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Effects of Myelopeptides on Immune Response and Morphometrical Manifestations of Inflammation in Experimental Penetrating Wound of the Eye

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Myelopid and MP-1 myelopeptide increase the count of antibody-producing cells, reduced under the effect of injury and standard therapy, and do not modify the suppression of delayed hypersensitivity. Injections of myelopid and MP-3 together with standard drugs optimized the traumatic inflammation processes.

Key Words: *myelopeptides; myelopid; penetrating wound of the eye*

Penetrating wound of the eye, despite the small volume of injury, is characterized not only by damage to ocular tissues with violation of the organ integrity and development of the wound process, but also by significant changes in the immune system [2,4,6,10]. For this reason, therapy in this condition is to be aimed at optimization of regeneration in the presence of traumatic inflammation, eradication of infection, and prevention of autoimmune reactions [2,6,7]. Myelopeptides [4,6] characterized by pronounced immunomodulating activity [5] are perspective agents stimulating healing of damaged eye tissues and formation of more mature structured cicatricial tissue.

We studied the effects of myelopid and MP-1 and MP-3 myelopeptides on immune response to a heterologous thymus-dependent antigen and morphological manifestations of inflammation in experimental penetrating wound of the eye.

MATERIALS AND METHODS

Experiments were carried out on 114 male Wistar rats (mean weight 216.1±2.7 g). Penetrating wound of the right eye (PWE) was injected under local anesthesia (2% procaine solution) in animals of experimental groups (n=89) [4,7], after which one of the drugs was injected. Controls (intact animals; n=25) were injected with 0.9% NaCl. The injections were started 6 h after the injury. Standard therapy drugs were injected daily subcutaneously: dexamethasone phosphate (0.1 mg/kg) daily, sodium diclofenac (0.5 mg/kg), ampicillin sodium salt (12.5 mg/kg), gentamicin sulfate (1.5 mg/kg) twice daily, and 3 injections of myelopeptides every other day (0.06 mg/kg) intraperitoneally. Immune response was induced in all rats by sensitization with sheep erythrocytes (10^8 cells subcutaneously into the right paw pad) 7 h after the start of experiment. On day 4, the resolving dose of the antigen (10^9 sheep

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erythrocytes) was injected subcutaneously into the right paw pad and 0.1 ml 0.9% NaCl into the left control paw. All animals were decapitated on day 5 under ether narcosis. Humoral response was evaluated by the number of antibody-producing cells (APC) in the lymph node nearest to the site of immunization (right popliteal) by local hemolysis in agarose gel [8], and the delayed-type hypersensitivity (DTH) reaction index was calculated [9]. Cell count in inflammatory infiltrate and pericicatricial zone and summary density of fibrous structures in the cicatrix were evaluated morphometrically, with 0.01 mm^2 of G. G. Avtandilov's stereometrical grid taken for a unit of area [1].

The data were statistically analyzed with consideration for log-normal distribution by the number of APC using analysis of dispersions, post-hoc Duncan's test, and post-hoc Fisher's LSD test for multiple comparison [3].

RESULTS

Antibody production was suppressed in rats with PWE (Fig. 1). Standard therapy augmented suppression of APC formation. Addition of myelopid or MP-1 to the standard therapy reduced the severity of antibody production suppression in comparison with that in animals receiving standard therapy alone. Injection of myelopid or MP-1 alone arrested the reduction of APC count, induced by the injury. Injection of MP-3 did not arrest the reduction of APC count. The development of DTH reaction was inhibited in animals with traumas in all groups, except the MP-1 group (Fig. 2). Myelopeptides did not abolish suppression of DTH reaction caused by the injury and standard therapy, which fact, considering the leading role of this type of reactions of cell-mediated immunity in the development of ophthalmic immunopathology [2], should be regarded as a positive result.

Macroscopic and histological studies of intact left eyes in animals of all groups detected no structural changes. The maximum reduction of inflammatory infiltration in the cicatrix was observed in the rats injected with myelopid and MP-3 alone and in combination with standard therapy (Table 1). This antiinflammatory effect of myelopeptides has never been described previously. An increment in the summary density of fibrous structures in the cicatrix was detected in the same animals ($p<0.05$). On day 5 of PWE, complete epithelialization of damaged focus was observed in rats treated with combinations of myelopid or MP-3 with standard therapy and receiving myelopid monotherapy. Changes in the cell composition of the pericicatricial

TABLE 1. Changes in the Inflammatory Infiltrate Cell Counts in the Cicatrix and Pericicatricial Zone (per 0.01 mm^2) in PWE ($M\pm m$)

