PHARMACOLOGY AND TOXICOLOGY

Immunocorrecting Properties of GB-115 Dipeptide

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We studied the effects of a new dipeptide with anxiolytic activity (GB-115, N-phenylhexanoyl-glycyl-L-tryptophan amide) on parameters of the immune in intact mice and in animals with secondary immunodeficiency caused by cyclophosphamide. GB-115 in doses of 0.1-10 mg/kg stimulated phagocytic activity of peritoneal macrophages and humoral immune response in intact mice. GB-115 exhibited immunocorrecting activity in animals with secondary immunodeficiency.

Key Words: *GB-115; phagocytosis; humoral and cellular immunity; immunophenotyping; cyclophosphamide*

Oligopeptides involved in homeostatic regulation of the nervous, endocrine, and immune system have a wide range of functions and exhibit anxiolytic, neuroprotective, antiulcer, antithrombotic, immunocorrecting, and other properties [2,5,9,10]. Peptide preparations are effective in low doses and have insignificant toxic activity [9]. A new dipeptide antagonist of cholecystokinin receptors (GB-115) was synthesized from the endogenous anxiogenic tetrapeptide cholecystokinin-4 (V. V. Zakusov Institute of Pharmacology) [4]. Experiments on mice and rats demonstrated antinociceptive and anxiolytic properties of GB-115 [6]. Taking into account the multicomponent effects of regulatory peptides, we can hypothesize that glycine-containing dipeptide GB-115 exhibits immunocorrecting activity.

Here we studied the effects of GB-115 on immune parameters in intact mice and in animals with secondary immunodeficiency induced by cyclophosphamide (CPA).

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MATERIALS AND METHODS

Experiments were performed on male C57Bl/6 mice (n=33), CBA mice (n=60), BALB/c mice (n=80), and F_1(CBA×C57Bl/6) hybrid mice (n=120) weighing 18-20 g and obtained from the Stolbovaya nursery (Russian Academy of Medical Sciences). GB-115 (N-phenylhexanoyl-glycyl-L-tryptophan amide) was synthesized at the Department of Chemistry (V. V. Zakusov Institute of Pharmacology). Pharmacological study showed that the dipeptide GB-115 is present in blood plasma after peroral administration and injection [3]. Taking into account the results of previous experiments, GB-115 was administered in doses of 0.1-10 mg/kg [6].

Immune parameters were estimated by the standard method [1]. Phagocytic activity of peritoneal macrophages (engulfment of ink particles) was evaluated in F_1(CBA×C57Bl/6) mice. For evaluation of the humoral immune response to thymus-dependent antigen sheep erythrocytes (SE), CBA and C57Bl/6 mice with the opposite reaction to this antigen were immunized intraperitoneally. Serum antibody titer was measured in the reaction of hemagglutination

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(RPHA) after 7 days. The cellular immune response was estimated from delayed-type hypersensitivity reaction (DTH) of F_1(CBA×C57Bl/6) mice [11]. Secondary immunodeficiency was induced by intraperitoneal injection of an alkylating agent CPA (Sigma) in a dose of 150 mg/kg [5]. GBA-115 was administered 3 times. The first treatment with GB-115 was performed 24 h after CPA injection. Immunization with SE was performed on day 4 after CPA injection to study the humoral and cellular immune response during secondary immunodeficiency. Subpopulation composition of lymphocytes was assayed on an EPICS XL 4 color flow laser cytometer (Beckman Coulter) [12]. On days 4 and 8 after CPA injection, spleen homogenates from BALB/c mice were stained with FITC-labeled monoclonal antibodies CD4 (Clone YTS191.1) and CD8 (Clone KT15) according to manufacturer's recommendations (non-washing method) and using a lysing reagent Optilyse C. All reagents were from Beckman Coulter. Data processing involved at least 10,000 events. The results were analyzed by Student's *t* test.

