THERAPEUTIC VALUE OF γ-GLOBULIN IN EXPERIMENTAL BURNS

B. E. Movshev, R. V. Nedoshivina, and I. K. Koryakina

UDC 617-001.17-085.373.6-036.8-092.9

KEY WORDS: γ-globulin; experimental burns; excretory-assimilative function of the liver.

The therapeutic value of immune preparations [11] has been proved by the study of functional parameters *in vivo* after burns: nitrogen metabolism [13], toxic properties of the serum [1], liver and kidney function [7], mineral and protein metabolism in calcified tissues, bacteriemia [6], erythrodiaeresis [10], and function of the reticuloendothelial system (RES) [8]. However, the mechanism of the therapeutic action of convalescent serum has not yet been explained. It has been postulated that the effect of sera obtained from burned subjects in the recovery period may be due to an increase in its content of hormones, antibacterial antibodies, nonspecific factors of immunity, and other substances possessing the properties of biostimulators. New data confirming the value of factors of autoimmunization and autointoxication in the pathogenesis of burns have recently been obtained [3, 12]. The important role of a high-molecular-weight toxin from burned skin in the development of the initial period of the disease has been demonstrated. In previous investigations the writers studied the principles of the immunotherapeutic effect on a model of intoxication caused by injection of extracts of burned skin into animals [5], the properties of antiserum against burn toxin [2], and also the function of the RES in severe burns treated with immune γ-globulin [9].

The object of the present investigation was to study primary autointoxication in burned animals during treatment with γ-globulin against toxin from burned skin.

EXPERIMENTAL METHOD

Experiments were carried out on 67 Wistar rats weighing 180-200 g and seven noninbred rabbits weighing 2.5-3 kg. Experimental burns were inflicted on rats (200 animals) under pentobarbital anesthesia (3 mg/100 g body weight) by means of a burning spirit swab on the depilated skin of the animal's back (20% of total body surface area, exposure 45-50 sec). Toxin was isolated from saline extracts of burned skin by a direct immunochemical method, as described previously [5]. The rabbits were immunized with toxic preparations by the scheme used at the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, with Freund's complete adjuvant (from Difco, USA) at the rate of 0.5 ml/kg body weight. In the course of 12 weeks each animal received four cycles, each consisting of 20 mg protein, intradermally and subcutaneously. The rabbits were exsanguinated after reimmunization. γ-Globulin was isolated by the method in [15] and injected subcutaneously in a dose of 2-3.5 mg protein per rat 30 min after burning. The functional parameters were studied in the animals on the 1st, 2nd, and 5th days of the experiment. The excretory-assimilative function of the liver was assessed from the half-elimination time of Ujoviridin* (from Bitterfield, East Germany) from the blood stream and described previously [14]. Parameters of the kallikrein-kinin system (total esterase activity, prekallikrein, prekallikrein inhibitor) were determined by the method in [16]. All animals were divided into four groups: 1) burned rats treated with immune γ-globulin, 2) burned rats treated with normal, nonimmune γ-globulin (in the same dose and at the same times as in group 1), 3) untreated burned rats, 4) intact animals (control).

*Indocyanine green.

Laboratory of Pathologic Physiology, Central Research Institute of Hematology and Blood Transfusion, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 93, No. 6, pp. 52-55, June, 1982. Original article submitted December 10, 1981.

0007-4888/82/9306-0755$07.50

TABLE 1. Assimilative-Excretory Function of the Liver in Normal Rats and Burned Rats Treated and Not Treated with γ-Globulin ($\bar{x} \pm m$)

