
PHYSIOLOGY

The Effect of Polyoxidonium on Immune Response and Morphological Parameters of Inflammation after Experimental Penetrating Eye Injury

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Unique physiological features of the organ of vision and its immune status make penetrating eye injury (PEI) and its therapy a multidisciplinary problem [1–3]. PEI is an example of local injury inducing both local responses with the disruption of immunosuppression in the organ and general changes in the immune system [1, 4]. The Russian immunostimulant Polyoxidonium is a promising drug for enhancing healing processes in the injured eye tissues, formation of more mature and structured scar tissue [5, 6]. This study has provided evidence for the anti-inflammatory effect of Polyoxidonium and its ability to attenuate inflammatory cell infiltration in the injured area. We have demonstrated that, despite stimulation of secretion of antibodies, the traumatic immunosuppression of the delayed-type hypersensitivity response (DHR) to thymus-dependent xenoantigen occurs shortly after the injury. This offers new prospects in discovering the mechanism of the immunomodulation effect of this drug.

The study was performed on 119 white male rats weighing 213 ± 4 g. Penetrating injury of the right eye was inflicted under 2% procaine anesthesia [6]. On the first stage of the study, the immunomodulation effect of polyoxidonium alone or together with standard therapeutic protocol for PEI during immune response to sheep erythrocytes was evaluated. The injured animals were divided into four groups (Table 1). The fifth group included control animals (the PEI was not inflicted, while the right eye was sham anesthetized with 0.9% NaCl). All drugs were administered 6 h after the injury. Standard therapeutic drugs were administered subcutaneously (0.1 mg/kg dexamethasone phosphate once a day; 0.5 mg/kg sodium diclofenac,

12.5 mg/kg sodium ampicillin, and 1.5 mg/kg gentamicin sulfate twice a day). Polyoxidonium (0.1 mg/kg) was administered 6 h, two days, and four days after the injury. To induce the immune response, all rats were sensitized with sheep erythrocytes (10^8 cells subcutaneously in the right foot sole) 7 h after the beginning of the experiment. On day 4, the antigen was administered subcutaneously (10^9 sheep erythrocytes in the right sole, and 0.1 mL of 0.9% NaCl in the left sole). On day 5, all animals were anesthetized with ether and decapitated. The endocrine response was evaluated by the number of antibody-forming cells in the regional (popliteal) lymph node, which was evaluated by local hemolysis in agarose gel [7]; the DHR was evaluated by the response index [8].

In the second part of the study, the effect of Polyoxidonium on the morphological manifestations of the traumatic injury in non-immunized rats was evaluated. Injured animals were divided into five groups (Table 2). Animals without injury were included into the control group. All drug administration protocols and the euthanizing of animals were performed as described above. Morphometric analysis of histological specimens was carried out using the Videotest Morphologia 5.0 morphometric software (St. Petersburg, Russia). Measurements of the structures in the injured area were performed in 10 fields of view of hematoxylin–eosin stained slices at a magnification of $400\times$. The densities of fibers, fibroblasts, and inflammation cells per unit injured area (0.01 mm^2) were calculated. The statistical analysis was performed using post-hoc Duncan's test for multiple analysis of samples with a log-normal distribution [9]. The results are presented as the mean value and the standard error of the mean ($M \pm m$).

Rats with PEI (the first group) had inhibited antibody production in the regional lymph nodes and inhibited DHR (Table 1). The standard therapeutic protocol (the second group) did not prevent these changes and, in addition, led to a decrease in the num-

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Table 1. The effects of Polyoxidonium and standard therapeutic protocol for penetrating eye injury on the number of anti-body-forming cells and nucleated cells in the regional lymph node and DHR in rats

Group	Experimental procedure	Number of animals	Regional lymph node		DHR index, %
			number of antigen forming cell, log	number of nucleated cells, $\times 10^6$	
1	PEI + 0.9% NaCl solution	18	3.0961 ± 0.1075 (1248)	23.47 ± 2.67	10.33 ± 0.94
	$p_{1,5}$		$=0.012687$	>0.05	$=0.001282$
2	PEI + standard therapeutic protocol	18	2.6671 ± 0.1606 (465)	10.92 ± 1.51	7.60 ± 0.92
	$p_{2,5}$		$=0.000109$	$=0.016114$	$=0.000043$
	$p_{2,1}$		>0.05	$=0.014854$	>0.05
3	PEI + standard therapeutic protocol + polyoxidonium	10	4.1915 ± 0.1462 (15543)	34.2 ± 5.63	9.87 ± 1.12
	$p_{3,5}$		$=0.032617$	$=0.030722$	$=0.000967$
	$p_{3,1}$		$=0.000073$	$=0.027553$	>0.05
	$p_{3,2}$		$=0.000051$	$=0.000007$	>0.05
	$p_{3,4}$		>0.05	>0.05	>0.05
4	PEI + polyoxidonium	10	4.2683 ± 0.2405 (18548)	38.76 ± 3.24	9.94 ± 1.31
	$p_{4,5}$		$=0.019001$	$=0.002587$	$=0.000870$
	$p_{4,1}$		$=0.000057$	$=0.003138$	>0.05
	$p_{4,2}$		$=0.000033$	$=0.000033$	>0.05
5	Control	12	3.6879 ± 0.1412 (4875)	22.78 ± 4.35	16.19 ± 1.72

