State of the Nonspecific Component of the Immune System during Combination Therapy for Experimental Bile Peritonitis

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Combined use of sodium hypochlorite and intravenous laser irradiation of the blood in the postoperation therapy of 24-h bile peritonitis improved recovery of engulfing and digestive activity of neutrophilic granulocytes in the early postoperation period. Enzyme activity of neutrophilic granulocytes in earlier postoperation periods returned to normal after treatment with sodium hypochlorite alone.

Key Words: *bile peritonitis; neutrophilic granulocytes; sodium hypochlorite; intravenous laser irradiation of blood*

Progression of 24-h bile peritonitis is accompanied by a decrease in total activity and functional reserve of neutrophilic granulocytes (NG) and suppression of the nonspecific immune system.

Published data show that administration of sodium hypochlorite (SHC) [2] and intravenous laser irradiation of the blood (ILIB) [1] activate the nonspecific component of the immune system. It contributes to the delayed response of leukocytes and oxygen-dependent phagocytosis.

Here we studied metabolic and enzyme activity of NG in postoperation therapy of 24-h bile peritonitis with combined use of SHC and ILIB.

MATERIALS AND METHODS

Experiments were performed on dogs weighing 16±4 kg. The animals were kept according to the Sanitary Regulations on Organization, Equipment, and Maintenance of Experimental and Biological Rooms (Vivariums).

The animals were divided into several groups. Group 1 included intact dogs (n=45). The animals with 24-h bile peritonitis entered group 2 (n=45). Group 3 (reference group, ILIB) comprised dogs with 24-h bile peritonitis (n=15) receiving combination therapy and ILIB (24 and 48 h after surgery). Combination therapy in group 3 animals included intraoperative sanation of the abdominal cavity with furacilin (1:5000, 400 ml) and intravenous injection of 200 ml 0.89% NaCl immediately and 12 h after surgery. Group 4 dogs with 24-h bile peritonitis (n=15, reference group, SHC) were subjected to combination therapy, which included intraoperative sanation of the abdominal cavity with furacilin (1:5000, 400 ml) and intravenous injection of 200 ml 0.04% SHC immediately and 12 h after surgery. Combination therapy (intraoperative sanation of the abdominal cavity with furacilin (1:5000, 400 ml) and intravenous injection of 200 ml 0.04% SHC immediately and 12 h after surgery) and ILIB (24 and 48 h after surgery) were applied to group 5 dogs (main group, n=15).

Experimental bile peritonitis was modeled as described elsewhere [4]. The bile (1.5 ml/kg) was administered intraperitoneally through a punction hole to animals with local aseptic inflammation. Treatment was performed 3 times at 8-h intervals. Local aseptic inflammation was modeled by injecting $CaCl_2$ (0.25 ml/kg) under the skin of the thigh.

The content of cationic proteins and count of NG were estimated in peripheral blood smears stained

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with amido black 10B [6]. Myeloperoxidase activity was measured by the standard benzidine method with modifications of I. V. Nesterova [3]. The mean cytochemical coefficient (MCC) for cationic protein and myeloperoxidase was calculated as follows: MCC= $(4a+3b+2c+d)/100$, where *a*, *b*, *c*, and *d* are the numbers of cells with very high, high, intermediate, and low activity, respectively (standard method).

Spontaneous and stimulated tests with nitroblue tetrazolium (NBT) were performed by the standard method with modifications of I. V. Nesterova [3]. Engulfing and digestive activity of NG was measured using laboratory strain of *Staph. aureus* P209. The percentage of phagocytosis, phagocytic number, phagocytic index, percentage of digestion, and digestion index were calculated [3].

RESULTS

In animals with 24-h bile peritonitis MCC for myeloperoxidase and cationic proteins decreased by 3.5 and 2.8 times, respectively ($p<0.05$). These changes reflected a decrease in total activity of NG (Fig. 1).

MCC for myeloperoxidase and cationic proteins of NG progressively increased starting from day 1. Group 4 animals were characterized by a more rapid recovery of the test parameters (day 3). Despite the progressive increase in MCC in animals of groups 3 and 5 (main group), they returned to normal only on day 10. It was probably associated with prolonged effect of ILIB.

