Effect of Ointment Containing King Crab Collagenase on Infected Wound

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 10, pp. 421-424, October, 1997
Original article submitted September 2, 1996

Hematological, biochemical, and morphological effects of ointment containing king crab collagenase on infected wounds are studied in albino rats. The concentration of collagenase in the ointment should be no higher than 0.2 mg/g ointment, the duration of treatment no longer than 3-5 days.

Key Words: *collagenase; king crab; infected wound; healing; blood components*

Proteolytic enzymes trypsin, chymotrypsin, etc., are often used in the treatment of wounds of different origins in order to stimulate the rejection of necrotic tissues [4]. Effective necrolytic action of collagenase derived from the hepatopancreas of king crab (*Paralithoides camtschatica*) has been demonstrated for infected wounds [1,3]. There are no commercial ointments based on this enzyme. Our purpose was to assess the effect of an ointment based on king crab collagenase on the healing of purulent wounds in rats. The development of this ointment is in progress at the *Vektor* State Research Center of Virology and Biotechnology.

MATERIALS AND METHODS

Collase is a crude enzyme preparation of the king crab hepatopancreas possessing collagen- and caseinolytic activities [2]. Polyethylene oxide gel (Novosibirsk Drug Plant) is the base for the ointment manufactured by *Vektor* company. Collase ointment with pH 7.0-8.0 retains its activity for at least 6 months at 5-8°C.

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The effect of collase ointment on the healing of infected wounds was studied in male Low rats weighing 180-200 g. After depilation and treatment of the skin, flat skin wounds 2 cm in diameter were made on the back of narcotized animals. Bacterial suspension (0.5×10^9 bacterial corpuscles) containing 24-h cultures of *P. aeruginosa* (ATCC-27853) and *S. aureus* (ATCC-25923) was applied to the wounds. All animals were divided into 6 groups, 10 per group. In controls (group 1), no drugs or suspension were used. In group 2 (control), Iruxol ointment with clostridium peptidase A (Pliva) was applied. This ointment is widely used for enzymatic clearance of wounds. In group 3 (control), only the base of the tested ointment was used. In groups 4, 5, and 6 (experiment) the original unguent containing 0.2, 0.4, and 0.8 mg collase/g was used, respectively. The animals were followed up from the moment of wound infection. The ointment was applied daily with sterile spatula starting from day 3 after infection and formation of pyonecrotic wounds. The signs of inflammation were recorded and the size of the wound measured. On days 3, 5, 8, and 14 of treatment blood was collected for hematological and biochemical analysis and tissue fragments were dissected from the bottom of the wound for histological analysis.

