection in a volume of 1 ml/kg body weight daily for 3 days before the operation and 3 days thereafter. Animals of group 4 (experiment) received a solution of dalargin, in a dose of 10 μg/kg body weight, in accordance with the same scheme. In the course of healing the area of the wounds was measured and the average time of complete healing (ATCH) was calculated.

On the 2nd, 4th, 7th, 10th, 20th, and 30th days areas of the skin wounds were excised from animals removed from the experiment for histological and histochemical investigation: sections were stained with hematoxylin and eosin, picrofuchsine by Van Gieson's method, with toluidine blue for acid glycosaminoglycans (GAG), the PAS reaction for glycoproteins, Brachet's reaction for RNA, and Feulgen's reaction for DNA. Mitotic activity of fibroblasts and endotheliocytes was studied on the 3rd, 5th and 7th days at 10 a.m., 4 h after preliminary injection of colchicine (1.5 mg/kg, intraperitoneally). The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

Intraperitoneal injection and local application of dalargin significantly accelerated contraction of the wounds at all times of investigation; on the 4th and 8th days the area of the wounds in the group with intraperitoneal injection of the peptide was significantly smaller. By the 12th day this difference had disappeared. ATCH for intraperitoneal injection was 19.3 ± 0.6 days (72%; $p<0.05$) and 21.8 ± 0.9 days for local application (80.1%; $p<0.05$); the corresponding figures in the control were 26.8 ± 1.1 and 27.2 ± 0.8 days.

Histological and histochemical study of the tissue in the wound region showed that proliferation of fibroblasts was greatly intensified already in the early inflammatory phase of healing (2nd day after the operation) in the experimental groups (especially receiving local

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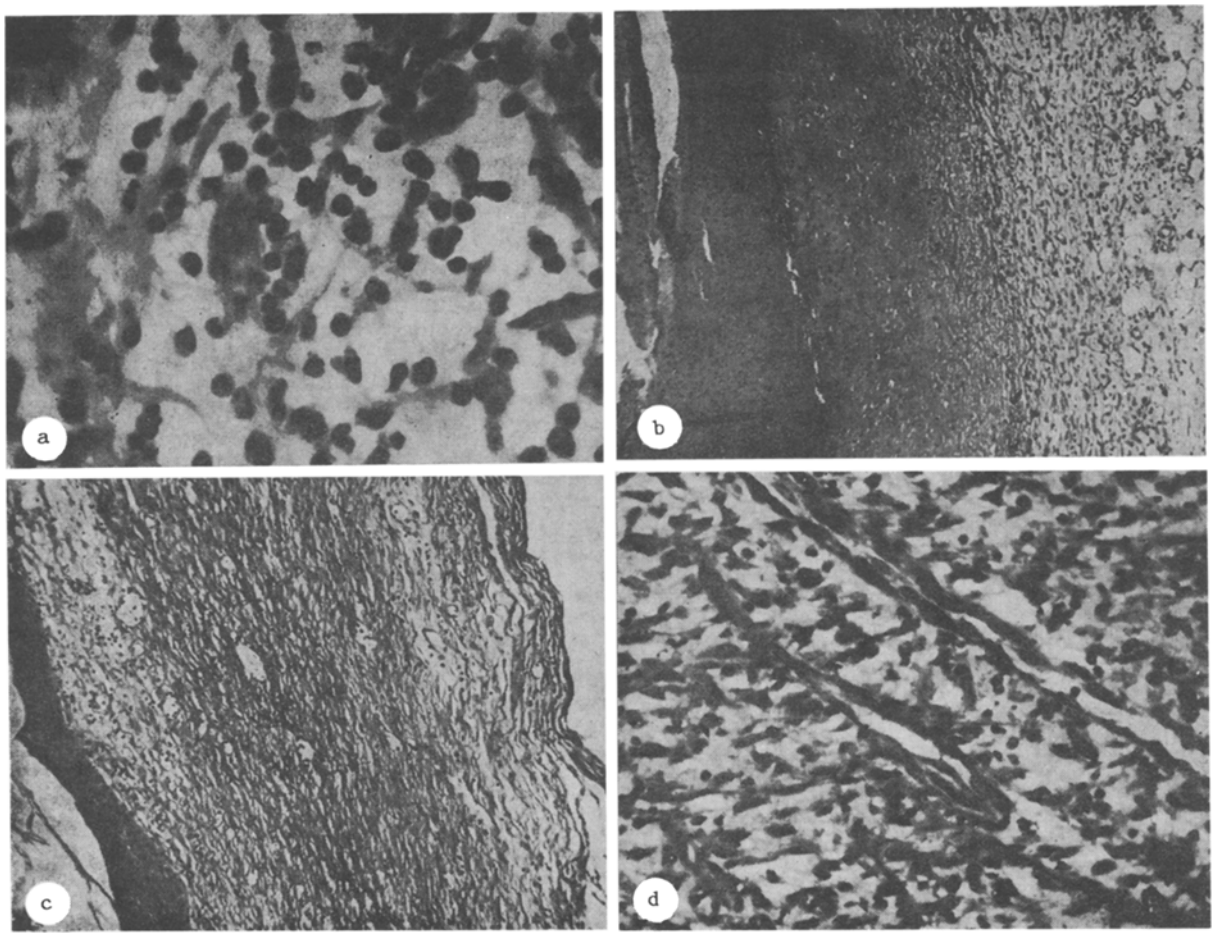