RESULTS

Peroral administration of GB-115 in doses of 0.1 and 10 mg/kg for 14 days was followed by a significant decrease in the weight of lymph nodes in F_1(CBA×C57Bl/6) mice (by 33.3 and 55.6%, respectively; Table 1). GB-115 had little effect on the weights of the spleen and thymus. Studying the function of peritoneal macrophages in mice showed that intraperitoneal injection of GB-115 in a dose of 1 mg/kg (3-fold treatment) increases the phagocytic index by 2.3 times compared to the control (21.5±5.5 and 9.3±1.0, respectively, $p<0.05$). However, injection of GB-115 in a dose of 0.1 mg/kg had little effect on phagocytosis by peritoneal macrophages (11.2±2.0). Our results are consistent with published data that some oligopeptides, including myelopeptide MP-3 (Leu-Val-Cys-Tyr-Pro-Gln) [8] and hexapeptide Semax (Thr-Lys-Pro-Arg Pro-Gly-Pro) [9], stimulate peritoneal macrophages.

Study of the humoral immune response showed that peroral administration of GB-115 to C57Bl/6 (0.1 and 10 mg/kg) and CBA mice (10 mg/kg) for 14 days significantly increases antibody production. However, the cellular immune response in F_1(CBA×C57Bl/6) mice remained unchanged after peroral administration of GB-115 in doses of 0.1 and 10 mg/kg for 14 days.

CPA-induced secondary immunodeficiency in control mice manifested in a significant decrease in the humoral and cellular immune response to SE. The immunocorrecting effect of GB-115 (0.1 mg/kg, 3 times, *per os*) was verified by a significant increase in antibody production and DTH, which did not differ from those in intact animals (Table 3).

In the next series, immunophenotyping of lymphocytes from BALB/c mice was performed in various stages of secondary immunodeficiency. Alkylation of DNA in proliferating and resting cells is accompanied by phasic changes in lymphopoiesis. The disappearance of mitoses is followed by the

TABLE 1. Weight of Lymphoid Organs in F_1(CBA×C57Bl/6) Mice after Peroral Administration of GB-115 for 14 Days (% of Body Weight×10^2, *M*±*m*)

Organ	Control	GB-115, 0.1 mg/kg	GB-115, 10 mg/kg
Spleen	27.5±0.7	29.3±1.0	28.0±0.5
Thymus	20.1±1.1	24.6±2.1	23.2±3.0
Popliteal lymph nodes	3.6±0.3	4.9±0.4*	5.6±0.3**

Note. Each group consisted of 10 animals. *$p<0.05$ and **$p<0.01$ compared to the control.

TABLE 2. Effect of Peroral Administration of GB-115 in Doses of 0.1 and 10 mg/kg for 14 Days on the Humoral (RPHA) and Cellular (DTH) Immune Response

Group of animals	Cellular immune response in F_1(CBA×C57Bl/6) hybrid mice, reaction index, %	Immune response to antigenic stimulation with 5×10^7 SE, mean $\log_2$ antibodies in RPHA	
		CBA	C57Bl/6
Control	29.2±2.6 (*n*=10)	7.4±0.3 (*n*=10)	6.0±0.3 (*n*=10)
GB-115, 0.1 mg/kg	25.2±3.8 (*n*=10)	8.2±0.4 (*n*=10)	7.5±0.4* (*n*=11)
GB-115, 10 mg/kg	31.7±2.3 (*n*=10)	8.6±0.2 (*n*=10)	8.8±0.4* (*n*=12)

Note. *n*, number of animals per group; *$p<0.01$ compared to the control.