The index of spontaneous NBT test decreased by 1.2 times in animals with 24-h peritonitis ($p<0.05$). These changes reflected a decrease in oxidative activity and effector capacity of NG. The index of spontaneous NBT test in animals of different groups remained low over the first 2 days after sanation ($p<0.05$), but returned to normal on days 3 (group 3) and 10 (groups 4 and 5). Rapid recovery of this index in group 3 dogs (ILIB, day 3) was related to activation of NG. Laser irradiation of the cell surface was followed by peroxidative photosensitizing modification

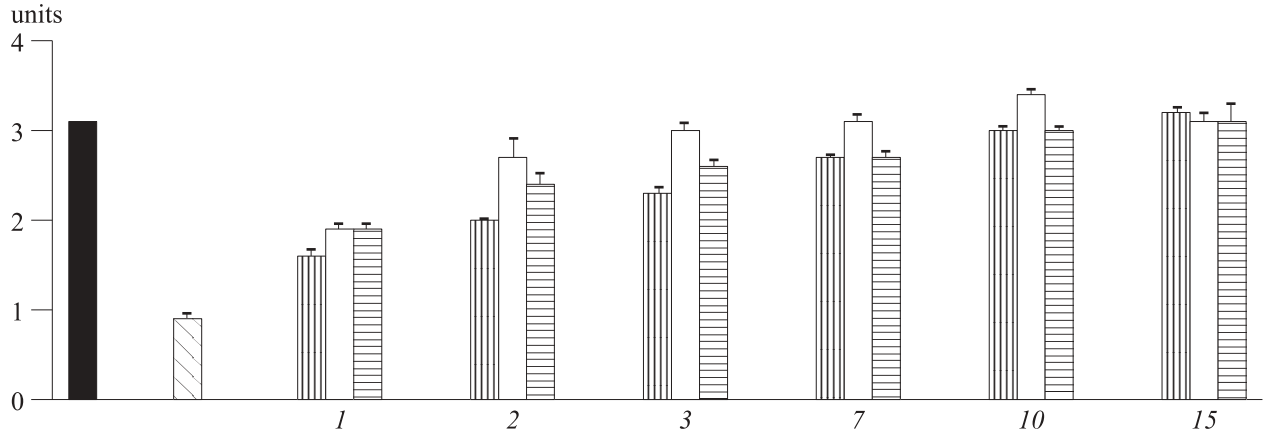

Fig. 1. Mean cytochemical coefficient for myeloperoxidase in neutrophilic granulocytes from animals with bile peritonitis. Here and in Figs. 2 and 3: dark bars, group 1; slant shading, group 2; vertical shading, group 3; light bars, group 4; and horizontal shading, group 5. Days 1 (*1*), 2 (*2*), 3 (*3*), 7 (*7*), 10 (*10*), and 15 (*15*).

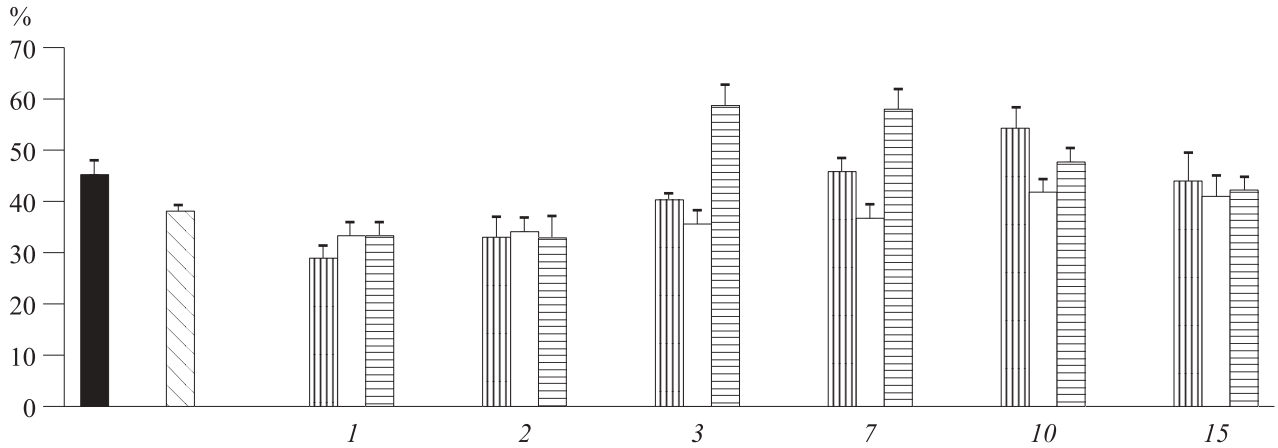

Fig. 2. Spontaneous NBT-test with NG from animals with bile peritonitis.

