Comparative Study of Immunocorrective Activity of Phenibut and Its Organic Salts in Experimental Immunodeficiency

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Phenibut and its derivatives normalized cellular (index of delayed-type hypersensitivity reaction) and humoral (antierythrocyte antibody titer) immunity and the lymphoproliferative processes in immunocompetent organs of animals with cyclophosphamide-induced immunosuppression. This indicates pronounced immunocorrective effects of these substances.

Key Words: *phenibut and its salts; immunocorrection; cyclophosphamide*

Modern immunophysiology proved the important role of neuroimmune relationships in normal and pathological functioning [2,3]. Based on the mutual dependence and functional integration of the nervous and immune systems, it was proven experimentally and clinically that congenital and acquired "defects" in one system inevitably manifested by changes in the other and in the entire regular (neuroimmunoendocrine) metasystem in general [5,6]. Hence, disorders in the function of the nervous system are associated with more or less pronounced changes in immunological reactivity [1,5]. Therefore, the search for substances with psychopharmacological effects and capable of correcting immune disorders in addition to their positive effects on the psychoemotional status is an important problem.

We compared immunocorrective activity of phenibut and its derivatives in experimental immunodeficiency.

MATERIALS AND METHODS

The study was carried out on 180 male and female CBA mice aged 3-4 months (18-20 g). The animals were divided into groups of 10 mice for each experimental series. Immunized animals receiving injection of 0.5 ml distilled water served as control group 1. In control group 2, immunological deficiency was induced by intraperitoneal injection of cyclophosphamide in a dose of 150 mg/kg (LD_{50}= 190 mg/kg) [4]. One hour after cyclophosphamide injection, the animals of experimental groups received a single therapeutic dose of phenibut (25 mg/kg) or its salts phenibut succinate (compound RGPU-149; 47.6 mg/kg), phenibut malate (RGPU-150; 49.2 mg/kg), or phenibut nicotinate (RGPU-151; 48.1 mg/kg). Doses of phenibut derivatives constituted $^1/_{10}$ molecular weight of the substance.

The effects of phenibut and its organic salts on the cellular component of the primary immune reaction to sheep erythrocytes (SE) were evaluated by delayed-type hypersensitivity (DTH) test. Their effects on humoral immunity were evaluated by the passive hemagglutination test (PHAT) [7]. The weights and cell composition of immunocompetent organs (thymus and spleen) were studied in order to evaluate the effects of phenibut and its derivatives on proliferative processes in these organs [7].

For DTH test, the animals were subcutaneously immunized with SE (2×10^8/100 μl saline; sensitization). On day 5, the resolving dose (10^8 SE in 50 μl saline) was injected under the aponeurosis of the right hind (experimental) paw and 50 μl solvent

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was injected into the left (control) paw. Local reaction was evaluated 24 h after injection of the resolving dose of the antigen and DTH index was estimated by the formula:

$$\text{Reaction index}=\frac{M_e-M_c}{M_c}\times 100\%,$$

where M_e and M_c are the weight of experimental and control paws, respectively.

The effects of phenibut and its organic salts on the formation of humoral immune response were studied as follows. Antigenic stimulation was carried out by single intraperitoneal injection of SE (5×10^8/100 μl saline) 1 h after injection of the studied substance. Seven days after immunization, the animals were sacrificed by chloroform overdosage and the serum was isolated. The complement was inactivated by heating the serum at 56°C for 30 min. PHAT was carried out in 96-well plates. In order to suppress nonspecific binding of antibodies, the test was carried out in 50 μl solvent (0.5% BSA in saline); serial double dilutions of the studied sera were made in this solvent. After serum dilution, SE (25 μl 1% suspension) was added to each well. Tentative evaluation of PHAT results was carried out after 1-h incubation at 37°C. The plates were then placed in a refrigerator (4°C) and after 18 h the

TABLE 1. Effects of Phenibut and Its Organic Salts on the Formation of Immune Response under Conditions of Experimental Immunosuppression (*M*±*m*)