0007-4888/97/0010-0992$18.00

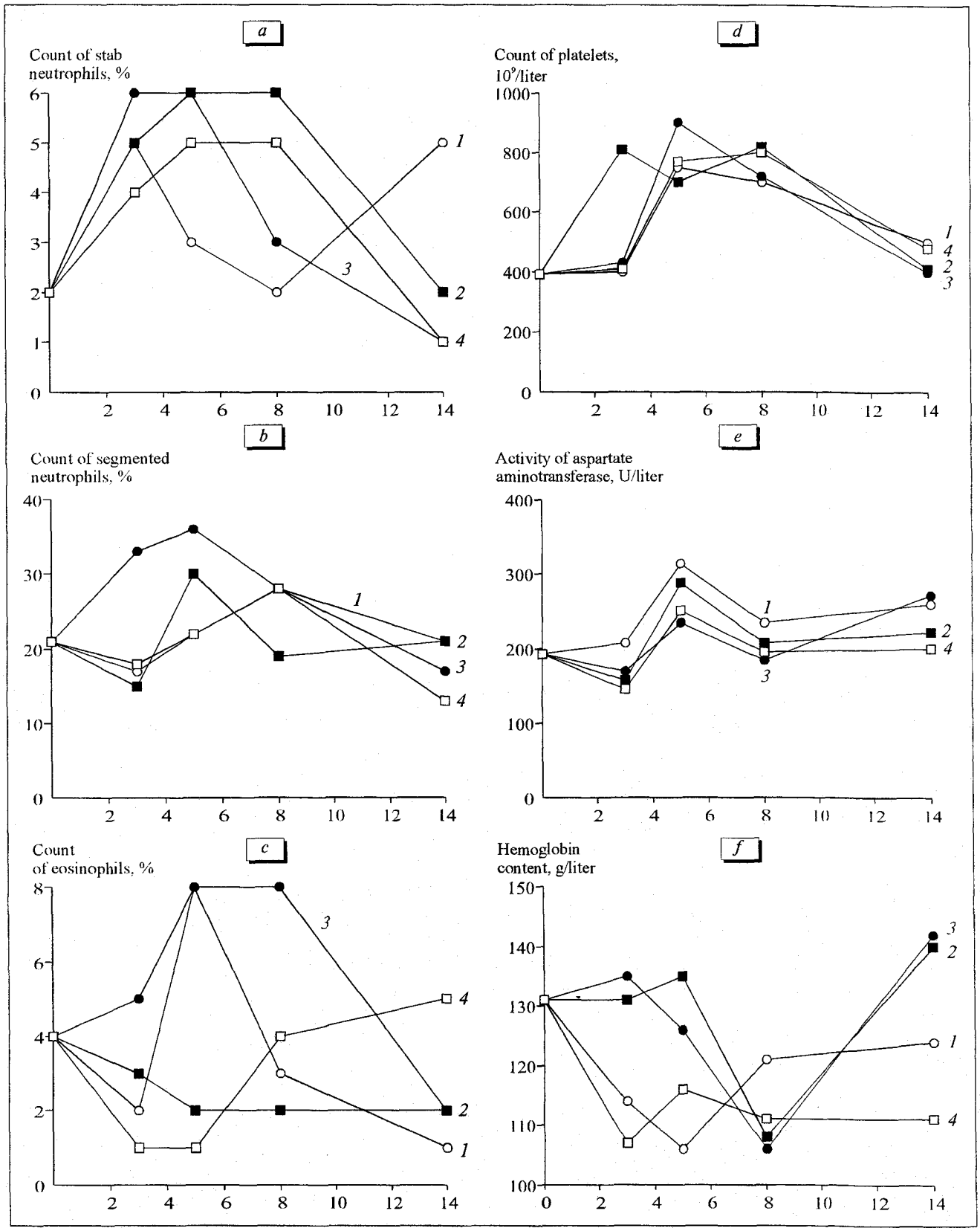

Fig. 1. Time course of some hematological and biochemical parameters of rat peripheral blood during treatment of infected skin wounds. *1*) animals treated with Iruxol ointment (group 2); *2-4*) rats treated with collase ointment in concentrations 0.2 (group 4), 0.4 (group 5), and 0.8 mg/g ointment (group 6), respectively. Abscissa: day of investigation.

TABLE 1. Contraction of Infected Skin Wounds under the Effect of Collagenase Ointments (*M±m*)

Group of animals	Ointment	Wound contraction after beginning of treatment, %				
		day 3	day 4	day 5	day 6	day 7
1	Control	41±6	75±10	64±9	74±9	88±11
2	Iruxol	40±5	66±9	65±8	78±10	91±11
3	Unguentum base	49±7	70±9	67±10	75±9	78±10
	Collase ointment, mg/g:					
4	0.2	54±8	70±10	77±10	74±10	90±11
5	0.4	52±8	50±6	47±6	69±10	71±8
6	0.8	45±7	66±9	65±9	67±9	69±8

For microscopic examination, tissue specimens from the bottom of the wounds were fixed in 10% formalin and then treated routinely. Paraffin sections were stained with hematoxylin and eosin.

Serum contents of collase and antibodies in experimental animals were assessed by solid-phase enzyme immunoassay.

RESULTS

Preliminary *in vitro* experiments showed the absence of bactericidal and bacteriostatic activities of collase for *P. aeruginosa* and *S. aureus* cultures, therefore we could rule out the probability of such effect of collase in infected wounds.

The time course of healing of pyonecrotic wounds varied in different experimental groups (Table 1). By the beginning of treatment the wounds were obviously pyonecrotic with the signs of local inflammation. After 4 days of treatment the size of the wounds decreased by 66-70% in groups 1-4. The wounds in groups 2 and 4 were covered with thin dry crusts, in many animals the crusts exfoliated, the wounds were clean and pink. In groups 1 and 3, the crusts were more coarse, in group 3 there was a marginal ridge. During the next 3 days the wound crust was completely detached, and the wounds virtually healed in groups 1, 2, and 4.

Application of the ointments for more than 4 days in groups 4-6 delayed the contraction of wounds. This was apparently caused by injury to the wounds and inhibition of regenerative processed by collase, particularly in groups 5 and 6.

