Comparative Study of Proteolytic Enzymes Used for Debridement of Purulent Wounds

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Necrolytic and fibrinolytic activity of 6 proteases used in surgery for debridement of purulent wounds is studied *in vitro*. The results are compared with the therapeutic effect elicited in a rat model of purulent wound. Crab collagenase, lysoamidase, and chymopsin are more active than protease C, terrilytin, and caripazyme. The results obtained agree with the data on the activity of proteases towards wound proteins and lay the basis for an original explanation of the mechanisms responsible for wound debridement with the help of collagenolytic proteases.

Key Words: *proteolytic enzymes; activity; purulent wounds; therapy*

Fibrin, a major protein of wound exudate, maintains the microcirculatory disorders during acute inflammation, and therefore should be removed from the wound [9,10]. Wound healing is often hampered by the presence of necrotic tissues composed predominantly of denatured collagen and elastin. These tissues are retained on the surface of granulations by native collagen fibers and contain no proteases. For this reason, their autolysis is very slow and prolonged, which leads to the prevalence of catabolic processes in the wound and the slowing down of maturation and reepithelialization of granulation tissue [5]. However, due to their various specificity, not all proteases used in surgery hydrolyze fibrin on the wound surface or collagen of necrotized tissue with equal efficiency. In the present study we have compared *in vitro* and *in vivo* several proteases used in surgery for debridement of purulent wounds.

MATERIALS AND METHODS

Six proteolytic enzyme preparations approved for use in Russia: chymopsin, terrilytin, caripazyme, protease C, lysoamidase, and crab collagenase, were studied. Crab collagenase is a relatively new preparation [7]; some of its properties were reported previously [1,3,6].

The necrolytic activity of a protease was evaluated from the amount of dissolved collagen and collagen degradation products after a 24-h incubation with 200 mg burn scab under optimal conditions [4]. For determination of fibrinolytic activity (FA), an enzyme solution (100 μl) was applied to fibrin gel and incubated for 24 h. The gel was prepared in graduated vials with parallel walls by mixing 2 ml 0.3% bovine fibrinogen and 0.04 ml 0.2% bovine thrombin (both preparations were from the Kaunas Plant of Bacterial Agents). For determination of direct FA the vials with gels were heated at 86°C for 30 min. The effectiveness of fibrinolysis was evaluated from the volume of lysed gel (percentage) [2].

Purulent wounds were modeled by the standard method [8] in 49 male albino rats. Native proteases (5 mg/ml) were applied to a standard 4-cm^2 three-layer gauze pad. Normal saline was applied on gauze pads to the wounds of control animals. The dressings were changed every day for 5 days.

Biopsy material for histological study was collected on the 3rd day after the start of treatment,

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TABLE 1. Necrolytic and Proteolytic Activity of Proteases *in Vitro* (*M±m*)

Enzyme	Enzyme concentration, mg/ml	Caseinolytic activity, PU/mg	Amount of hydrolyzed protein, %			
			scab		fibrin	
			in 3 h	in 24 h	direct activity	total activity
Chymopsin	0.3	0.06	0[1]	0.5±0.4		
	1.0		0.4±0.2	1.2±0.1	45±3	70±5
Terrilytin	0.3	9.0	0	0		
	1.0		0	0	38±3	50±1
Caripazyme	0.3	7.0	0.6±0.2	4.3±0.2		
	1.0		2.1±0.1	5.4±0.5	12±1	47±2
Protease C	0.3	1.6	0.5±0.1	3.9±0.2		
	1.0		2.5±0.2	5.9±0.4	50±3	60±4
Lysoamidase	0.3	2.4	0.7±0.1		3.9±0.2	
	1.0		2.1±0.4	5.4±0.3	26±2	65±1
Crab collagenase	0.3	0.06	2.9±0.2*		6.8±0.1*	
	1.0		4.8±0.3*	13.2±0.3*	66±4*	87±3*

Note. Data of 5 experiments with each enzyme are shown. [1]Minus the scab autolysis data. PU: proteolytic unit. *$p<0.01$.

fixed in 10% neutral Formalin, dehydrated in absolute alcohol, and embedded in paraffin. Sections 7 μ thick were stained with hematoxylin-eosin. The specimens were examined at magnifications of 90 and 160.

RESULTS

Of the proteases studied, crab collagenase proved to have the highest necrolytic activity. The activity of a 0.03% solution of collagenase manifested itself after just 3 h of incubation, whereas the other proteases in the same amount at this time displayed an activity not much higher than that of scab autolysis (Fig. 1).

When the enzyme concentration was increased to 0.1%, most preparations showed an enhanced ability to hydrolyze the necrotic detritus, although the range of variation was quite wide — from 0.5% (terrilytin and chymopsin) to 3-5.3% destroyed scab protein in 3 h of incubation (protease C and collagenase). After 24 h a higher degree of hydrolysis was recorded, indicating an increase in the necrolytic capacity of the proteases. Crab collagenase again showed the highest activity.

