Effect of Perftoran on Macroglobulin Content in the Plasma and Peritoneal Exudate of Rats with Acute Exudative Inflammation (Peritonitis)

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 138, No. 10, pp. 397-399, October, 2004
Original article submitted April 23, 2004

The effect of blood substitute perftoran on the content of α_1- and α_2-macroglobulins in the plasma and exudate was studied in rats with acute exudative inflammation. After intravenous injection of perftoran macroglobulin content increased in the plasma, but remained unchanged in the peritoneal fluid.

Key Words: *macroglobulins; perftoran; experimental peritonitis*

Inflammation in the abdominal cavity (peritonitis) is the most severe complication of surgical diseases. The mortality rate from peritonitis is high. The search for new drugs for the treatment of pathological processes during inflammation and development of effective therapeutic methods are urgent problems.

Macroglobulins are involved in the pathogenesis of inflammation. They inhibit various proteolytic enzymes, including elastase-like and chymotrypsin-like proteinases. These enzymes are released from granulocytes into the blood and inflammatory exudate and produce secondary tissue damage [4]. Macroglobulins play a role in the regulation of blood proteolytic systems responsible for hemostasis, fibrinolysis, and kininogenesis. Being endogenous antiproteinases, macroglobulins modulate a variety of immune reactions determined by protease activity. Macroglobulins form complexes with cytokines, growth factors, and hormones and perform intracellular transport of these substances by receptor-mediated endocytosis. Hence, macroglobulins are involved in the regulation of proliferation and differentiation of various cells [11,13].

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Here we studied the effect of blood substitute perftoran possessing gas-transport function and polyfunctional properties on the content of α_1- (MG-1) and α_2-macroglobulins (MG-2) in the plasma and exudate from rats with acute exudative inflammation (peritonitis).

MATERIALS AND METHODS

Experiments were performed on 72 male and female outbred albino rats weighing 170-250 g. The animals were divided into 3 groups of 24 specimens each. Acute exudative inflammation in group 1 and 2 rats was produced by intraperitoneal injection of 0.2% $AgNO_3$ (1 ml) [3]. Perftoran in a dose of 5 ml/kg was simultaneously injected into the femoral vein of group 1 animals (main group). Group 2 rats received an equivalent volume of physiological saline and served as the control. Group 3 animals were treated only with physiological saline. The rats were decapitated 3, 6, and 24 h after induction of experimental peritonitis under ether anesthesia. Blood samples were taken from the decapitation wound. The abdominal cavity was opened to obtain the exudate.

The concentration of MG-1 and MG-2 in blood samples and exudate was measured by rocket immunoelectrophoresis [1] with monospecific rabbit anti-

0007-4888/04/1384-0351

sera [10] and expressed in rel. units/ml. Pooled from pregnant rats served as the standard. The amount of macroglobulins in the pooled serum was taken as 100 rel. units. Albumin content in the exudate was estimated by the standard method on a STATFAX biochemical analyzer.

The results were analyzed by means of InStat2 software. We calculated the arithmetic mean and standard error (SEM). Intergroup differences were evaluated by Mann—Whitney test. The relationship between test parameters was estimated by Spearman correlation test.

RESULTS

The amount of MG-1 in rats is maximum under physiological conditions. MG-1 concentration in group 1 rats increased by 1.5 times 3 h after administration of $AgNO_3$ and surpassed that in animals of other groups (Table 1). Cytokine-induced activation of macroglobulin biosynthesis occurs at the late stage [9]. Therefore, the increase in protein concentration was probably related to its release from tissues. MG-1 concentration in group 1 rats decreased by 3 times 6 h after treatment, but tended to increased by the 24th hour. These changes reflect activation of MG-1 biosynthesis in macroglobulin-producing cells (hepatocytes, macrophages, and fibroblasts). MG-1 concentration remained practically unchanged in control animals. We revealed only a slight increase in MG-1 concentration in control rats by the end of observations.