Immune response parameter	Control 1	Control 2: CP (150 mg/kg)	Experiment 1: phenibut (25 mg/kg)+CP (150 mg/kg)	Experiment 2: RGPU-149 (47.6 mg/kg)+CP (150 mg/kg)	Experiment 3: RGPU-150 (49.2 mg/kg)+CP (150 mg/kg)	Experiment 4: RGPU-151 (48.1 mg/kg)+CP (150 mg/kg)
DTH index, %	11.4±0.5	8.2±0.6 t_1=4.2 at p_1<0.05	19.7±2.7 t_1=3.0 at p_1<0.05 t_2=4.2 at p_2<0.01	21.5±1.5 t_1=6.4 at p_1<0.001 t_2=8.3 at p_2<0.001	31.7±2.4 t_1=8.3 at p_1<0.001 t_2=9.5 at p_2<0.001	26.5±4.4 t_1=3.4 at p_1<0.05 t_2=4.2 at p_2<0.01
Antibody titer in PHAT, lg	1.20±0.06	0.50±0.03 t_1=10.4 at p_1<0.001	1.24±0.13 t_1=0 t_2=4.5 at p_2<0.01	1.30±0.03 t_1=1.7 at p_1<0.05 t_2=20 at p_1<0.001	1.35±0.12 t_1=1.2 at p_1<0.05 t_2=7.1 at p_1<0.001	1.90±0.12 t_1=5.4 at p_1<0.001 t_2=11.6 at p_1<0.001
Spleen weight, mg	102.3±3.2	84.8±3.4 t_1=3.7 at p_1<0.05	123.8±11.6 t_1=1.6 at p_1<0.05 t_2=3.3 at p_2<0.05	140.0±10.6 t_1=3.4 at p_1<0.05 t_2=5.0 at p_2<0.01	110.0±7.7 t_1=0.9 at p_1<0.05 t_2=3.0 at p_2<0.05	125.9±7.6 t_1=2.9 at p_1<0.05 t_2=5.0 at p_2<0.01
Count of NC/mg spleen, $\times10^5$	2.7±0.2	2.0±0.2 t_1=2.5 at p_1<0.05	3.5±0.4 t_1=1.8 at p_1<0.05 t_2=3.4 at p_2>0.05	3.2±0.4 t_1=1.1 at p_1<0.05 t_2=2.7 at p_2>0.05	3.2±0.6 t_1=0.8 at p_1<0.05 t_2=1.9 at p_2>0.05	2.5±0.3 t_1=0.6 at p_1<0.05 t_2=1.4 at p_2>0.05
Thymus weight, mg	38.5±1.0	31.0±1.4 t_1=4.4 at p_1<0.01	49.4±2.5 t_1=4.0 at p_1<0.01 t_2=6.5 at p_2<0.001	38.6±1.9 t_1=0 t_2=3.2 at p_2>0.05	47.6±3.5 t_1=2.5 at p_1<0.05 t_2=4.4 at p_2<0.01	42.6±2.4 t_1=1.6 at p_1<0.05 t_2=4.2 at p_2<0.01
Count of NC/mg thymus, $\times10^4$	3.5±0.2	2.8±0.2 t_1=2.5 at p_1<0.05	8.0±1.3 t_1=3.5 at p_2>0.05 t_2=4 at p_2>0.01	10.8±2.8 t_1=2.6 at p_2>0.05 t_2=2.9 at p_2>0.05	2.7±0.9 t_1=0.9 at p_2>0.05 t_2=0.1 at p_2>0.05	3.7±0.9 t_1=0.2 at p_2>0.05 t_2=1.0 at p_2>0.05

Note. Statistical values in comparison with control 1 (t_1 and p_1) and control 2 (t_2 and p_2). CP: cyclophosphamide.

reaction was evaluated. Antibody titer (maximum dilution of the serum, at which SE were agglutinated) was expressed in geometric mean values.

In order to detect changes in the weights and cell composition of immune organs, laboratory animals were immunized with SE (5×10^8/100 μl saline) 1 h after injection of the test substance. The animals were sacrificed after 24 h. The thymus and spleen were removed and weighed; cells suspensions were prepared in medium 199 (50 mg/ml for the spleen and 10 mg/ml for the thymus), filtered, washed in medium 199 (2×10 min at 1500*g*) to remove adipose tissue admixtures, and resuspended in medium 199 to initial concentration. The suspensions of the lymphoid organ were mixed with 3% acetic acid (1:1) stained with methylene blue and nucleated cells (NC) were counted in a Goryaev chamber. The counts of NC were expressed in absolute values and percents.

The results were processed statistically. The significance of differences between the parameters was evaluated using Student's *t* test.

RESULTS

Single intraperitoneal injection of cyclophosphamide in a dose of 150 mg/kg suppressed cell-mediated delayed-type hypersensitivity reaction, inhibited the formation of antierythrocyte antibodies in PHAT, and blocked the lymphoproliferative processes in the thymus and spleen. All values in mice of control group 2 were significantly lower than in animals receiving distilled water (Table 1).

Comparison of the effects of phenibut and its derivatives on the cellular component of immunogenesis showed that all these substances not only restored ($p_2<0.001$), but appreciably stimulated DTH (from $p_1<0.05$ to $p_1<0.001$). Phenibut malate and nicotinate exhibited a more pronounced immunostimulatory effect than phenibut (Table 1).

Evaluation of the humoral immunoreactivity revealed a statistically significant increase in the titer of antierythrocyte antibodies after injection of phenibut nicotinate in comparison with the immunosuppression model ($p_2<0.001$) and with animals receiving placebo ($p_1<0.001$). This indicates obvious immunostimulatory effects of the substance. Phenibut malate (RGPU-150) injected after cyclophosphamide progressively stimulated antibody production. Injection of these substances eliminated the immunosuppressive effect of the cytostatic (from $p_2<0.01$ to $p_2<0.001$) and the values reached the levels observed in animals receiving distilled water ($p_1>0.05$; Table 1).

The studied substances caused changes in the morphometric parameters of the immune organs in animals with immunodeficiency (Table 1). Phenibut abolished involution of the spleen and increased its weight ($p_1<0.05$; $p_2<0.05$) and cellularity ($p_1<0.05$; $p_2<0.05$). The drug acted on the thymus as an immunostimulator ($p_1<0.05$ and $p_2<0.05$). In animals treated with phenibut succinate we observed a significant increase in the weights of the lymphoid organs ($p_1<0.05$ and $p_2<0.05$) and NC counts ($p_1<0.05$ and $p_2<0.05$). Phenibut malate (RGPU-150) virtually did not change the cell count ($p_1<0.05$ and $p_2<0.05$), but significantly increased the weights of immunogenesis organs ($p_1<0.05$ and $p_2<0.05$; Table 1).

Hence, phenibut and its organic salts exhibited a corrective effect on the humoral and cell-mediated components of the primary immune response to thymus-dependent antigen in cyclophosphamide-induced immunosuppression. Comparison of the experimental results showed that phenibut derivatives induced pronounced positive shifts in all studied parameters of immune reactivity, similar (or superior by some parameters) to the effects of the reference drug.

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