Immunopharmacological Properties of Noopept

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Noopept, a peptide analog of piracetam, enhanced phagocytic activity of mouse peritoneal macrophages, stimulated humoral and cellular immune response to various antigens, and markedly increased spontaneous proliferative activity of splenocytes. In animals with secondary immune deficiency caused by cyclophosphamide, noopept exhibited immunocorrector properties.

Key Words: *noopept; cyclophosphamide; humoral and cellular immunity; phagocytosis; proliferative activity of splenocytes*

Glyproline oligopeptides exhibit a wide spectrum of functional activities acting as regulators of the nervous, endocrine, and immune systems [1]. Previous studies demonstrated immunoregulatory and neurotrophic activities of heptapeptide Semax [4,8] and antiviral and cytokine-modulating properties of Selank [11]. Noopept (ethyl ether of N-phenylacetyl-L-prolylglycine) by its characteristics is a peptide analog of piracetam possessing pronounced nootropic and neuroprotective effects [2,14]. Antiinflammatory effects of noopept were demonstrated on models of acute and chronic inflammation [5].

Here we studied immunotropic properties of noopept administered in different doses and through different routes to CBA, C57Bl/6, and (CBA×C57Bl/6)F_1 mice and in animals with secondary immunodeficiency caused by cyclophosphamide (CP) treatment.

MATERIALS AND METHODS

Experiments were carried out on male CBA, C57Bl/6, and (CBA×C57Bl/6)F_1 mice weighing 18-20 g (n=274, Stolbovaya nursery, Russian Academy of Medical Sciences).

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The dose—effect relationship for noopept is described by a double-humped dome—shaped curve with peaks of antiamnestic effects at doses of 0.5-0.8 and 10 mg/kg [9]. The resistance of the dipeptide to enterocyte brush border enzymes determines its efficiency after oral administration; the neuroprotective effects of noopept are more pronounced after parenteral administration (injections) [3]. In light of this, noopept was administered intravenously, intramuscularly, and *per os* in doses of 0.5, 5 and 10 mg/kg. The effects of noopept on cellular immune response, specifically, on delayed-type hypersensitivity (DTH) reaction and phagocytic activity of peritoneal macrophages (absorption of Indian ink particles) were studied in experiments on (CBA×C57Bl/6)F_1 mice [10]. For evaluation of spontaneous and mitogens-induced proliferation of splenocyte from (CBA×C57Bl/6)F_1 mice, aseptically prepared splenocyte suspension was cultured in a CO_2-incubator for 72 h and the cells were transferred onto fiberglass filters (Whatman) using a harvester (Flow). Radioactivity was counted on a scintillation β-counter (in collaboration with N. F. Gamaleya Institute of Epidemiology and Microbiology, Moscow).

The effects of noopept on humoral immunity was studied in 3 experimental series after immunization with sheep erythrocytes (SE) in different concentrations using Erne method and reaction of direct agglutination carried out in a Takachi micro-

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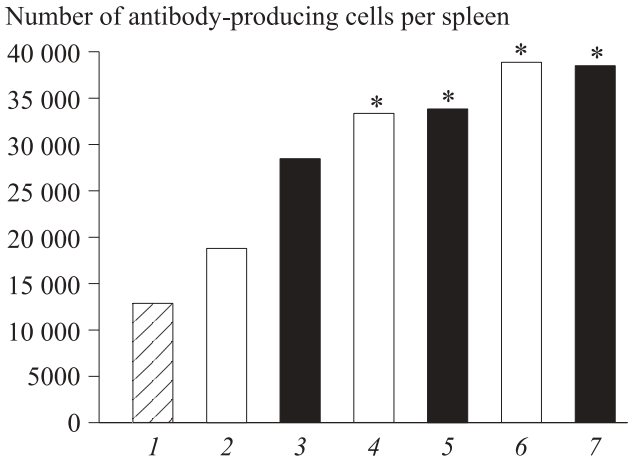
titration device. The amounts of antibody-producing cells and antibodies were measured in two mouse strain demonstrating opposite reaction to SE: CBA and C57Bl/6 [10]. For induction of secondary immunodeficiency, the mice received intraperitoneal injection of CP (Sigma) in a dose of 150 mg/kg 1 day before SE injection.

