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EXPERIMENTAL INVESTIGATION OF THE IMMUNOTHERAPY OF BURNS

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The effect of convalescent burn serum on the toxic properties, level of activity of proteolytic enzymes, and morphological changes after burns was studied in experiments on rats. After burns the serum and organ extracts were found to acquire toxic properties, proteolytic enzyme activity was increased, and marked morphological changes developed. Injection of convalescent burn serum promoted detoxication, reduced the proteolytic enzyme activity distinctly, and reduced the severity of the morphological changes. Serum of healthy animals gave a much smaller therapeutic effect.

KEY WORDS: burns; convalescent burn serum; immunotherapy.

The acute period of burns is characterized by toxic manifestations, disturbances of the function of various organs, and predominance of catabolic processes [1, 3, 7, 8].

Because of the combination of disturbances associated with burns, a method of treatment must be used which can influence the pathogenetic basis of the functional changes.

The pathogenesis and methods of treatment of burns have now been studied for many years. The theory of the noninfectious immunology of burns has been formulated and a method of immunotherapy, consisting of treatment of burned patients with the serum of persons recovering from burns, has been developed. Although the method of immunotherapy has been widely acclaimed [2, 4-10], the study of the mechanism of action of convalescent serum still continues. The investigation of whether the processes of increased catabolism can be influenced by immunotherapy is of great interest, for increased proteolysis may be one of the sources of the toxic effects.

The object of the present investigation was to study the effect of convalescent burn serum on the toxic properties, the level of proteolytic enzyme activity, and the morphological changes in the organs of rats after burns.

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EXPERIMENTAL METHOD

Experiments were carried out on 38 Wistar rats weighing 200-250 g, divided into the following groups: 1) intact; 2) with burns, untreated; 3) animals with burns treated by injection of convalescent burn serum; 4) animals with burns treated by injection of serum from intact rats.

An alcohol flame burn measuring 15-20% of the body surface, with an exposure of 50 sec, was inflicted on the epilated surface of the back. Convalescent serum was obtained from the rats 1.5-2 months after burning. The injection of convalescent serum and of normal serum began 2 h later and continued daily for 5 days after burning in a dose of 1 ml/100 g body weight.

The toxic properties of the serum and of saline extracts of liver and kidneys were studied by the blood culture method, using the index of migration of leukocytes in a buffy coat culture [2, 3].

Proteolytic enzyme activity was studied in glycerol extract. Cathepsin D activity was studied by Anson's method, activity of cathepsin B and trypsin-like proteases against N-L-benzoyl-argininamide at pH 5.3 and 8.2, respectively, and activity of leucine-aminopeptidase against L-leucinamide. The protein content was determined by Lowry's method. The skin, liver, kidney, and the organs of immunogenesis (thymus, lymph nodes, spleen) were investigated histopathologically.

Material was fixed in Carnoy's fluid and in 10% formalin solution and embedded in paraffin wax. Sections 5 μ thick were stained with hematoxylin-eosin, with Schiff's reagent for neutral mucopolysaccharides, by Brachet's method for RNA, and with Sudan III and IV for lipids. The usual controls were set up. Special attention was paid to the depth of burning of the skin as the principal source of stressor factors, to changes in the liver hepatocytes, and to the degree of plasmatization of the cells in the organs of immunogenesis.

EXPERIMENTAL RESULTS

The results of the study of the toxic properties and proteolytic activity in the serum and extract of liver and kidneys are given in Table 1. Serum and extracts have no toxic action in intact animals. Burns caused the appearance of toxic properties both in the serum and in organ extracts, as shown by marked inhibition of leukocyte migration in blood cultures.

Proteolytic enzyme activity in the serum varied within wide limits. In liver extract the levels of cathepsin D and trypsin-like proteases were considerably increased. In kidney extract the increase in leucine-aminopeptidase activity was greatest.