Fig. 1. Histological changes in tissues of healing wounds. a) Proliferation of fibroplasts (mitoses can be seen), macrophagal response and new capillary formation in granulation tissue on 2nd day after operation and local application of dalargin (400 ×); b) beginning of granulation tissue formation under scab and thick layer of fibrinous exudate in control group on 4th day after operation (100 ×); c) relatively mature granulation tissue with vertical capillaries and differentiating fibroblasts at the same time with intraperitoneal injection of dalargin (200 ×); d) mature, fully epithelized scar on 15th day after operation, with intraperitoneal injection of dalargin (100 ×). Hematoxylin and eosin.

treatment) compared with the corresponding controls in tissues in the floor of the wound defect (in subcutaneous fatty areolar tissue and muscles). Under these circumstances, unlike in the control relatively differentiated forms of quite large cells with fusiform or stellate, RNA-rich cytoplasm and with a large nucleus and nucleoli could be seen. Growth of endothelial bands and the formation of new capillaries, accompanying bands of fibroblasts, were observed. Mitotic figures in fibroblasts and endothelium were much less common than in the control (Fig. 1a). In some places foci of immature granulation tissue appeared, from which fibroblasts migrated and capillaries spread into the superficial fibrinous-leukocytic layer. At sites of proliferation of fibroblasts, the formation of a network of immature argyrophilic collagen fibrils and accumulation of acid GAG were observed in the experimental animals.

Dalargin also had a marked effect on chemotaxis and activation of macrophages. These cells were more numerous than in the control and they varied in shape: monocytelike, immature and mature activated macrophages with phagocytic inclusions and with PAS-positive granules in the cytoplasm. The number of macrophage–fibroblast junctions also was significantly increased, evidence of active interaction between these cells. Undoubted stimulation of regeneration of the epidermis was noted: Even at this early stage growth of the epithelial layer was observed at the edges of the wound.

The morphological manifestations of inflammation in the tissues of the floor of the wound varied in their importance. Edema and neutrophilic infiltration were virtually identical in

the control and experimental groups. In the treated animals, however, especially those receiving dalargin intraperitoneally before and after the operation, a more active response of the microcirculatory bed was observed, with dilatation and congestion of capillaries and venules and with more rapid new capillary formation. Under these circumstances the number of mast cells was considerably increased near vessels, with morphological evidence of their functional activity: vacuolation of their cytoplasm, degranulation, the appearance of cells with orthochromatic granules, and so on. On the 4th day after the operation the granulation tissue in the experimental groups, but not in the control (Fig. 1b), already formed a continuous layer on the floor of the wound, with relatively high granulation. The capillaries now were arranged in vertical vascular loops, whereas the differentiated fibroblasts were arranged horizontally and were spindle-shaped (Fig. 1d). In the ground substance the content of acid GAG and the number of mature fuchsinophilic (Van Gieson-positive) collagen fibers were considerably increased. After local application of dalargin the total number of fibroblasts (including cells in a state of mitosis) and also the number of newly formed capillaries with mitoses in the endotheliocytes were greater than after intraperitoneal injection. Prolongation of the proliferative effect, with some degree of inhibition due to this cellular differentiation, collagen biosynthesis, and general maturation of the granulation tissue, evidently took place due to the continued action of the locally applied dalargin.