MG-2 is the acute-phase protein. Three hours after induction of inflammation MG-2 concentration in group 1 and 2 rats did not differ from that in animals receiving physiological saline. MG-2 concentration in group 1 and 2 rats increased in the follow-up period and exceeded the baseline level by 64 and 35 times, respectively (24 h after treatment). Different changes in the concentration of MG-1 and MG-2 in rats receiving perftoran illustrate that the effects of this preparation on plasma protein content are realized via different mechanisms. A short-term increase in MG-1 concentration 3 h after induction of inflammation was probably related to perftoran-produced changes in cell membranes properties. Published data show that perfluoroorganic compounds have affinity for phospholipids (major component of cell membranes) [2]. Moreover, proxanol entering the composition of perftoran interacts with hydrophobic regions in membrane proteins [7]. Components of perftoran produce conformational changes in the cell membrane, which probably provides conditions optimal for MG-1 diffu-

TABLE 1. Content of MG-1 and MG-2 in Rat Plasma (rel. units/ml, *M*±*SEM*, *n*=8)

Group	MG-1			MG-2		
	3 h	6 h	24 h	3 h	6 h	24 h
Physiological saline		49.66±4.78			67.84±12.27	
$AgNO_3$ and perftoran	76.07±6.87$^{**+++bc}$	25.99±3.44^{**++}	40.90±3.83	78.04±15.00	119.50±36.33	4953±1294^{*+ab}
$AgNO_3$ and physiological saline	49.41±3.03	45.90±3.61	55.20±12.04	68.15±16.54	119.70±43.32	2362±709^{+ab}

Note. $^{*}p<0.001$ and $^{**}p<0.01$ compared to the $AgNO_3$+physiological saline group; $^{+}p<0.001$ and $^{++}p<0.05$ compared the physiological saline group; $p<0.001$ compared to the parameter recorded 3 (^{a}), 6 (^{b}), and 24 h (^{c}) after induction of peritonitis.

TABLE 2. Content of MG-1 and MG-2 in the Peritoneal Exudate (rel. units/ml, *M*±*SEM*, *n*=8)

Group	MG-1			MG-2		
	3 h	6 h	24 h	3 h	6 h	24 h
Control ($AgNO_3$ and physiological saline)	25.63±1.37	32.49±2.57	34.25±2.50^{*a}	74.07±17.33	58.16±10.18	736.4±113.7^{ab}
Main ($AgNO_3$ and perftoran)	24.62±1.27	32.16±3.80	24.43±1.81	57.18±13.20	57.75±7.59	775.20±112.56^{ab}

Note. $^{*}p<0.05$ compared to the main group; $p<0.001$ compared to the parameter recorded 3 (^{a}) and 6 h (^{b}) after induction of peritonitis.

sion from the cell or surface into the circulation. The increase in MG-2 concentration 24 h after treatment reflects the increased biosynthesis of this substance and is probably associated with the stimulatory effect of perftoran on phagocytosis [6].

MG-1 and MG-2 were detected in the peritoneal exudate (Table 2). MG-1 content remained practically unchanged in group 1 rats, but increased by 34% in control animals 24 h after $AgNO_3$ administration. Changes in MG-2 concentration in the exudate were similar to those in the plasma. MG-2 concentration in the exudate from group 1 and 2 rats increased less significantly than in the blood 24 h after treatment (by 12 times). No differences were revealed in MG-2 concentration in the exudate from group 1 and 2 rats. It should be emphasized that by the end of observations plasma protein concentration in group 1 rats 2-fold surpassed that in group 2 animals. The observed changes were probably related to the effect of perftoran on vascular permeability. Under normal conditions the distance between capillary endotheliocytes is 4-5 Å. However, the hydrodynamic radius of MG-2 is 93.5 Å [10]. These features make diffusion of macroglobulins into the extravascular space impossible. The presence of macroglobulins in the peritoneal fluid under these conditions results from their synthesis or secretion by peritoneal macrophages. The distance between endotheliocytes increases to 100 Å during inflammation [8], which is sufficient for the release of high-molecular-weight proteins from vessels. These changes are accompanied by the increase in plasma macroglobulin content in the peritoneal exudate. Our results were confirmed by correlations between the contents of albumin and MG-2 in the exudate. The Spearman coefficients in control rats were -0.72, 0.21, and 0.55 (3, 6, and 24 h after the induction of peritonitis, respectively). A weak correlation was revealed between the amount of albumin and MG-2 in group 1 animals 24 h after treatment (r=0.3). Probably, perftoran decreases the severity of damage to the vascular endothelium and suppresses the release of high-molecular-weight proteins from vessels.

These data show that intravenous pretreatment with perftoran is followed by an increase in the concentration of circulating macroglobulins. The concentrations of MG-1 and MG-2 in the plasma from perftoran-receiving rats increase 3 h and 1 day after the induction of inflammation, respectively. Perftoran has no effect on protein content in the exudate.

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