Group of animals	Experimental conditions	Time of experiment, days		
		1	2	5
	Uptake of Ujoviridin (T/2, min)			
1	Burns, treated with immune γ-globulin	4,3±0,5*** (n=5)	4,8±0,14*** (n=5)	3,3±0,3** (n=5)
2	Burns, treated with nonimmune γ-globulin	5,5±0,11*** (n=6)	5,9±0,1*** (n=5)	4,2±0,29*** (n=5)
3	Burns, untreated	8,2±0,74*** (n=4)	9,6±0,57*** (n=4)	5,9±0,06*** (n=5)
4	Intact animals (control)		2,8±0,4 (n=10)	
	Hepatic blood flow (in ml/min/g)			
1	Burns, treated with immune γ-globulin	1,8±0,2** (n=5)	1,5±0,1*** (n=6)	2,4±0,2** (n=6)
2	Burns, treated with nonimmune γ-globulin	1,3±0,02*** (n=6)	1,2±0,05*** (n=6)	1,7±0,2 (n=6)
3	Burns, untreated	0,9±0,1*** (n=5)	0,8±0,05*** (n=5)	1,4±0,01*** (n=6)
4	Intact animals (control)		2,9±0,6 (n=10)	

Legend. Here and in Table 2, asterisks denote $P \leq 0.05$ (* — compared with group 2, ** — with group 3, *** — with group 4).

TABLE 2. Kallikrein-Kinin System in Normal Rats and in Burned Rats Untreated and Treated with γ-Globulin Preparations ($\bar{x} \pm m$)

Group of animals	Experimental conditions	Time of experiment, days		
		1	2	5
	Esterase activity			
1	Burns, treated with immune γ-globulin	13,4±3,9 (n=10)	7,2±2,6 5	9,0±2,8 5
2	Burns, treated with nonimmune γ-globulin	10,8±4,7 10	12,9±3,9 5	11,6±2,5 5
3	Burns, untreated	13,8±4,7 9	9,9±2,2 5	8,5±2,5 5
4	Intact animals (control)		7,7±2,0 11	
	Prekallikrein			
1	Burns, treated with immune γ-globulin	57,5±2,2*** 10	63,7±5,4 5	61,9±9,8 5
2	Burns, treated with nonimmune γ-globulin	42,9±3,3*** 10	61,4±5,6 5	46,2±10 5
3	Burns, untreated	39,6±2,6*** 9	60,1±9,8 5	49,6±8,2 5
4	Intact animals (control)		60,2±4,9 12	
	Prekallikrein inhibitor			
1	Burns, treated with immune γ-globulin	1,0±0,2 10	1,2±0,1 5	1,2±0,1 5
2	Burns, treated with nonimmune γ-globulin	1,1±0,1 10	1,1±0,1 5	1,0±0,2 5
3	Burns, untreated	1,1±0,1 9	1,3±0,2 5	1,2±0,2 5
4	Intact animals (control)		1,21±0,10 12	

Legend. Activity expressed in μmoles hydrolyzed TAME (N-tosyl-L-arginine-methyl ester)/ml plasma/h, *** with group 4.

EXPERIMENTAL RESULTS

When injected into burned rats immune γ-globulin gave a significant therapeutic effect (Table 1). The excretory function of the liver, determined by the ability of the hepatocytes to eliminate the dye from the blood stream, in the animals of group 1 on the 1st day after burning was almost twice as high as in the corresponding untreated rats. The half elimination period of Ujoviridin was reduced by 52% ($P < 0.001$). Meanwhile the blood supply to the liver cells was improved: The velocity of the hepatic blood flow reached 1.8 ml/min/g, significantly higher than that in untreated animals (0.9 ml/min/g; $P < 0.001$).

In the animals of group 2, as a result of treatment of the burn with normal, nonimmune γ-globulin, stimulation of hepatocyte function also was observed, although by a lesser degree than in the rats of group 1. The half-elimination period of the dye from the blood stream was reduced by 37% compared with its level in untreated rats, and the velocity of the hepatic circulation was increased to 1.3 ml/min/g. Both these parameters demonstrate that nonimmume γ-globulin is less effective in its action on liver function than immune γ-globulin. Compared with the untreated rats, however, the animals of this group showed a marked improvement in hepatocyte function.

In the rats of group 3 (with untreated burns) the most marked inhibition of the assimilative-excretory function and hepatic circulation was found. On the first day after burning the half-elimination period of Ujoviridin was increased by 310% compared with that in intact rats. The velocity of the hepatic blood flow also was sharply reduced in the burned rats (0.9 ml/min/g compared with 2.7 in normal animals).

In the course of the experiments liver function of burned rats treated with γ-globulin improved as a rule on the 2nd day and regained its normal level on the 5th day after burning. The advantage of immune over nonimmune γ-globulin was demonstrated significantly at all times of the investigation.