TABLE 3. Immunocorrecting Effect of GB-115 (0.1 mg/kg Perorally) on Humoral (RPHA) and Cellular (DTH) Immune Response in Mice with CPA-Induced Immunodeficiency

Group of mice	Cellular immune response in F_1(CBA×C57Bl/6) hybrid mice, reaction index, %	Immune response to antigenic stimulation with 5×10^6 SE, mean $\log_2$ antibodies in RPHA
Intact	49.5±6.0	3.9±0.3
Control (CPA, 150 mg/kg)	15.6±1.7*	1.6±0.3*
CPA (150 mg/kg)+ GB-115 (0.1 mg/kg)	53.6±3.5+	3.3±0.4+

Note. Each group consisted of 10 animals. $p<0.01$: *compared to intact animals; +compared to the control.

stage of regeneration. The number of mitoses increases in the initial stage, but decreases in the follow-up period [7]. Our experiments showed that the weights of the thymus and spleen significantly decrease on day 4 after CPA administration. These parameters returned to normal after treatment with GB-115. Compensatory hyperplasia of the spleen was observed on day 8. Involution of the thymus persisted during this period. The dipeptide (3 intraperitoneal injections) had a protective effect and normalized the weight of the thymus. However, GB-115 had little effect on the weight of the spleen (Fig. 1). Immunophenotyping of spleen lymphocytes showed that $CD4^+/CD8^+$ lymphocyte ratio significantly increases on day 4 after CPA injection. GB-115 had no effect on the number of these cells (Fig. 1). Secondary immunodeficiency was manifested in a decrease in all parameters on day 8 after CPA injection. GB-115 had a strong correcting effect on these changes (Fig. 2).

Our results are consistent with published data on immunocorrecting activity of chemically different anxiolytics. Similar changes were described previously [1,5]. For example, the dipeptide Noopept (N-phenyl-acetyl-L-propyl-glycine, peptide analogue of piracetam) exhibits nootropic, neuroprotective, and anxiolytic properties [1]. This compound increases phagocytic activity of peritoneal macrophages and stimulates the humoral and cellular immune response in mice of various strains during antigenic stimulation. Noopept was effective during secondary immunodeficiency due to CPA administration [5]. Some peptide preparations (delta sleep-inducing peptide, Cerebrolysin, and Noopept) significantly stimulate proliferative activity of various cells, including splenocytes (Noopept) and neurons (Cerebrolysin). These changes contribute to the increase in immunomodulating and neuroprotective properties [5].

Chronic stress is accompanied by immunosuppression, which probably contributes to the development of diseases with various immune disorders. GB-115 normalizes the humoral and cellular immune response, restores the $CD4^+/CD8^+$ T

