EFFECT OF TRYPSIN INHIBITOR ON THE TOXICITY AND PROTEOLYTIC ACTIVITY OF THE SERUM AND ORGANS IN THERMAL BURNS

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The toxic properties and proteolytic enzyme activity after burns were studied in experiments on albino rats treated with the protease inhibitor Contrical. On the 8th day after burning, the proteolytic activity in the liver and kidneys of the rats was increased and extracts of these organs possessed marked toxicity. The serum also exhibited toxic properties. Injection of Contrical diminished or, in some cases, completely abolished the toxic action. The proteolytic enzyme activity was lowered in the case of those organs and enzymes whose activity had been increased after burning. The results indicate definite correlation between the increased preteolytic activity and the formation of the toxemic process.

KEY WORDS: proteolytic activity; trypsin inhibitors; thermal burns.

After thermal trauma in animals, correlation is found between the increased rate of protein breakdown and changes in the toxic properties of the serum detected by the hemoculture method [5]. To restore the normal protein, metabolism inhibitors of trypsin and kallikrein – Trasylol and Contrical – are used [1-3, 8-10]. Trasylol, in particular, if injected in large doses into burned mice, reduces the excretion of amino nitrogen with the urine [9]. With the inhibition of protein breakdown and a decrease in the quantity of protein breakdown products entering the bloodstream, there is a corresponding decrease in the intensity of autointoxication in burned patients under treatment with Trasylol [7, 10]. However, as yet no concrete evidence is available of changes in the toxic properties of the serum and organ extracts in burns during the action of inhibitors of trypsin-like enzymes.

The object of the present investigation was to compare the proteolytic activity and toxicity of the serum and tissue extracts after thermal burns during Contrical treatment.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred albino rats weighing 150-200 g. Ten animals were intact (control); a flame burn covering 20% of the body surface with an exposure of 45 sec was inflicted on 20 animals. Ten of these 20 animals received intraperitoneal injections of Contrical (VEB Arzneimittelwerk, East Germany) in a dose of 10,000 antitrypsin units per rat daily from the time of burning for the next 7 days. The other 10 rats did not receive Contrical. The animals were decapitated on the 8th day after burning. Under sterile conditions blood was collected and the liver and kidneys removed. The protein content (by Lowry's method) and the activity of cathepsin D [6], cathepsin B, trypsin-like proteases (by their behavior toward benzoylargininamide at pH 5.3 and 8.2 respectively), and of leucine-aminopeptidase (by its action on leucinamide [1]), were investigated in the serum and also in saline and glycerol ex-

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TABLE 1. Effect of Contrical on Proteolytic Enzyme Activity and Toxicity of the Serum and Organs of Burned Rats (M ± m)

Test object	Factor studied	Control (I)	Burns (II)	P_{I-II}	Burns + Contrical (III)	P_{II-III}
Serum	Cathepsin D	35±5	34±5	>0,2	30±7	>0,2
	Trypsin	28±4	22,4±1	>0,2	29±2	>0,2
	Leucine-aminopeptidase	282±2	200±33	>0,2	200±5	>0,1
	Toxicity	+20±5,9	—19±2,2	<0,001	+18±4,7	<0,001
Liver	Cathepsin D	4,2±0,47	8,1±0,7	<0,001	4,5±0,23	<0,001
	Cathepsin B	1,7±0,13	2,0±0	>0,2	1,8±0,09	>0,1
	Trypsin	1,8±0,2	2,8±0,3	<0,05	2,3±0,3	>0,2
	Leucine-aminopeptidase	10,3±0,6	15±1,1	<0,05	15±1,2	>1
	Toxicity	+22±5,7	—18±2,4	<0,001	+26±4,7	<0,001
Kidneys	Cathepsin D	9,2±0,3	11,6±0,8	<0,05	7,8±0,3	<0,05
	Cathepsin B	1,86±0,9	2,1±0,1	>0,05	2,0±0,03	>0,05
	Trypsin	3,4±0,6	4,2±0,1	>0,2	3,2±0,04	<0,05
	Leucine-aminopeptidase	19,6±1,1	25±1,2	<0,05	15,8±0,8	<0,05
	Toxicity	+18±3,3	—14±2,9	<0,001	+21±3,6	<0,001

Legend. Activity of cathepsin D shown in μg tyrosine/mg protein; activity of cathepsin B, trypsin, and leucine-aminopeptidase shown in ‰ N_2/mg protein; toxicity given in conventional units [5].

tracts of the organs. The toxic effect of the serum and of saline homogenates of the organs was determined at the same time with respect to changes in migration of the leukocytes in a culture of peripheral blood leukocytes [4].

EXPERIMENTAL RESULTS AND DISCUSSION

As Table 1 shows, on the 8th day after burning, the activity of the proteolytic cathepsin D and leucine-aminopeptidase in the liver and kidneys of the rats increased and saline extracts of these organs showed marked toxicity. Similar toxicity was exhibited also by the serum, but no increase in proteolytic enzyme activity was observed in the serum at this period.

In the rats receiving Contrical the toxic effects of the serum and organs were reduced, or in some cases completely abolished, and the proteolytic enzyme activity was increased by a lesser degree: during administration of Contrical the cathepsin D activity in the liver was restored to normal; cathepsin D and leucine-aminopeptidase activity in the kidneys also returned to normal.

The activity of cathepsin B and of the trypsin-like proteases in the organs and the activity of all the proteolytic enzymes studied in the serum that had not increased as a result of the burns likewise remained unchanged under the influence of Contrical.

Contrical, an inhibitor of trypsin and kallikrein, when administered to a burned animal thus inhibited the proteolytic activity of enzymes that differ considerably from trypsin: cathepsin D and leucine-aminopeptidase. However, the action of Contrical on leucine-aminopeptidase is not universally consistent. It restores the activity of this enzyme to normal only in the kidneys and not in the liver, where it is also increased after burning. Under these circumstances Contrical completely abolished the toxicity both in the serum and in the liver and kidneys.

The differences in the intensity of the action of Contrical on the enzyme systems in different organs and tissues may be due to the pattern of its distribution between the organs, for it mainly enters the kidneys [11]; another possibility is that Contrical may act not so much on the tissue cathepsins as on factors causing their increased activity in burn trauma.

Since Contrical, an inhibitor of proteolytic enzymes, reduces toxicity in burns it can be postulated that the appearance of toxicity in burned rats is connected with an increase in their proteolytic activity. However, the toxicity evidently cannot be produced entirely by tissue proteinases, for there is no precise parallel between the action of Contrical on toxicity and on proteolytic enzyme activity in burns.

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