Hematological analysis of peripheral blood carried out over the course of treatment showed increased counts of stab and segmented neutrophils, eosinophils, and platelets (Fig. 1) in comparison with the initial values (2.0±0.5%, 21.0±6.6%, 4.0±1.4%, and 392±112×10^9/liter, respectively). In group 6, hemoglobin concentrations were lowered (Fig. 1, *f*). Biochemical analysis of the blood showed increased activity of aspartate aminotransferase (Fig. 1, *e*) *vs.* the initial value.

Changes in the above characteristics of the blood in controls (groups 1-3) and experimental (groups 4-6) animals were caused mainly by intoxication and painful irritation of infected wound but not by toxic effect of collase and other components of the ointment. Changes in the blood were less expressed in the animals treated with collase-containing ointment (the least in groups 4 and 5) than in those treated with Iruxol ointment (group 2).

Analysis of the sera of animals treated with collase-containing ointments (groups 4-6) showed that the protein was not absorbed through the wound surface (the sensitivity of the method 250 ng collase/ml blood serum), and antibodies to collase were produced on days 3, 5, 8, and 14.

Examinations of skin and underlying tissue specimens at the site of infected wound on days 3, 5, 8, and 14 showed edema and plethora of the derma on days 3 and 5 with lymphocytic infiltration, numerous plasma cells and macrophages, and necrotic zones in the wound in all the groups. After 8 days, many fibroblasts and young vessels appeared at the wound edges, and necrotic zones disappeared in all animals. The granulation tissue was more mature in group 4 than in other groups. This tissue contained collagen bundles, and the wound surface was lined with epithelium. After 14 days, mature granulation tissue was formed in all animals. In groups 2 and 4, the course of wound epithelialization and healing were much better in general.

Thus, collase-containing (0.2 mg/g) ointment has a pronounced necrolytic and healing effect on infected experimental wounds when applied for 3-5 days. Higher concentrations of collase and longer treatment are not recommended, because wound healing is slower. The time course of hematological and biochemical parameters of peripheral blood and histological observations indicate a therapeutic activity of collase ointment cleansing the wounds similarly as the enzymatic ointment Iruxol.

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Isolation and Characterization of Soluble β-Amyloid and Apolipoproteins from Cerebrospinal Fluid

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 124, No. 10, pp. 425-428, October, 1997
Original article submitted June 24, 1996

An original method of isolation and purification of soluble β-amyloid and apoproteins from the cerebrospinal fluid of healthy donors is developed. The method consists of purification of high density lipoproteins by centrifugation of cerebrospinal fluid and reverse phase high-performance liquid chromatography of isolated lipoproteins. The obtained β-amyloid and apoproteins from cerebrospinal fluid are characterized immunologically and by mass-spectroscopy.

Key Words: *β-amyloid; Alzheimer disease; high density lipoproteins; cerebrospinal fluid; protein purification*

β-Amyloid (Aβ) is a major component of amyloid deposits in the cerebral tissue in Alzheimer disease, Down syndrome, and normal aging. Since 1984, when this Aβ was first discovered, and until recently this protein was regarded as pathological. However, in 1992 Aβ in a soluble form (sAβ) was detected in conditioned media of some cell strains and in the plasma and cerebrospinal fluid (CSF) not only in patients but also in healthy subjects [3,10,12]. The concentration of sAβ in the plasma is about 1 ng/ml [10], while in CSF it varies from 4 to 20 ng/ml [9,11,12].

However, simple methods of quantitative isolation of native sAβ from biological fluids so far have not been developed, and therefore synthetic analogs are used for evaluating its structural and biological properties. Isolation of sAβ from human plasma and CSF by affinity chromatography and immunoprecipitation with anti-Aβ antibodies has been reported [10,12]. However, these protocols are suitable for analytic rather than preparative purposes. Moreover, these methods require expensive and not always available antibodies. In light of this, the development of a simple method of preparative isolation of sAβ is of great importance for understanding the structure and metabolism of this protein and its role in the pathogenesis of Alzheimer disease. It was found that sAβ in the plasma and CSF is associated with high density lipoproteins (HDL) [6], which served as the basis for the development of isolation and characterization of native sAβ from CSF HDL. Apart from sAβ, various apolipoproteins were isolated from HDL. Apolipoprotein composition of CSF is little studied, therefore, we identified these apoproteins using immunoblot analysis and mass-spectroscopy.

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0007-4888/97/0010-0995$18.00