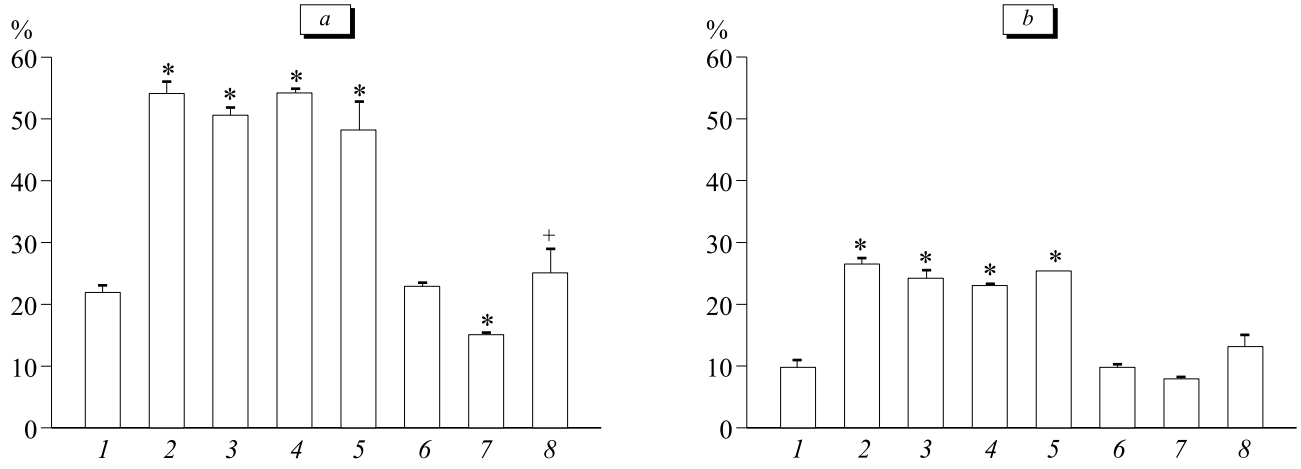

Fig. 1. Effect of intraperitoneal injection of GB-115 on the number of $CD4^+$ (*a*) and $CD8^+$ lymphocytes in the spleen (*b*) of BALB/c mice with cyclophosphamide-induced secondary immunodeficiency (days 4 and 8 after CPA administration). Here and in Fig. 2: intact animals, day 4 (*1*); CPA, day 4 (*2*); CPA+GB-115 (0.1 mg/kg), day 4 (*3*); CPA+GB-115 (1 mg/kg), day 4 (*4*); CPA+GB-115 (10 mg/kg), day 4 (*5*); intact animals, day 8 (*6*); CPA, day 8 (*7*); and CPA+GB-115 (1 mg/kg), day 8 (*8*). *$p<0.01$ compared to intact mice (control); +$p<0.05$ compared to control mice with cyclophosphamide-induced immunodeficiency.

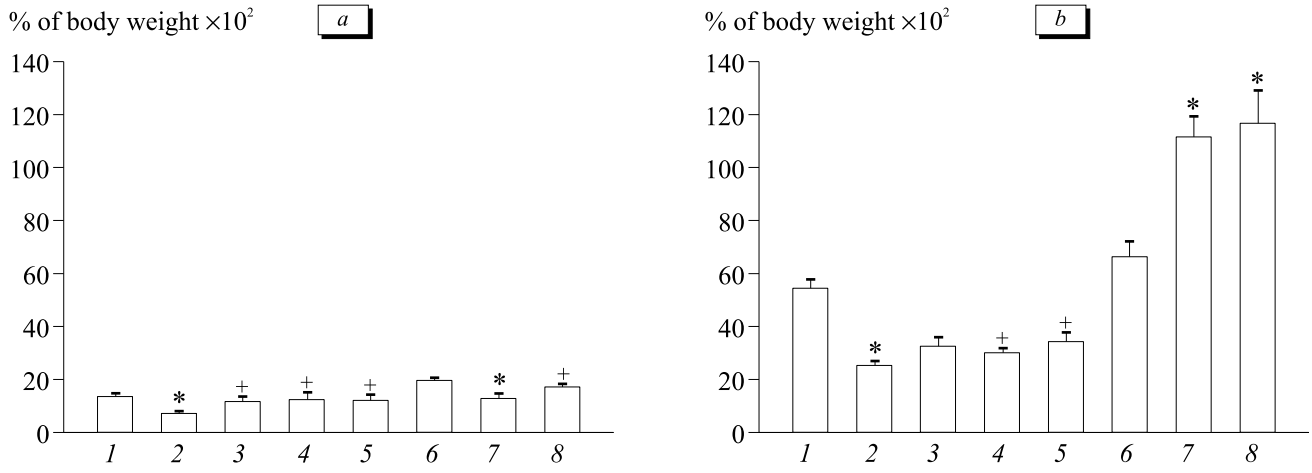

Fig. 2. Effect of intraperitoneal injection of GB-115 on the weights of the thymus (*a*) and spleen (*b*) in BALB/c mice with cyclophosphamide-induced secondary immunodeficiency (days 4 and 8 after CPA administration).

lymphocyte ratio, and prevents involution of the thymus during secondary immunodeficiency. These changes play an important role in the multicomponent effects of GB-115.

Thus, the detection of immunocorrecting properties of the dipeptide preparation GB-115 extends our knowledge on biological activity of GB-115 and suggests that GB-115 holds much promise for the correction of neuroimmune disorders accompanied by immunosuppression.

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