

# DETOXICATION AND IMMUNOCORRECTIVE PROPERTIES OF SOME SORPTION METHODS OF TREATMENT OF SEPTICOPYOTOXEMIA INDUCED BY EXPERIMENTAL SPREADING PERITONITIS

N. K. Manucharov, Sh. K. Makharadze, E. N. Akhvlediani,  
Yu. D. Tavdidishvili, M. D. Sychev, and M. M. Kiladze

UDC 616.381-002.3-092.9-085.246.9

**KEY WORDS:** septicopyotoxemia; immunocorrective methods of treatment.

Spreading suppurative peritonitis is often septicemic in character because of the sorption superposed on a background of massive microbial inflammation and disturbance of the defensive mechanisms of the peritoneum [2, 8]. After operation, when surgical methods have exhausted their possible value, attention is focused on treatment of the septicopyotoxemia [1]. Recently a number of sorption methods of treatment of the toxemic syndrome have become widely used in surgical practice, although the mechanisms of their therapeutic effect have not been adequately studied.

The aim of this investigation was to study the detoxication and immunocorrective effect of the mechanisms of the most commonly used sorption methods of treatment.

## EXPERIMENTAL METHOD

Experiments were carried out on 40 male mongrel dogs weighing 13-15 kg, in which acute spreading suppurative (fecal) peritonitis was produced. The animals were divided into four groups in accordance with the methods of treatment used: hemoperfusion, plasmapheresis, ultraviolet irradiation of autologous blood, and extracorporeal connection to a xenogeneic spleen. The efficacy of treatment was evaluated periodically by clinical and laboratory tests, and considerable attention was paid to tests of general toxicity of the blood, with recording of the paramecium time (PT), the leukocytic index of toxemia (LIT), the leukocytic shift index (LSI), molecules of average mass (MAM), and immunologic parameters. The relative (in percent) and absolute (per liter) numbers of T and B lymphocytes in the dogs' blood were determined by the spontaneous rosette-formation test with sheep (E-RFC) and mouse (M-RFC) erythrocytes, following the instructions given in "Technical Recommendations of the Ministry of Health of the USSR" [10]. The rosette-forming ability of the neutrophils also was determined in the test with sheep erythrocytes, in order to ascertain their functional activity [4-7]. The ability of neutrophils to form rosettes reflects their phagocytic activity, i.e., it determines the adhesive properties of the cell membranes and characterizes one phase of phagocytosis: the attraction phase. Lysozyme activity was judged from the change in the degree of light transmittance of a bacterial suspension of *Micrococcus lysodeikticus*, under the influence of the test blood, and compared with transmittance of the original suspension [5]. Complementary activity of the blood serum was determined by the 50% hemolysis method. The test material was the dogs' venous blood. Chemoperfusion was carried out on commercial models of apparatus under the UAG-01 and AKST marks, by the usual method. As sorbents, activated charcoal of the SKN-M, SKN-2M, SKN-4N, and SKN-1K brands (USSR/CIS) were used. Volume plasmapheresis was carried out by the discrete method, consisting essentially of successive collection and centrifugation of the blood and its separation

---

K. D. Eristavi Research Institute of Experimental and Clinical Surgery, Tbilisi. (Presented by Academician of the Russian Academy of Medical Sciences A. D. Ado.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 114, No. 10, pp. 395-398, October, 1992. Original article submitted April 15, 1991.

TABLE 1. Dependence of Changes in Parameters of Toxemia of Dogs with Spreading Peritonitis on Methods of Treatment

Criterion	Hemoperfusion		Plasmapheresis		UV irradiation of blood		Xenogeneic spleen	
	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment	before treatment	after treatment
Pulse rate (beats/min)	145—150	100—102	138—142	90—94	142—148	124—126	140—144	92—94
Respiration rate (cycles/min)	Over 30	Under 20	Over 30	Under 20	Over 30	From 20 to 30	Over 30	Under 20
Temperature	38,7±1,5	37,6±2,04	38,9±2,6	37,1±1,99	38,8±2,53	37,9±2,61	38,7±3,05	37,3±2,86
BP	Unstable	Stable	Unstable	Stable	Unstable	Stable	Unstable	Stable
CVP	Negative	Normovolemia	Negative	Normovolemia	Negative	Negative	Negative	Normovolemia
Intestinal particles	Negative	Normovolemia	Negative	Normovolemia	Negative	Negative	Negative	Normovolemia
Diuresis	Absent	Sluggish	Absent	Sluggish	Absent	Absent	Absent	Sluggish
	Oliguria	Adequate with stimulation	Oliguria	Adequate with stimulation	Oliguria	Oliguria	Oliguria	Adequate with stimulation
Toxicity of blood, per cent (biological)	98±15,3	67±13,6	102±16,9	54,7±12,1	100,7±10,3	98,6±18,5	69,4±5,7	58,5±
LIT	9,3±1,4	4,6±0,7	8,9±1,12	2,7±0,65	9,1±4,6	7,5±1,9	5,7±0,1	4,9±0,69
NAM	0,735±0,3	0,362±0,012	0,815±0,041	0,347±0,02	0,804±0,036	0,695±0,031	0,793±0,04	0,385±0,02