Experimental treatment	Number of animals	Number of inflammatory infiltrate cells in cicatrix	Pericicatricial zone					
			macrophages	lymphocytes	eosinophils	neutrophils	all granulocytes	plasma cells
PWE+0.9% NaCl	19	15.84±1.87	2.16±0.22	0.26±0.04	0.66±0.17	1.68±0.16	2.34±0.26	0.16±0.02
PWE+standard therapy	10	11.3±0.22^{a}	2.13±0.14	0.48±0.06^{a}	0.60±0.06	1.03±0.11^{a}	1.63±0.15^{a}	0.13±0.03
PWE+myelopid+standard therapy	10	6.40±0.24^{ab}	0.07±0.03^{ab}	0.12±0.03^{b}	0.02±0.01^{ab}	0.02±0.01^{ab}	0.03±0.02^{ab}	0.083±0.020
PWE+MP-1+standard therapy	10	7.07±2.07^{ab}	2.29±0.42	0.46±0.10	0.56±0.11	0.94±0.13^{a}	1.50±0.15^{a}	0.25±0.04^{b}
PWE+MP-3+standard therapy	10	1.89±0.14^{ab}	0.60±0.09^{ab}	0.30±0.05	0.11±0.02^{ab}	0.18±0.02^{ab}	0.29±0.07^{ab}	0.12±0.04
PWE+myelopid	10	8.96±0.23^{a}	1.92±0.16^{c}	0.68±0.08^{abc}	0.38±0.08^{ac}	1.06±0.14^{ac}	1.44±0.21^{ac}	0.18±0.03
PWE+MP-1	10	20.00±1.88^{abd}	2.14±0.25	0.50±0.04^{a}	0.64±0.09	1.52±0.12^{bd}	2.16±0.16^{bd}	0.14±0.02^{d}
PWE+MP-3	10	4.63±1.12^{abe}	1.10±0.19^{ab}	0.26±0.06^{b}	0.21±0.04^{ab}	0.39±0.08^{ab}	0.60±0.07^{ab}	0.18±0.03

Note. $p<0.05$ according to Duncan's test compared to: group 1, b2, c3, d4, and e5.

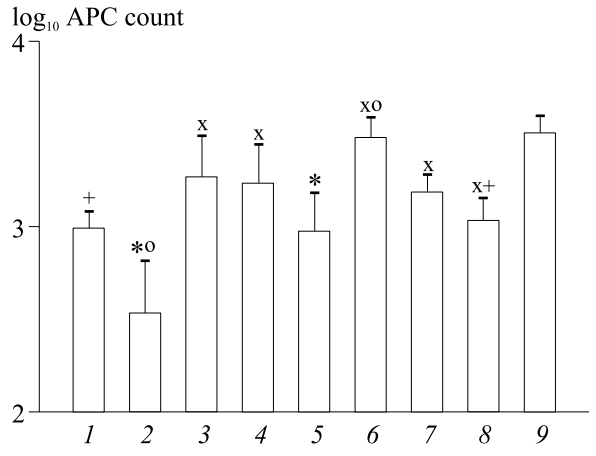

Fig. 1. Effects of myelopeptide and standard therapy on APC count in the regional lymph node in PWE. Here and in Fig. 2: *1*) PWE+0.9% NaCl; *2*) PWE+standard therapy; *3*) PWE+myelopid+standard therapy; *4*) PWE+MP-1+standard therapy; *5*) PWE+MP-3+standard therapy; *6*) PWE+myelopid; *7*) PWE+MP-1; *8*) PWE+MP-3; *9*) intact rats+0.9% NaCl. $p<0.05$: according to post-hoc Duncan's and LSD tests compared to: *group 9, xgroup 2; according to LSD test compared to: +group 9, ogroup 1.

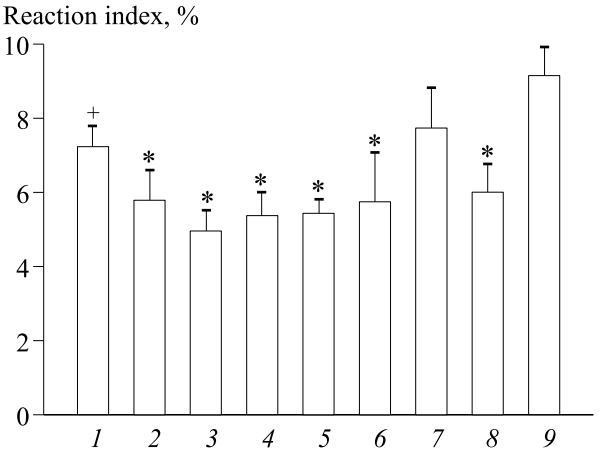

Fig. 2. Effects of myelopeptides and standard therapy for PWE on the intensity of DTH reactions

zone, depending on the therapeutic protocol, were noted (Table 1). Reduced count of granulocytes at the expense of neutrophils was detected in animals receiving standard therapy compared to this parameter in group 1. This effect was presumably mediated by dexamethasone and sodium diclofenac, exhibiting the maximum antiinflammatory effects [2,7]. A significant decrease in the counts of macrophages and granulocytes (neutrophils and eosinophils) was detected in rats treated with myelopid or MP-3. This effect was enhanced in animals receiving combinations of these drugs with standard therapy. It was found that MP-1 exhibited no antiinflammatory effect of its own and did not change the granulocyte count.

Lymphocyte count in tissue adjacent to the cicatrix increased under the effect of standard therapy. Myelopid used in complex with standard therapy abolished its effect and reduced lymphocyte count in the infiltrate, while injection of myelopid alone resulted in an appreciable increase of lymphocyte count. Lymphocyte count also increased in response to MP-1. The count of plasma cells increased only in animals receiving MP-1 in combination with standard therapy.

Hence, myelopid and MP-1 increased APC count reduced as a result of PWE and did not modify the suppression of DTH reaction. The best optimization of traumatic inflammation by inflammatory infiltration, density of cicatricial fibrous structures, and epithelialization of damaged zone were observed in animals treated with myelopid and MP-3 in complex with standard therapy.

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