TOXICITY AND PROTEOLYSIS IN GERMFREE RATS WITH THERMAL BURNS

T. L. Zaets, I. K. Koryakina, T. I. Kotkina, and G. I. Podoprigora

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The toxic properties of the organs and activity of proteolytic enzymes in them were studied in experiments on germfree rats after thermal burns. In the absence of a pathogenic microflora a state of toxemia was found to develop and the proteolytic enzyme activity was increased. The intensity of the toxic and catabolic reactions depended on the severity of the burns. The toxic manifestations and increase in proteolysis in the germfree rats were in some cases more marked than in ordinary animals. It is concluded that tissue sources of toxic products play an important role in burns.

KEY WORDS: burns; toxemia; proteolysis; germfree animals.

Burns and, in particular, the period of toxemia have been shown to be accompanied by increased breakdown of tissue proteins [2, 7-9]. Not only is the activity of proteolytic enzymes in the organs increased under these circumstances, but extracts of the organs have marked toxicity [1, 3, 5, 10]. The view that in burns increased breakdown of organ proteins is due to activation of tissue proteinases requires special evidence in its support, for proteolytic enzymes may also be bacterial in origin. The toxicity of serum and organ extracts may also be due to products of bacterial metabolism.

In this investigation the dynamics of the toxemia and the level of proteolysis were studied in germfree rats after burns.

EXPERIMENTAL METHOD

Germfree Wistar rats were obtained from the Center for Selection and Breeding of Germfree Animals (Orleans, France). The germfree rats were reared in germfree isolators and given sterile diets [11]. The diets were sterilized in a vacuum autoclave at 123°C for 25 min. The germfree rats were subjected to microbiological control tests in accordance with the usual scheme [12]. Parallel experiments were carried out on noninbred ordinary rats of the same weight (150-200 g). A flame burn covering 20% of the body surface was inflicted on some animals of both groups for an exposure of 50 sec, and on other animals for 20 sec. The animals were decapitated on the eighth day after burning. Blood was taken and organs removed under sterile conditions. The protein concentration (by Lowry's method) and activity of cathepsin D, cathepsin B, trypsin-like proteases (against benzoyl-argininamide at pH 5.3 and 8.2 respectively), and of leucine aminopeptidase [1] were determined in the serum and in saline and glycerol extracts from the organs. The toxic effect of the serum and saline homogenates of the organs was determined at the same time on the basis of changes in migration of leukocytes in a leukocyte film culture [4, 6].

EXPERIMENTAL RESULTS AND DISCUSSION

As Table 1 shows, on the eighth day after burns, activity of several proteolytic enzymes was increased in nearly all the organs and in the blood serum of the ordinary rats. The changes were greatest in the liver,

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TABLE 1. Effect of Thermal Burns on Proteolytic Enzyme Activity and Toxicity of Blood Serum and Organs of Germfree and Ordinary Rats

Organs	Index studied	Ordinary control rats (15)	Ordinary rats burned for 50 sec (15)	Germfree rats (5)	Germfree rats burned for 50 sec (5)	Germfree rats burned for 20 sec (7)
Blood serum	Toxicity	+20	—19*	+10	—29*	—16*
	Cathepsin D	7,3±2,8	26,0±7,7*	5,0±0,8	21±7*	21±3*
	Trypsin-like proteases	19,4±3,2	20,5±2	20,0±2	24,4±3	24±1
	Leucine aminopeptidase	257±5	254±47	219±14	243±8	238±11
Liver	Toxicity	+20	—18*	+14	—27*	—8*
	Cathepsin D	4,5±0,4	8,2±0,6*	2,4±0,1	8,0±0,5*	2,8±0,5
	Trypsin-like proteases	2,5±0,3	4,0±0,9	1,7±0,1	2,0±0,2	1,6±0,2
	Leucine aminopeptidase	15,8±2	20,3±1,9	15,4±0,2	19,8±0,6*	13,3±1,2
Kidneys	Toxicity	+18	—14*	+17	—30*	—13*
	Cathepsin D	8,6±0,7	11,4±0,9*	5,3±0,7	10,3±0,8*	8,6±0,8*
	Trypsin-like proteases	4,7±0,4	4,1±0,5	1,5±0,4	1,4±0,4	2,4±0,4
	Leucine aminopeptidase	20,1±2	28,7±2,4	14,6±0,2	14,3±1,1	19,8±3
Pancreas	Toxicity	+22	—16*	—	—	—
	Cathepsin D	6,8±1,2	11,2±1,7*	3,5±0,2	8,0±1,3*	10,9±2,2*
	Trypsin-like proteases	3,5±0,5	9,1±2,4*	1,0±0,5	1,0±0,6	2,4±0,4
	Leucine aminopeptidase	17,3±2,9	19±3,8	7,7±0,2	9,0±3	11,6±0,5*
Spleen	Cathepsin D	11,9±0,9	13±1,1	9,0±1,3	21,6±2,4*	8,5±0,6
	Trypsin-like proteases	2,8±1	4,2±1,5	1,8±0,5	3,6±0,6*	2,5±0,4
	Leucine aminopeptidase	17,4±2,1	18,6±4,6	14,1±0,8	22,4±1,4*	15,2±0,2
Heart	Cathepsin D	5,8±0,2	7,6±1,5	5,3±0,1	14,2±1,9*	3,5±0,2
	Trypsin-like proteases	1,7±0,5	3,7±0,2*	2,4±1	1,5±0,2	2,8±0,2
	Leucine aminopeptidase	8,6±1,3	9,3±1,5	7,7±0,4	12,8±1,4*	4,3±0,4
Muscles	Cathepsin D	4,1±0,3	6,9±0,5	6,9±0,8	8,3±0,9	8,1±0,5
	Trypsin-like proteases	2,6±0,5	3,4±0,6	1,1±0,4	3,6±0,8*	2,7±0,2
	Leucine aminopeptidase	11,2±2	16,9±2,9	9,2±1,1	12,2±1,8	6,9±0,9