The study of the state of the kallikrein-kinin system showed the clearest changes in the prekallikrein level (Table 2).

In the rats of group 1 the prekallikrein level on the 1st day of the experiment remained at its highest (95.5% of normal). This is evidence that as a result of treatment of the burned animals with immune γ-globulin only slight activation of the kallikrein-kinin system took place.

In the rats of group 2 the level of activation of the system under the influence of normal, nonimmune γ-globulin was a little higher, and prekallikrein utilization was greater, namely 71.2%. The most profound disturbances of the kallikrein-kinin system were observed in the animals of group 3 (with untreated burns), namely 65.7% of prekallikrein remained inactivated compared with the normal level.

The difference between the parameters of the experimental animals was regular in character and confirmed by the results of an investigation on the 5th day after burning. The prekallikrein level in the burned animals was completely restored to normal under the influence of immune γ-globulin (102.8%, $P > 0.05$), whereas under the influence of nonimmune γ-globulin and also in the untreated animals restoration of the prekallikrein level by a statistically significant degree was not found (76.7 and 82.3%, respectively).

The values obtained for esterase activity and prekallikrein inhibitor showed no significant differences between the experimental groups. The results thus indicate that immune γ-globulin has a protective action on the burned organism. This effect is assessed quantitatively and significantly by normalization of the assimilative-secretory function of the liver, restoration of the velocity of the hepatic blood flow, and also the absence of activation of prekallikrein in the blood of the burned animals. The regular character of the effect also is confirmed by the time course of the process, which was most marked on the 1st day after burning. The favorable therapeutic effect of immune γ-globulin is evidently determined by the specificity of its action on the toxin from burned skin.

The therapeutic effect of normal, nonimmune γ-globulin, arising after its injection into burned rats, must also be noted. The functional changes in this case were less marked than in the untreated burned animals, but the values were not restored practically to the normal level as they were a result of treatment with immune γ-globulin.

The results demonstrate the value of measures of specific detoxication in the combined treatment of burns.

LITERATURE CITED

1. I. K. Koryakina, Patol. Fiziol., No. 5, 73 (1964).
2. B. E. Movshev, in: Acute Burn Toxemia [in Russian], Kiev (1976), pp. 74-76.
3. B. E. Movshev, "Pathogenesis of autointoxication in thermal burns (isolation and properties of burn toxin)," Author's Abstract of Doctoral Dissertation, Moscow (1977).
4. B. E. Movshev, Vopr. Med. Khim., No. 4, 560 (1981).
5. B. E. Movshev and R. V. Nedoshivina, Patol. Fiziol., No. 5, 61 (1976).
6. N. S. Murashova, R. V. Nedoshivina, and I. K. Koryakina, in: Abstracts of Proceedings of the 5th Scientific Conference on Burns [in Russian], Part 2, Leningrad (1967), p. 44.
7. R. V. Nedoshivina, "Effect of immunotherapy on functional state of the kidneys and liver in the acute period of burns," Author's Abstract of Candidate's Dissertation, Moscow (1965).
8. R. V. Nedoshivina, Patol. Fiziol., No. 2, 91 (1971).
9. R. V. Nedoshivina and B. E. Movshev, Probl. Gematol., No. 2, 30 (1978).
10. Z. G. Pozdnyakova, Patol. Fiziol., No. 5, 48 (1966).
11. L. N. Pushkar', "Immunotherapy in acute burn toxemia," Author's Abstract of Doctoral Dissertation, Moscow (1968).
12. N. A. Fedorov, Vestn. Akad. Med. Nauk SSSR, No. 3, 33 (1979).
13. N. A. Fedorov and V. V. L'vova, Patol. Fiziol., No. 4, 26 (1962).
14. N. A. Fedorov, R. V. Nedoshivina, I. K. Koryakina, et al., Patol. Fiziol., No. 3, 45 (1980).
15. J. S. Baumstar et al., Arch. Biochem., 108, 514 (1964).
16. R. W. Colman, L. Mattler, and S. Sherry, J. Clin. Invest., 48, 11 (1969).