On the 7th day after the operation the degree of maturity of the granulation tissue in all the experimental animals was considerably higher than in the control: its structure became more regular, the total number of cells and vascular elements was reduced, and mature fibroblasts and collagen fibers predominated. However, many macrophages still remained in the surface layer of granulation tissue and junctions appeared between macrophages and fibroblasts, especially after local application of dalargin. An active mast cell response, hypermia of the blood vessels, and certain morphological manifestations of their permeability were observed as before in these animals, and these also were evidently associated with the continuing action of dalargin.

By the 10th day the primary scab had been shed by most of the experimental animals, the layer of fibrin and leukocytes was much thinner, and the granulation tissue was formed into mixed fibrous and granulation tissue; differences between the groups with local application and intraperitoneal injection of dalargin had almost disappeared. A particular feature of the granulation tissue in the experimental animals compared with the control was an increase in the number of lymphocytes and plasma cells in them, possibly on account of activation of immune mechanisms. Peripheral epithelization took place more rapidly than in the control.

On the 15th day the area of the wounds in the rats of the experimental groups was greatly reduced by contraction and in many animals complete epithelization was observed (Fig. 1c). Fibrous granulation tissue was transformed into fibrous scar tissue. By the 20th day the defect was epithelized in all the rats treated with dalargin; unepithelized areas of different sizes were still present in the control animals, and the granulation tissue showed some signs of immaturity. By the 30th day epithelization was now observed in the majority of the control animals, but the scar in the experimental rats was more mature and was showing signs of regression. The skin appendages and elastic fibers were not restored.

The study of mitotic activity of the cells indicates that dalargin, whether injected intraperitoneally or applied locally, increased by 2.5-3 times the percentage of cells commencing the mitotic cycle on the 3rd-5th day of the experiment. By the 7th day the level of mitotic activity after intraperitoneal injection reached the control values, whereas in animals treated by local application it still remained above the control value. This corresponded to the morphological picture of maturation of granulation tissue.

Dalargin thus has a marked action on repair processes during skin wound healing. This action is evidently mediated through its effect on the microcirculatory system, which plays an important role in nutrition of regenerating tissues [6, 8]. Under the influence of dalargin an increase was observed in the number and intensity of functional activity of the mast cells, whose vasoactive factors play a decisive role in regulation of the regional hemodynamics [4]. Dalargin evidently has no specific anti- or counter-inflammatory action, but appreciably shortens the duration of the inflammatory phase of wound healing by direct stimulation of repair processes (and, consequently, promoting an earlier transition to the proliferative phase of healing). Proliferation of fibroblasts and endotheliocytes with new capillary formation took place much earlier in the experimental groups. This was clearly reflected also in the value of the mitotic index of these cells. A very important role in the induction of their proliferation is played by macrophages, which produce a number of growth factors, including

fibroblast growth factor [4]. In fact, under the influence of dalargin, the intensity of chemotaxis and the phagocytic and secretory activity of the macrophages were appreciably increased, and junctions between macrophages and fibroblasts also were more numerous. The role of dalargin in stimulation of the immune mechanisms of wound healing, expressed morphologically as infiltration of the granulation tissue with lymphocytes and plasma cells at certain periods, likewise cannot be ruled out. Early proliferation of fibroblasts under the influence of dalargin leads to their more rapid differentiation, proteoglycan and collagen biosynthesis, fibril formation, maturation of granulation tissue, and its conversion into fibrous scar tissue. Dalargin also has an undoubted stimulating effect on epithelial regeneration.

It is evidently unnecessary to use dalargin for a long period of time, for even when a 6-day course of local application was tested, some slowing of maturation of granulation tissue was observed on the 4th-7th day as a result of prolonged proliferation of fibroblasts and endothelium. Dalargin, with its trigger mechanism of action, evidently induces a cascade of inflammatory-reparative reactions, and shortens all phases of wound healing.

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EFFECT OF ACETYLCHOLINESTERASE INHIBITORS ON REINNERVATION OF MOUSE SKELETAL MUSCLE

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Acetylcholinesterase inhibitors (AChEI) are widely used in clinical medicine to potentiate synaptic transmission. It is well known that many neuromuscular disorders are accompanied by processes of denervation and reinnervation [2]. Consequently it is important to understand the action of AChEI on the time course of reinnervation of muscle fibers. No special electrophysiological investigations have hitherto been undertaken to study the effect of AChEI on the course of reinnervation in functionally mature muscles of animals.

This paper gives data on the action of AChEI on the state of neuromuscular transmission under conditions of evoked denervation and reinnervation.

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