It should be emphasized that the results of *in vitro* studies do not reflect the true activity of proteases in wound content rich in inhibitors. It is important, however, that the necrolytic activity of the enzymes determined in relation to the wound substrate did not correspond to their commercial activity, which is traditionally determined in relation to milk protein (casein).

Our procedure for studying the FA of proteases involved the separate determination of the total effect of the enzymes on the fibrin clot and direct hydrolysis of fibrin (Table 1). The total FA was the sum of direct hydrolysis of fibrin and hydrolysis mediated by plasminogen, which is present in small amounts in a commercial fibrinogen preparation.

Chymopsin, crab collagenase, protease C, and lysoamidase exhibited the highest total activity: the fibrin clot was completely lysed by these enzymes after 36-48 h. The activity of terrilytin and caripazyme was much lower. Similar results were obtained in the direct FA assay.

Analysis of the results of both experiments shows that crab collagenase is the most potent fibrinolytic and plasminogen-activating agent. It is noteworthy that crab collagenase has a relatively low specific activity towards casein. The FA activity of chymopsin and lysoamidase was to a great extent mediat-

TABLE 2. Effectiveness of Native Proteases in Debridement of Purulent Wounds *in Vivo* (*n*=7)

Protease	Days of therapy		Contraction rate, % per day
	debridement	healing	
Chymopsin	4.8±0.3	-	7.8±0.2
Terrilytin	6.9±0.1	-	3.9±0.1
Caripazyme	7.3±0.3	-	2.8±0.3
Protease C	6.3±0.1	-	6.1±0.2
Lysoamidase	4.8±0.2	-	7.6±0.2
Collagenase	3.2±0.4*	14.2±0.4*	8.9±0.1*
Untreated wound	11.3±0.6	27.3±3.7	2.2±0.3

Note. *$p<0.05$. A dash signifies not determined.

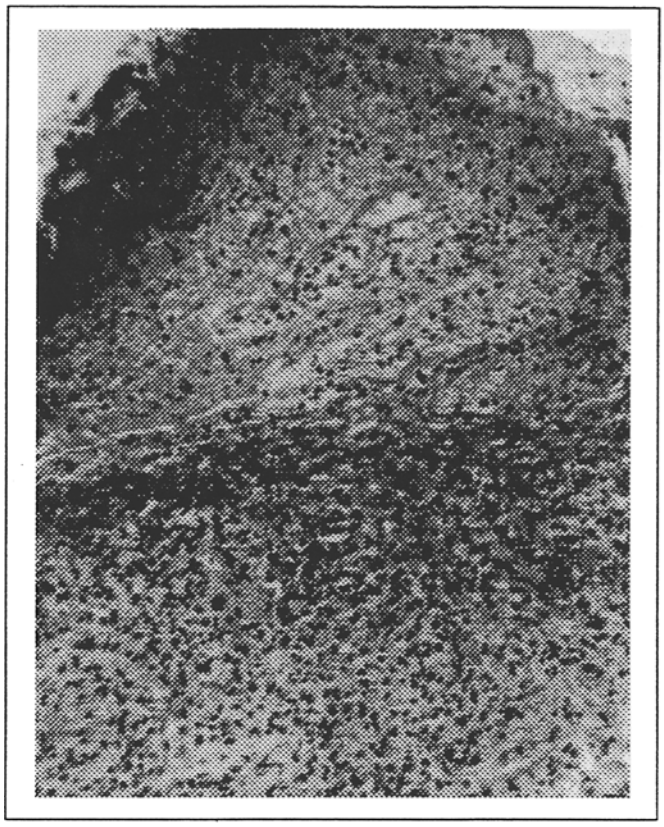

Fig. 1. Bottom of untreated wound on day 5 of observation. Necrotic detritus firmly attached by native collagen fibers to underlying granulations is seen on the wound surface. Here and in Figs. 2 and 3: histophotogram, hematoxylin and eosin staining, × 160.