Note: The geometric mean of the antibody-forming cell numbers (the antilogarithm of the mean logarithm of antibody-forming cell number) is indicated in brackets. Here and in Table 2, $p_{1,5}$, $p_{4,1}$, $p_{4,2}$, etc. indicate the numbers of groups whose differences were estimated using Duncan's test.

Table 2. The quantitative analysis of structures in an injured area 6 h and five days after the injury and administration of Polyoxidonium and standard drugs (per 0.01 mm² of the injured area)

Group	Experimental procedure	Number of animals	Time	Fibers, number	Fibroblasts, number	Inflammatory infiltrate, number of cells
1	PEI + 0.9% NaCl solution	7	5 day	40.98 ± 0.79	6.99 ± 0.53	7.42 ± 0.67
2	PEI + standard therapeutic protocol	8	5 day	45.03 ± 2.44	6.81 ± 0.35	5.57 ± 0.32
	$p_{2,1}$			>0.05	>0.05	$=0.015567$
3	PEI + standard therapeutic protocol + polyoxidonium	8	5 day	36.92 ± 0.98	6.16 ± 0.38	5.25 ± 0.16
	$p_{3,1}$			>0.05	>0.05	$=0.007009$
	$p_{3,2}$			$=0.001133$	>0.05	>0.05
4	PEI + polyoxidonium	8	5 day	34.00 ± 2.23	5.85 ± 0.40	2.75 ± 0.29
	$p_{4,1}$			$=0.004398$	$=0.042569$	$=0.000053$
	$p_{4,2}$			$=0.000079$	>0.05	$=0.000657$
	$p_{4,3}$			>0.05	>0.05	$=0.001601$
5	PEI	10	6 h	8.92 ± 0.46	1.70 ± 0.10	12.98 ± 0.72
	$p_{5,1}$			$=0.000053$	$=0.000031$	$=0.000121$
	$p_{5,2}$			$=0.000031$	$=0.000053$	$=0.000063$
	$p_{5,3}$			$=0.000063$	$=0.000063$	$=0.000053$
	$p_{5,4}$			$=0.000121$	$=0.000121$	$=0.000031$

ber of nucleate cells in the organ. This could be induced by the inhibiting action of dexamethasone included in the standard protocol on the recruiting of antigen-specific B- and T-lymphocyte clones and their proliferation in situ, which was earlier described for glucocorticoids [8]. Administration of Polyoxidonium along with the standard therapy (the third group) induced an increase in the number of antigen-forming and nucleate cells in the regional lymph nodes; however, the suppression of DHR did not change. This is a positive result, considering the leading role of Th1 type immune response in the immunopathology of the eye [10]. Similar changes were found in the fourth group.

The morphological study showed that, 6 h after the injury, alteration prevailed in inflammatory processes of the cornea, while proliferation and synthesis in the connective tissue were absent, since the morphometric parameters of animals from the fifth group were different from the rest (Table 2). In animals that received 0.9% NaCl instead of drugs (the first group), formation of scar started in the cornea five days after the PEI. The experimental therapy (groups from the second to the fourth) decreased inflammatory infiltration. This decrease was the most prominent in the group that received only Polyoxidonium (the fifth group). The number of fibroblasts was decreased only in animals receiving Polyoxidonium (the fourth group); the other therapeutic protocols did not affect this parameter. At the same time, the synthetic activities of fibroblasts were different in these groups. The formation of fibers in the cornea did not change in the group with the standard treatment in comparison with the untreated group. A significant decrease in fiber density was found in rats of the fourth group receiving only Polyoxidonium in comparison with animals of the first and second groups. In animals of the third group receiving Polyoxidonium along with the standard therapy, the density of fibers was lower than in rats of the second group. We demonstrated earlier that simultaneous administration of Polyoxidonium and standard drugs led to the formation of a more structured scar tissue 12 days after the PEI [6]. In sham-

control animals, there were no morphological changes in eye tissues.

Polyoxidonium reduces inflammatory cell infiltration in the injured area and does not prevent the traumatic immunosuppression of DHR while stimulating the antibody formation in response to a xenoantigen.

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