Morphological Evaluation of the Effect of King Crab Collagenase on Experimental Thermal Burn

N. A. Markovich, V. V. Omigov, C. M. Balakhnin, E. G. Malygin, V. V. Zinov'ev, and L. S. Sandakhchiev

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The effect of crab collagenase on the healing of thermal burns of uninfected rats is studied morphologically. The enzyme is applied to the wounds using a polyethylene oxide gel with antibiotics. Protease with antibiotics is more effective than Laevomecol ointment.

Key Words: *king crab; collagenase; thermal burn; healing; rats*

Proteolytic enzymes, such as trypsin, chymotrypsin, and collagenase, are often used in the treatment of thermal burns to accelerate the rejection of scab and necrotic tissues [2]. An enzymatic agent exhibiting both collagenolytic and caseinolytic activities [3,4] has been prepared from the hepatopancreas of the king crab *Paralithodes camtschatica*. The reported effect of crab collagenase on wound healing [1] prompted us to investigate the effect of collagenase ointment on the healing of uninfected thermal burn in rats.

MATERIALS AND METHODS

The wound-healing effect of collase ointment on thermal burns was assessed in male Low albino rats weighing 160-200 g. All the animals were fed a full-value diet and kept in a vivarium. Thermal burn was caused by a 15-sec application of an electrical heating element 10 mm in diameter set up at 100°C on a thoroughly shaved suprascapular area.

Collase is a partially purified enzyme preparation from the hepatopancreas of king crab (*Paralithodes camtschatica*). It is produced at the Vektor State Research Center of Virology and Biotechnology (Kol'tsovo, the Novosibirsk region). The preparation shows collagenolytic and caseinolytic activities. Polyethylene oxide (PO) gel produced at the Novosibirsk Chemical and Pharmaceutical Plant served as the base for collase ointment. The collase-PO mixture can be stored for at least 6 months at 5-8°C.

Two series of experiments were carried out. In the first series, we studied the effect of an ointment containing PO and collase in a dose of 0.1 mg/g gel (pH 7.0-8.0). Control animals were treated with PO gel without collase. In the second series, Henampicol ointment was tested. This ointment consists of collase (pH 7.0-8.0), antibiotics (ampicillin, gentamycin), and trimecaine. Control groups included intact rats and those treated with Laevomekol, or PO with antibiotics and trimecaine. In all animals, burns were treated with 0.02% Furacillin during the first 3 days. Treatment with the ointments was started on day 4. Changes in the wound size were recorded, and skin specimens for histological analysis were collected for microscopic examination on day 1 after burn (without treatment) and on days 8, 14, 21, and 30 of the therapy. The material was fixed in 10% Formalin and routinely processed for light microscopy. Paraffin sections were stained with hematoxylin and eosin.

RESULTS

An earlier rejection of the scab was observed in rats treated with PO and PO+collase, starting from day

Vektor State Research Center of Virology and Biotechnology, Ministry of Health of Russia; Research Institute of Molecular Biology, Russian Academy of Sciences, Novosibirsk

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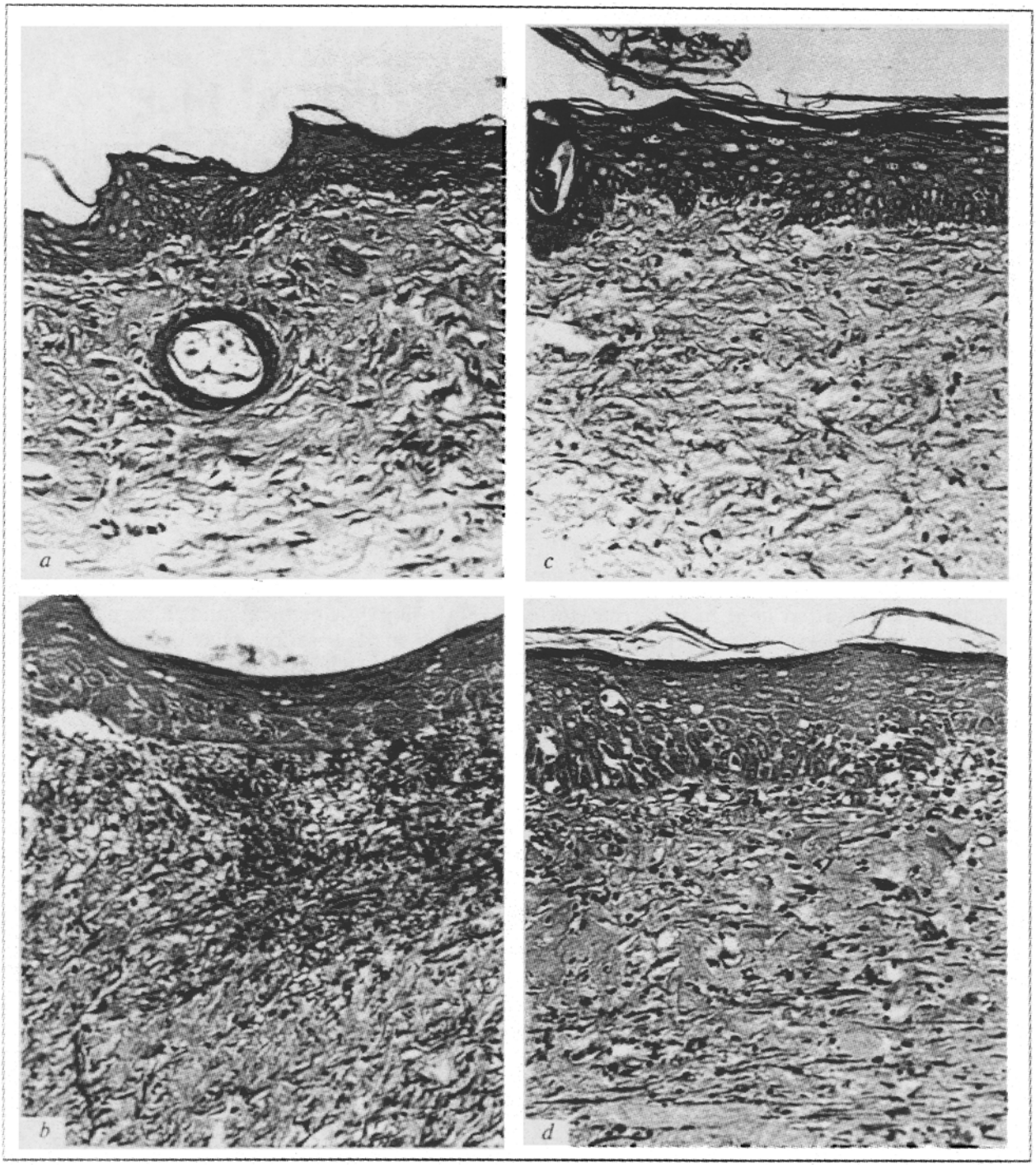