After injection of convalescent burn serum into animals with primary burns a decrease or, in some cases, complete abolition of the toxic action of the serum and organ extracts was observed. The content of all proteolytic

TABLE 1. Toxic Properties and Proteolytic Enzyme Activity in Burned Animals (M \pm m)

Group of animals	Liver				Kidneys				Serum			
	toxicity	Cath D	Tp	LAP	toxicity	Cath D	Tp	LAP	toxicity	Cath D	Tp	LAP
Intact	+22 \pm 5,7	4,8 \pm 0,7	1,6 \pm 0,4	20 \pm 3	+18 \pm 3,3	7,8 \pm 0,3	3,4 \pm 0,6	19,6 \pm 1,1	+20 \pm 5,9	7,3 \pm 2,8	19,4 \pm 3,2	257 \pm 5
Burns, untreated	-18 \pm 2,4	10,5 \pm 1,4	4,4 \pm 1,2	27 \pm 2	-14 \pm 2,9	10,5 \pm 0,4	4,0 \pm 0,5	31,8 \pm 2	-19 \pm 2,2	26 \pm 7,7	20,5 \pm 2	254 \pm 17
Injection of convalescent serum	+13 \pm 3,2	3,2 \pm 0,42	1,6 \pm 0,3	20,1 \pm 1,7	+6 \pm 4,3	7,8 \pm 1,5	4,9 \pm 1,7	26,8 \pm 6	+8 \pm 5,7	3,5 \pm 0,8	18,8 \pm 4,0	291 \pm 1
Injection of normal serum	-10 \pm 4,4	6,6 \pm 1,3	2,0 \pm 0,4	21,2 \pm 2,2	-16 \pm 3,2	12,2 \pm 0,8	3,2 \pm 0,7	27,5 \pm 4,0	-15 \pm 4,0	—	21,5 \pm 3,5	277 \pm 9
P ₁₋₂	<0,001	<0,01	>0,05	>0,05	<0,001	<0,001	>0,05	<0,001	<0,001	<0,05	>0,05	>0,05
P ₃₋₄	<0,001	<0,05	>0,05	>0,05	<0,001	<0,05	>0,05	>0,05	<0,01	—	>0,05	>0,05
P ₃₋₂	<0,001	<0,001	>0,05	>0,05	<0,001	<0,05	>0,05	>0,05	<0,001	<0,02	>0,05	>0,05

Legend. Cath D) Cathepsin D, Tp) trypsin-like proteases; LAP) leucine-aminopeptidase. Toxicity expressed in conventional units, cathepsin D activity in μ g tyrosine/g protein of extract, trypsin and LAP in μ g N₂ of substrate/g protein of extract.

enzymes - cathepsin D, trypsin-like proteases, and leucine-aminopeptidase - in the liver extract returned to normal. The cathepsin D level in kidney extract returned to normal but the decrease in leucine-aminopeptidase activity was not significant.

After injection of serum from healthy animals a therapeutic effect also was observed but it was less marked than after injection of convalescent burn serum. The results of the histochemical tests showed that a large area of necrosis appeared in the skin of the rats after burning and spread gradually into the depth of the tissue. In the liver changes in the histophysiology of the hepatocytes were discovered. The deposition of glycogen in them was sharply reduced but the lipid content was increased, so that fatty degeneration of some of the hepatocytes resulted.

After injection of convalescent serum no changes were found in the content or distribution of glycogen in the hepatocytes in the liver on the 5th day after burning. Large quantities of glycogen were present in all hepatocytes. Lipid inclusions in the hepatocytes were absent and the liver had the same appearance as in the intact animals. A different picture was observed after injection of the serum of intact rats: Disturbances of carbohydrate metabolism developed and were similar to the changes in the untreated rats with burns, in which a zonal distribution of glycogen and a decrease in its content in the hepatocytes were observed. In the organs of immunogenesis a sharp response to burn trauma was observed on the 5th-7th day after burning. The response increased in intensity as the zone of tissue necrosis grew larger. The number of pyroninophilic cells in these organs was increased. In the thymus the thickness of the cortical zone was reduced and the mass of the medulla increased. Following injection of convalescent burn serum into the burned animals the histological picture into the organs of immunogenesis differed from that found in the burned animals which were untreated with serum from intact animals. On the 5th day after the injection of convalescent burn serum no reduction in the lymphocyte population of the thymus could be observed, the cortical layer was increased in thickness, the mass of the medulla was reduced, and the degree of pyroninophilia of the cells of the thymus, spleen, and lymph nodes was depressed.

Analysis of these results shows that injection of convalescent burn serum considerably reduced the severity of the toxic changes, prevents the increased catabolism, and minimizes the morphological disturbances of the organs. In all probability the therapeutic effect of convalescent serum is due to the wide spectrum of antibodies, binding toxic products of both tissue and bacterial origin, which it contains. This could be the reason for the lower reactivity of the organs of immunogenesis of the burned animals after injection of convalescent burn serum compared with the groups of untreated rats and the rats receiving serum of intact animals after burning.

It can be concluded from this investigation that convalescent serum is a highly effective agent for the treatment of burns and that it can be recommended as a component of the combined treatment of burns.

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