Noopept was administered to CBA and C57Bl/6 mice simultaneously with the antigen (for evaluation of its effects on the induction phase of the immune response) or on days 3 or 5 after immunization with SE (for evaluation of its effects on the productive phase). The presence of IgM and IgG in samples was assayed 30 min after inactivation of blood serum at 56°C by adding 1 M 2-mercaptoethanol (1:10 v/v) followed by 30-min incubation at 37°C.

The results were expressed as mean $\log_2$ antibody titer in the reaction of direct hemagglutination.

Significance of differences was evaluated using Student *t* test.



Effect of single intramuscular injection of noopept on humoral immune response in C57Bl/6 male mice (*n*=7-8) after intravenous injection of SE in a dose of 5×10^6. *1*) control; *2*, *3*) noopept 24 h before SE, *4*, *5*) noopept simultaneously with SE, *6*, *7*) noopept 24 h after SE. Light bars: 0.5 mg/kg noopept, dark bars: 5 mg/kg noopept. **p*<0.05 compared to the control.

RESULTS

Oral administration of noopept in a dose of 10 mg/kg considerably increased phagocytic index (by 3.6 times, to 18.1±2.9 vs. 5.1±1.4 in the control). After single intravenous injection of the preparation in a dose of 5 mg/kg phagocytic activity of peritoneal macrophages tended to increase (to 10.1±2.0), while spontaneous proliferative activity of splenocytes increased by 70.4%. After peroral treatment with noopept in a dose of 10 mg/kg for 14 days, spontaneous proliferation of lymphoid cells also tended to increase. Proliferative response of T cells to concanavalin A significantly increased (by 16.2%) only in mice receiving the preparation for 14 days (Table 1).

Single intramuscular injection of noopept in doses of 0.5 and 5 mg/kg to CBA mice did not stimulate antibody formation, while in C57Bl/6 mice administration of noopept in these doses considerably increased humoral immune response by 16.7 and 22.7%, compared to the control. In C57Bl/6 mice, 2.6- and 3-fold increase in IgM-response compared to the control was observed after administration of noopept simultaneously with SE and on the next day after antigenic stimulation, respectively (Fig. 1).

Intravenous injection of noopept in a dose of 5 mg/kg to CBA mice during the productive phase of IgM-response stimulated antibody production compared to the control (Table 2). The effect of noopept on the synthesis of IgG was studied on CBA and C57Bl/6 mice. Similarly to previous experiments, the more pronounced stimulation of humoral immune response (during both inductive and proliferative phases) was observed in C57Bl/6 mice characterized by lower immune response to SE. In animals with CP-induced immunodeficiency, IgM-response was suppressed by 38% compared to the control. Intravenous injection of the dipeptide to mice 1 or 6 days after CP treatment prevented the development of immunodeficiency (Table 2). Administration of CP to CBA and C57Bl/6 mice suppressed production of IgG antibodies by 69.2 and 59.1% compared to the control, while noopept markedly stimulated antibody production.

TABLE 1. Effect of Noopept on Proliferative Activity of Splenocytes (*M*±*m*; *n*=10)

Group	^{3}H-thymidine incorporation (cpm) in cultures	
	without mitogen	with concanavalin A
Control	26,484±2443	744,567±1155
Noopept, 5 mg/kg intravenously	45,129±6283*	72,257±5165
Noopept, 10 mg/kg *per os*	33,753±5633	86,676±5759*

Note. **p*<0.05 compared to the control.