into plasma and globular components in plastic containers. A Mark K-70 refrigeration centrifuge was used. Continuous-flow ultraviolet irradiation of the blood was carried out on a serially produced "Izol'da" (USSR/CIS) apparatus, blood being passed through its working part continuously at a volume velocity of 50-60 ml/min for 30-40 min. The dose of irradiation was 0.6-0.9 J/ml. Extracorporeal connection of the donor's spleen was carried out by the method developed at the Research Institute of Transplantology and Artificial Organs, Ministry of Health of the USSR [9].

## EXPERIMENTAL RESULTS

Connection to extracorporeal methods of detoxication gave a marked beneficial clinical effect in almost two-thirds of the animals. Usually after a single session the temperature, pulse rate, respiration rate, arterial, and central venous blood pressure returned to normal. The animals became more active, they slept and ate better, signs of intestinal paresis disappeared, and signs of encephalopathy were reduced.

Improvement of the animals' clinical state was accompanied by a positive change in the morphological composition of the blood: a decrease in the degree of leukocytosis, reduction of the nuclear shift of the neutrophils and of their toxigenic granulation, as well as lowering of the general toxicity of the blood by 45% (MAM, LSI, LIT). According to our observations, plasmapheresis and hemoperfusion had the best detoxicating effect in septicopytoxicemia; connection to a xenogeneic spleen and ultraviolet irradiation of the blood were somewhat inferior to these methods (Table 1). Considerable changes took place in the immune system. Immunobiological activity is known to play an important role in the course and outcome of pyogenic inflammatory diseases of the abdominal organs. Factors of nonspecific resistance, responsible both for primary, early protection and for the T and B systems of immunity, and ultimately responsible for all immune reactions in the body and for performance of immunoregulatory functions, also are involved in the mechanism of pathogenesis of these diseases. By nonspecific factors of resistance we include several substances, of which complement and lysozyme attracted our attention. We know that complement determines the bactericidal quality of the blood and is involved in antigen-antibody reactions. Lysozyme can carry

TABLE 2. Changes in Immune Status of Dogs during Treatment of Acute Spreading Peritonitis

Group tested	Leuko- cytes, 10 <sup>9</sup> /liter	Lympho- cytes, 10 <sup>9</sup> /liter	Neutro- phils, 10 <sup>9</sup> /li- ter	T-lympho- cytes, 10 <sup>9</sup> /liter	B-lympho- cytes, 10 <sup>9</sup> /liter	O-Lympho- cytes, 10 <sup>9</sup> /liter	E-RFC, 10 <sup>9</sup> /liter	Comple- mentary activ- ity, U/ml	Lysozyme activity, per cent
Intact animals	7.2±0.56	1.57±0.12	66.2±5.74	23.2±1.95	50±2.37	26.25±2.7	34.7±3.12	45.8±3.67	41.07±3.05
Height of the disease	6.5±1.49	2.01±0.18	77.9±6.81	24.0±2.06	44.7±0.86	32.7±2.8	36.9±4.9	54.1±4.53	46.5±3.92
Methods of treatment	8.4±0.76	1.89±0.076	72.4±6.13	27.6±2.14	53.8±4.9	18.6±3.4	42.0±3.9	48.5±4.21	42.7±3.94
Xenogenic spleen		22.1±2.07	6.7±0.55	0.5±0.032	1.0±0.08	0.34±0.04	1.9±0.205		
Ultraviolet irradiation	11.8±1.06	1.9±0.084	75.9±6.73	25.4±2.17	47.9±3.16	26.5±2.7	30.3±5.1	52.8±4.7	46.7±4.01
Hemoperfusion	10.1±1.028	2.0±0.11	72.0±6.45	24.5±2.06	49.3±2.3	26.2±4.0	37.6±4.1	50.4±4.82	43.9±3.87
Plasmapheresis	10.3±1.028	1.6±0.94	77.5±6.98	24.1±2.03	41.6±4.02	34.4±2.97	41.3±2.86	53.1±4.9	46±4.3
		13.7±1.19	9.5±0.86	0.40±0.032	0.7±0.038	0.58±0.1	1.3±1.01		

Legend. Significance of differences compared with initial values  $p < 0.05$ .