Legend. 1) Toxicity expressed in conventional units, content of cathepsin D in μg tyrosine/mg protein of extract, activity of trypsinlike proteases and leucine aminopeptidase in μg nitrogen/mg protein of extract; 2) Indices differing significantly ($P < 0,05$) in burned and control animals marked by asterisk.

pancreas, and muscles. The increase in cathepsin D activity in the serum was significant but variable. Activity of proteolytic enzymes in the kidneys was increased by 30-40%. In the spleen and heart muscle only a small increase was found in the activity of cathepsin B and trypsin-like proteases, respectively.

Besides the increase in enzyme activity, considerable toxicity was detected in the serum and in extracts from the liver, kidneys, and pancreas of these rats. Neither the serum nor the organs of the control rats had any toxic properties.

Proteolytic enzyme activity of the germfree rats was identical with or a little lower than that of the ordinary animals. After severe burns (exposure 50 sec) of the germfree rats considerable toxicity developed and proteolytic enzyme activity increased; in some cases, moreover, these changes were more severe than in ordinary animals (for example, a much greater increase in cathepsin D activity was observed in the kidneys of the germfree rats). Furthermore, activity of many more enzymes appeared in the germfree animals. For example, activity of leucine aminopeptidase, which was not increased in ordinary rats after burns, increased in the liver and pancreas of the burned germfree animals, and activity of trypsin-like proteases increased in their skeletal muscles. Proteolytic enzyme activity also was increased in organs of germfree rats whereas virtually no increase in enzyme activity could be detected in the corresponding organs of the ordinary rats. For instance, activity of all enzymes tested – cathepsin D, cathepsin B, trypsin-like proteases, and leucine aminopeptidase – increased in the spleen.

The immunologically intact organism thus responded more intensively to burn trauma. One possible explanation is that mechanisms of limitation of the catabolic reaction develop during the formation of the antimicrobial resistance of the animal. The intensity of the catabolic reaction of the germfree rats depended quite clearly on the severity of the lesion: After the milder burn (exposure 20 sec) no such sharp increase in proteolytic activity was observed in some organs.

Toxicity in the burned germfree rats was discovered both in the serum and in extracts of the liver and kidneys; the toxicity was much greater after burning for 50 sec than after burning for 20 sec. Since toxicity appeared in the organs and proteolytic enzyme activity increased after burning irrespective of whether the animals possessed microflora or not, but in proportion to the severity of the burn, it can be concluded that the proteolytic enzymes whose activity is usually increased in the tissues of the burned animal are true tissue enzymes. The toxic properties of the organs during this period are also evidently due in the same way to changes in tissue metabolism and not to products of bacterial metabolism.

The results described above do not, of course, rule out the role of infection in burns. Very probably when immunity is weakened as it characteristically is in burns, proliferation of bacteria in the body aggravates

the course of the toxemia and intensifies tissue breakdown. However, corrective therapy must take into account not only the bacterial, but also the tissue character of the toxemia in thermal burns.

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PROPERDIN AND PROTEIN COMPOSITION OF THE LYMPH AND BLOOD IN BURNS

V. I. Kurochkin and Yu. B. Lishmanov

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Experiments on 25 dogs showed that burns are accompanied by a regular redistribution of plasma proteins between the body fluids and by increased lymphatic resorption. Retention of properdin, albumins, and α globulins in the tissues during burns was demonstrated indirectly. The degree of burning was found to depend on the initial properdin level.

KEY WORDS: burns; properdin; proteins; lymph.

The concentration of properdin and proteins in the lymph and blood of dogs with thermal burns was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on 25 dogs of both sexes weighing 8-25 kg. A burn of the hind foot was produced in 20 dogs by immersing it in hot water (80°C) for 30 sec. Lymph from the afferent and efferent vessels of the popliteal lymph node and blood were obtained before burning and 3 and 24 h thereafter. Lymph and blood were obtained from five control dogs under the same conditions, but without burning. The properdin concentration was determined by a method based on its binding with inulin and subsequent mineralization of the properdin–inulin complex followed by isometric distillation of ammonia in Conway dishes, total protein by the IRF-22 refractometer, and the protein composition by electrophoresis in agar gel. The results were subjected to statistical analysis by the Fisher–Student method.

Department of Pathological Physiology, N. P. Ogarev Mordovian University, Saransk.

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