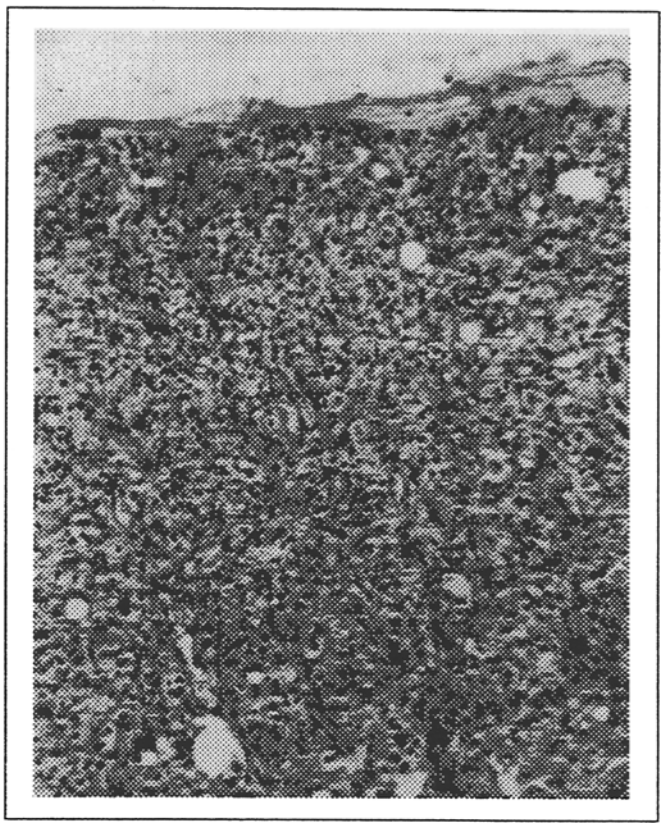

Fig. 2. Bottom of wound two days after treatment with collagenase. Mature granulations contain large amounts of collagen and fibroblasts. There is no necrotic detritus on the wound surface.

ed by plasminogen activation. The effectiveness of direct fibrin hydrolysis by chymopsin, which has a high total FA, was comparable to that of terrilytin. Caripazyme showed a relatively low direct FA activity. Consequently, FA of proteases employed for wound necrolysis is due primarily to direct hydrolysis of wound fibrin. At the same time, wound exudate contains proteases that after exogenous activation by plasminogen (or other enzymes) may play a certain role in wound cleansing.

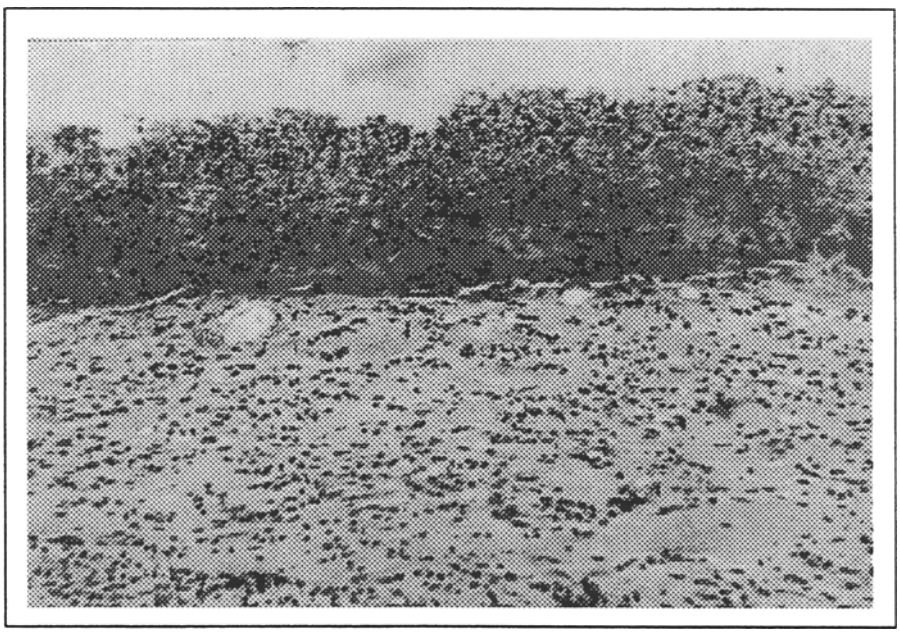

Fig. 3. Bottom of wound two days after treatment with lysoamidase. Granulation tissue contains large amounts of collagen; inflammation is moderate. The wound surface is covered with a thin layer of necrotic detritus with a well-pronounced demarcation line.

Thus, expanded analysis of the substrate specificity of enzymes used for wound debridement revealed a discrepancy between their activity towards wound proteins (necrotic scab and fibrin) and towards the standard substrate casein. It can be concluded that the conventional approach to the comparison of proteases is not informative enough; and therefore the specific activity of enzymes towards wound proteins should be considered when enzyme-containing preparations for the control of wound healing are developed.

To validate our conclusion that a fundamentally new approach to the comparison of proteases for wound debridement is necessary, namely the use of wound proteins as a substrate, we studied the effects of native proteases on wound healing in laboratory animals.