TABLE 2. Effect of Noopept on Inductive and Proliferative Phases of Humoral Immune Response in CBA and C57Bl/6 Mice after Intraperitoneal Injection of 5×10^6 SE ($M\pm m$)

Experimental Conditions	IgM	IgG	
	CBA	CBA	C57Bl/6
SE	6.25±0.74 (n=9)	2.63±0.86 (n=8)	2.22±1.00 (n=9)
SE+noopept, 5 mg/kg intravenously	6.75±0.59 (n=9)	4.89±1.12* (n=9)	4.60±0.97* (n=10)
SE+noopept, 5 mg/kg intravenously (on days 3-5)	7.50±0.45* (n=8)$^+$	3.20±0.34 (n=10)$^{++}$	4.44±0.78* (n=9)$^{++}$
CP, 150 mg/kg intraperitoneally, after 24 h			
SE	3.88±0.70 (n=9)	0.75±0.27 (n=8)	0.86±0.22 (n=8)
SE+noopept, 5 mg/kg intravenously	5.13±1.37 (n=9)	4.13±0.37* (n=9)	4.33±0.27* (n=8)
SE+noopept, 5 mg/kg intravenously (on day 5)	5.75±1.07* (n=9)	2.88±0.56* (n=8)	4.43±1.21* (n=9)

Note. $p<0.05$ compared to: *control, $^+$administration of noopept on day 3, $^{++}$administration of noopept on day 5 after SE.

Intravenous injection of noopept in a dose of 5 mg/kg to $(CBA\times C57Bl/6)F_1$ mice simultaneously with antigenic stimulation with SE sharply increased DTH reaction (Table 3). Injection of CP 24 h before antigenic stimulation considerably suppressed cellular immune response. Dipeptide administered to mice with secondary immunodeficiency not only abolished the suppressive effect of CP, but also stimulated DTH reaction compared to the corresponding values in the control group. Hence, noopept exhibited an immunocorrective effect on cellular and humoral immune responses in mice of various strains with CP-induced secondary immunodeficiency.

These results are consistent with published data on immunocorrective activities of regulatory peptides. A series of experiments performed by A. A. Mikhailova *et al*. showed that myelopeptides act as immunocorrectors, in particular, hexapeptide MP-1 (Phe-Leu-Gly-Phe-Pro-Thr) restored immune response suppressed by irradiation, antibiotics, cytostatics, while myelopeptide MP-3 stimulated functional activity of macrophages [6,7]. Administration of noopept to NMRI mice selectively increased the titer of antibodies to β-amyloid$_{(25-35)}$ peptide prefibrils [12], which is an important factor of the neuroprotective effect of this preparation in neurodegenerative disorders.

The efficiency of noopept in experimental ischemic stroke was demonstrated [3]. It is well known that changes in the immune response during cerebral ischemia lead to apoptotic death of lymphocytes and shift the T-helper response towards Th2

TABLE 3. Effect of Noopept on Cellular Immunity in $(CBA\times C57Bl/6)F_1$

Experimental conditions	Index of reaction	DTH reaction, % of control
SE, 5×10^7 subcutaneously	37.1±2.8 (n=8)	100
Noopept, 5 mg/kg intravenously	54.1±9.1* (n=8)	145.9
CP, 150 mg/kg intraperitoneally, after 24 h		
SE, 5×10^7 subcutaneously	31.6±1.7* (n=8)	85.3
SE, 5×10^7 subcutaneously+ noopept, 5 mg/kg intravenously	59.4±11.7* (n=9)	160.2

Note. *$p<0.05$ compared to the control.

[13]. Stroke induces long-lasting depression of the cell-mediated immunity (monocyte deactivation, lymphopenia, Th1/Th2 shift) and promotes inflammation, which leads to aggravation of brain injury and widening of the ischemic area [13,15]. In our experiments, noopept exhibited maximum activity under conditions of CP-induced immunodeficiency and genetically determined low response to SE in C57Bl/6 mice, which also attests to immunocorrective properties of this preparation.

Thus, the combination of immunotropic activity and pronounced antiinflammatory properties in noopept suggests the possibility of using this preparation for the treatment of neurodegenerative disorders associated with the development of immunodeficient states caused by age-related disturbances in humoral and cellular immunity, physical and psychoemotional traumas.

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