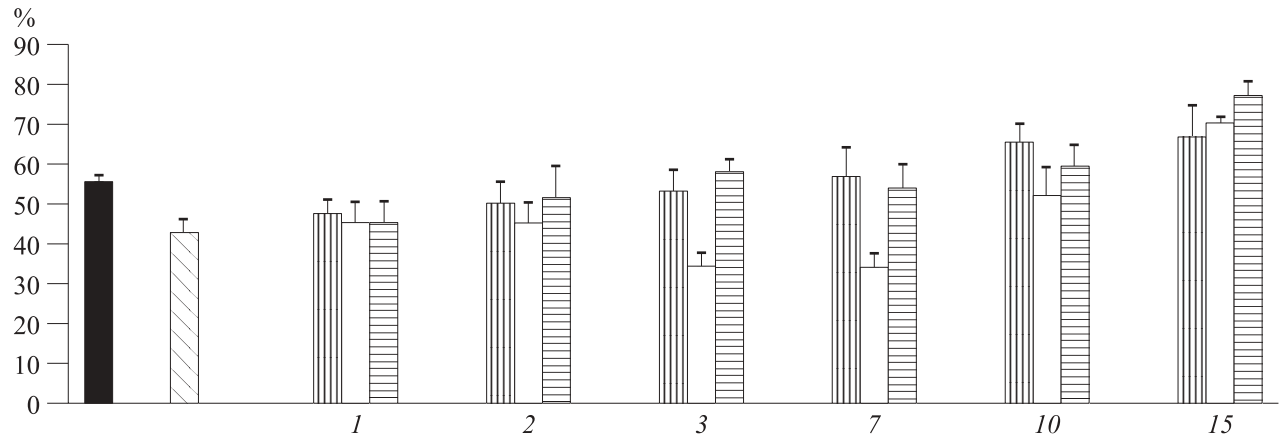

Fig. 3. Stimulated NBT-test with NG from animals with bile peritonitis.

of membrane lipids and activation of oxygen-dependent metabolism in NG. Our results are consistent with published data (Fig. 2) [5].

Functional and metabolic reserves of phagocytic cells were estimated in stimulated NBT test with NG. The index of stimulated NBT test in dogs with 24-h bile peritonitis was 23% lower compared to intact animals. These changes reflected a decrease in functional and metabolic reserves of phagocytic NG (Fig. 3).

The index of stimulated NBT test in animals of different groups tended to normal starting from day 1. On day 2 the index of stimulated NBT test in group 3 and 5 dogs did not differ from that in intact animals. This effect is probably associated with activation of NG due to laser irradiation of the blood. The index of stimulated NBT test in group 4 dogs returned to normal on day 10.

The intensity of phagocytosis decreased by 19% in animals with 24-h bile peritonitis ($p<0.05$). We revealed a decrease in the phagocytic number (by 24%, $p<0.05$), phagocytic index (by 31%, $p<0.05$), percentage of digestion (by 22%), and digestion index (by 35%, $p<0.05$).

Phagocytic activity of blood NG tended to normal starting from day 1. The test parameters most rapidly returned to normal in group 5 dogs. It manifested in recovery of digestive function, phagocytic number (day 2), and phagocytic index (day 3). Group 3 animals were characterized by delayed recovery of digestive function, phagocytic number (day 3), and phagocytic index (day 7). In group 4 dogs these parameters returned to normal on day 7.

Our results indicate that postoperation therapy of bile peritonitis with SHC and ILIB accelerates and improves the recovery of digestive activity in NG. It should be emphasized that enzyme activity of NG returns to normal in the earlier postoperation period only after treatment with SHC.

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