Prior to the application of protease, the wounds had inflamed surrounding tissues, were covered with grayish necrotic detritus which was firmly attached to granulation tissues, and contained moderate amounts of pus. By day 3 of treatment with collagenase, the wounds were free of detritus and covered with red plethoric granulations. It is noteworthy that after collagenolytic hydrolysis, the detritus from the wound surface could be removed in a continuous layer like epidermis from surface burns. The wounds rapidly contracted at a mean rate of 9% of the initial surface per day and healed on day 14. Lysoamidase and chymopsin cleansed the wounds after 4-5 days of application. Unlike with collagenase, the detritus was lysed and removed tangentially or focally. The wounds were covered with pink granulations and contracted at a mean rate of 7.7% per day. Wounds treated with protease C, terrilytin, and caripazyme took longer to be freed of necrotic detritus (after 6-7 dressings). They were covered with pink granulations and contracted at a mean daily rate of 4.3%. In untreated animals the wounds for a long time remained covered with necrotic detritus, which was firmly attached to the underlying tissues. Granulations formed on days 9-11, when the wounds were free of pus and necrotized tissues (Table 2).

These observations were confirmed by histological studies. Prolonged and pronounced inflammatory processes occurred in untreated wounds. Granulations were covered with a thick layer of necrotic detritus, which was firmly attached by native collagen fibers to the wound surface (Fig. 1). Differences in the morphology of modeled wounds were noticeable on day 3 of therapy with the enzyme. The bottom of collagenase-treated wounds was covered with mature granulations with a large number of fibroblasts and an abundance of newly synthesized collagen. There was no necrotic detritus on the wound surface (Fig. 2).

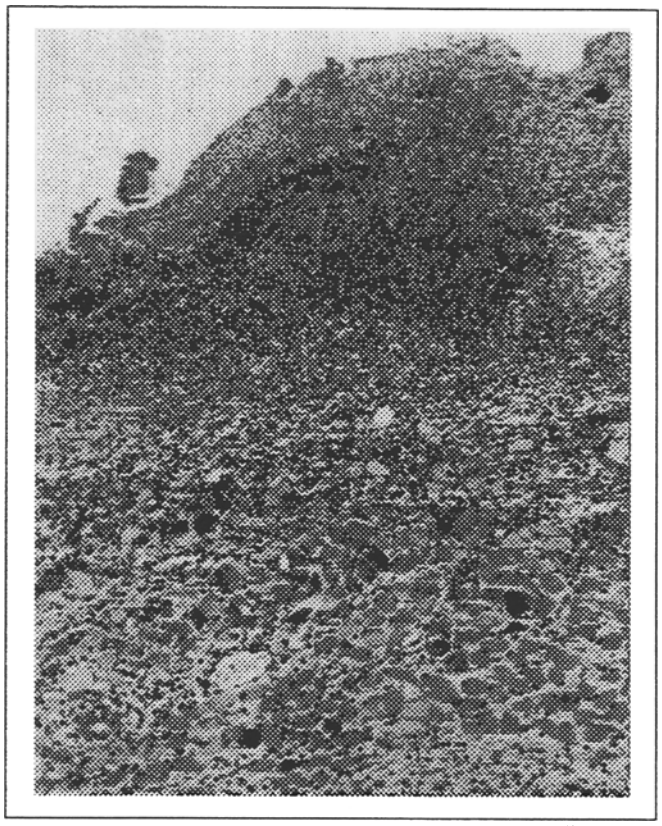

Fig. 4. Bottom of wound two days after treatment with terrilytin. Granulations are covered with a thick layer of necrotic detritus firmly attached to the underlying tissues. Granulations are immature, and proliferative and synthetic processes are weak. Histophotogram, staining with hematoxylin and eosin, × 90.

On day 3 of treatment with lysoamidase or chymopsin, the granulation tissue on the bottom of the wounds contained large amounts of collagen and was moderately infiltrated by polymorphonuclear neutrophils. The granulations were covered with a 100-μ thick layer of detritus with a well-pronounced demarcation line (Fig. 3). On day 3 of therapy, the wounds treated with protease C, terrilytin, or caripazyme were in the worst condition. Histologically, they looked alike. Granulations were covered with detritus (up to 600 μ thick), which was firmly (as in the controls) attached to them by native collagen fibers. The granulation tissue was immature, and proliferative and synthetic processes in it were weak (Fig. 4).

On the basis of clinical observations, histological studies, and previously reported immunochemical data obtained after application of crab collagenase [8] we propose an original interpretation of the mechanism of wound debridement with proteases possessing various specificity. Crab collagenase interacts with the detritus both from the outside and from its borders with healthy tissues, where it hydrolyses native collagen fibers or fibrin deposits, thus

separating a layer of necrotic masses and detritus and promoting their rejection. Proteases not possessing collagenolytic activity carry out necrolysis layer-by-layer, making it longer for wounds to be cleansed and to heal. The comparatively high proteolytic and therapeutic activity of crab collagenase prompts its further investigation for use in clinical practice for the debridement of purulent wounds.

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