out lysis of microorganisms and can also take part in antigen-antibody reactions. Since lysozyme is produced by neutrophils, its level is an indirect indicator of their functional activity. In all the dogs at the height of acute spreading peritonitis (2-3 days after creation of the model) a decrease in immunologic reactivity was observed, manifested first as a significant decrease ( $p < 0.05$ ) in the relative concentration of T lymphocytes (from the initial  $50.5\% \pm 2.9$  to  $44.2\% \pm 1.4$ ), with a simultaneous increase in the number of O-cells (from the initial  $26.2\% \pm 2.3$  to  $32.7\% \pm 2.7$ ). This is in all probability connected with reduction of expression of E-receptors of the T lymphocytes under the influence of endogenous factors inducing general toxemia. Lymphocytes with reduced avidity (rosettes with three or four erythrocytes) predominated. Reduction of T-cell activity at this stage, against the background of a high blood neutrophil count, was perhaps also attributable to factors stimulated by the latter, for there is evidence of mutual regulation of the numbers of neutrophils and of T lymphocytes under the influence of these factors [7]. The total level of B lymphocytes and of rosette-forming neutrophils had a tendency to fall.

The complementary activity was reduced (from an initial  $45.8 \pm 3.4$  to  $54.1 \pm 3.9$  units/ml), possibly indicating weakening of nonspecific reactivity in the animals, but possibly due to an increase in the consumption of complement in the body. The lysozyme level was raised a little, a characteristic feature of the acute phase of inflammation.

Methods of detoxication used in this study in peritonitis were found to have a beneficial immunocorrective effect, which varied in degree (Table 2).

The greatest degree of normalization, or even some increase in the parameters of immunologic reactivity in the dogs were observed after connection to a xenogeneic spleen and after UV irradiation of autologous blood. In this case the relative and absolute numbers of T and B lymphocytes actually exceeded the background values. Simultaneously with increased avidity of the lymphocytes (rosettes with 5-10 erythrocytes) a sharp decrease was observed in the number of O lymphocytes to  $18.5\% \pm 5.0$ , which is itself a favorable prognostic sign. The adhesive ability of the neutrophils and activity of complement also increased, but the lysozyme level returned close to normal.

The most marked immunocorrective action was exhibited after the use of plasmapheresis. The number of T lymphocytes and their avidity continued to fall, whereas the number of B lymphocytes and of rosette-forming neutrophils rose a little, and the number of O lymphocytes in the blood was high ( $34.3\% \pm 3.4$ ), with an absolute value of  $0.58 \pm 0.055$  thousand/liter. The serum complementary and lysozyme activity did not differ significantly from the same parameters at the height of the disease, probably due to the specific nature of the method.

The following conclusions were thus deduced from the results described above. In septicopytoxemia resulting from spreading peritonitis there is an increase in severity of the toxic effect, accompanied by the development of circulatory and volemic disorders: renal-hepatic failure, cerebral edema, paralytic ileus [2, 3]. Meanwhile considerable disturbances of the immune status are observed, and in particular, affecting the cellular component of immunity (primarily the T system).

The use of sorption methods of treatment differ in their effect on the pathological process. Extracorporeal connection to a xenogeneic spleen and ultraviolet irradiation of autologous blood considerably enhance immune reactivity, whereas plasmapheresis and hemoperfusion, while possessing a powerful detoxicating action, have a weaker immunocorrective effect [8, 9]. It is probable that a combination of these methods of detoxication which we used will be indicated for the treatment of septicopytoxemia arising as a result of spreading peritonitis.

## REFERENCES

1. V. G. Bochorishvili, Sexology with the Principles of Infectious Pathology [in Russian], Tbilisi (1988).
2. V. P. Bashtan, Morphologic and Functional Disturbances in Peritonitis [in Russian] (1988).
3. R. A. Grigoryan and F. S. Drapyan, Klin. Med., No. 8, 29 (1989).
4. A. E. Gromov, L. V. Potashov, N. P. Nikonchuk, et al., Effect of Blood on Man and Animals [in Russian], Leningrad (1986), pp. 20-22.
5. E. V. Gembitskii, Revmatologiya, No. 3, 3 (1987).
6. V. G. Dorofeichuk, Lab. Delo, No. 3, 28 (1987).
7. I. I. Dzerzhinskaya, Immunologiya, No. 1, 64 (1982).
8. A. I. Lobakov, V. L. Chernyakov, and O. N. Vetchinnikova, Vestn. Khir., No. 10, 109 (1987).
9. A. M. Karyakin, V. V. Kucher, P. A. Susla, and B. L. Kofman, Khirurgiya, No. 4, 109 (1983).
10. D. I. Strukov and V. I. Petrov, Acute Spreading Peritonitis [in Russian], Moscow (1987), pp. 142-161.
11. R. V. Petrov and Yu. M. Lopukhin, Evaluation of the Human Immune Status. Technical Recommendations [in Russian], Moscow (1984).
12. A. B. Tsy-pin and V. I. Shumakov, Treatment of Septic Diseases by Connection to a Xenogeneic Spleen. Technical Recommendations [in Russian], Moscow (1988).