Fig. 1. Microscopic examination of the effect of various ointments with collase on healing of thermal burns in rats (day 21 of experiment). *a*) intact rat skin (control); *b*) skin of rat treated with polyethylene oxide (PO) gel; *c*) skin of rat treated with PO gel with collase; *d*) skin of rat treated with Henampicol. Hematoxylin and eosin staining, ×250.

10 after burn. In the control group the scab was rejected 3-4 days later.

Morphological changes (coagulation necrosis of epidermis and the superficial layer of the derma) were similar in all the animals on day 1 of experiment. The healing process was longer in the group of untreated animals, where necrotic fragments were often seen on the damaged skin.

The most intensive formation of the connective tissue (seen as abundant granulations) was observed in PO-treated rats. It was attended by pronounced vascular hyperemia and inflammatory cell infiltra-

TABLE 1. Decrease in the Surface of Thermal Burns and Times of Wound Healing in Rats Treated with Ointments Containing Antibiotics and Collase

Preparation	Decrease in the wound size, day from the start of treatment, %				Healing time, days from the start of treatment
	day 8	day 12	day 21	day 27	
Control (no treatment)	18±6 (9)	66±6 (8)	92±2 (7)	98±2 (6)	26.5±0.3 (4)
Control (Laevomecol)	15±9 (9)	65±8 (8)	93±2 (6)	98±1 (6)	26.5±0.7 (6)
Control (ointment base)	22±14 (6)	78±5 (5)	97±1 (5)	100±0 (4)	23.2±1.5 (5)
Henampicol	10±8 (7)	60±13 (6)	90±5 (6)	99±1 (5)	25.0±1.0 (6)

Note. The number of animals is given in parentheses.

tion. However, these processes were developed later than in other animals (Fig. 1, *a*, *b*).

PO gel with collase provided a better wound debridement and faster formation of granulations, which did not differ from the normal connective-tissue derma in morphological characteristics and tinctorial properties (Fig. 1, *c*).

In the second series of experiments we examined the effects of ointments containing gentamycin and ampicillin with and without collase. A decrease in the wound surface was observed from day 2 to day 14 of treatment with the ointments. However, there were no significant differences between experimental and control animals in the wound surface reduction and acceleration of healing (Table 1).

Morphologically, on day 1 after burn, a pronounced coagulation necrosis of the skin caused by direct exposure to thermal factor prevailed in animals of all groups. The necrosis was accompanied by marked polymorphic cell infiltration.

The activation of the reparative process was noted on day 8, when granulations were seen. On day 14, the granulations were covered with newly formed epithelium.

In addition, considerable differences in the state of the skin were observed on day 8. In untreated rats (control), necrotic inflammatory changes predominated, while the initial signs of regeneration were obvious in rats treated with the ointments containing antibiotics.

Starting from day 21 until the end of experiment (day 30), the wound healing process was practically complete in Henampicol-treated animals (Fig. 1, *d*). Normally differentiated epidermis formed over the wound surface; its thickness varied from animal to animal, and regenerating skin appendages were seen. However, dermal edema of different severity, hyperemia, and inflammatory cellular infiltration were preserved in all the animals. Sometimes foci of sclerotic granulations were present.

Some peculiarities of skin morphology were observed in Laevomecol-treated rats at the late stage of the experiment. In these animals, wound healing was often characterized by regenerating epithelium and numerous fresh granulations infiltrated with numerous neutrophilic leukocytes, which was observed at the early stages of the wound healing process occurring in other groups of rats.

Thus, although there were no significant differences in the size of burns treated by the ointments containing gentamycin, ampicillin, and collase and in those treated with Laevomecol, morphological findings indicate that ointments with antibiotics and collase have a better effect on wound healing.

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