Study of Anti-Inflammatory Effects of GB-115, a Glycine-Containing Retropeptide Cholecystokinin Analog

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Anti-inflammatory effects of GB-115 compound (N-phenylhexanoyl-glycyl-L-tryptophan amide) injected intraperitoneally in doses of 0.1, 1, and 10 mg/kg were demonstrated on the model of ConA- and carrageenan-induced inflammation. Intraperitoneal injection of GB-115 in a dose of 1 mg/kg to C57Bl/6 female mice with experimental autoimmune encephalomyelitis significantly alleviated the pathological symptoms, improved spontaneous locomotor activity, promoted recovery of thymus weight, and reduced edema and neutrophil infiltration of the perivascular space of the brain tissue. Intraperitoneal injection of GB-115 in a dose of 1 mg/kg suppressed generation of active oxygen forms by neutrophils in the chemiluminescence test.

Key Words: *GB-115; ConA; carrageenan; experimental encephalomyelitis; neutrophils*

A new dipeptide cholecystokinin receptor antagonist GB-115 has been created at V. V. Zakusov Institute of Pharmacology [2]. Anxiolytic and antinociceptive effects of the compound were demonstrated in *in vivo* experiments [5,6]. Immunocorrective effects of GB-115 was detected in secondary immunodeficiency induced by cyclophosphamide injection [10]. Since the polycomponent effects of some regulatory oligopeptides include their immunopharmacological activity [1,3,4], we studied the anti-inflammatory effect of GB-115 in experimental inflammation, autoimmune encephalomyelitis (EAE), and the effect of the compound on generation of reactive oxygen species (ROS) by neutrophils.

MATERIALS AND METHODS

Experiments were carried out on 36 C57Bl/6 female mice (12-15 g), 50 CBA and 30 F_1(CBA×C57Bl/6) males (18-20 g), and 50 outbred male rats (150-200 g) from Stolbovaya Breeding Center of the Russian Academy of Medical Sciences. Compound GB-115 (N-phenylhexanoyl-glycine-L-tryptophane amide) was synthesized at Department of Chemistry of V. V. Zakusov Institute of Pharmacology [9].

The choice of GB-115 doses was based on the results of previous studies [5,6].

ConA-induced inflammatory reaction was simulated as described previously [8]. Compound GB-115 in doses of 0.1, 1, and 10 mg/kg was injected intraperitoneally to CBA mice 30 min before injection of ConA (Sigma).

In order to evaluate the anti-inflammatory effect of GB-115 on carrageenan-induced exudative edema of the paw [8], 20 min before carrageenan (Sigma) administration the rats were intraperitoneally injected with GB-115 in doses of 0.1, 1, and 10 mg/kg and the reference drug diclofenac (Chemopharm) in a dose of 6.75 mg/kg, which corresponds to the daily dose of 75 mg for humans [7] converted for rats after E. J. Freilich *et al.* [12].

Autoimmune encephalomyelitis was modeled as described previously [11]. Rat myelin oligodendrocyte

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glycoprotein 35-55 (MOG_{35-55}) emulsified in 100 μl Freund's complete adjuvant (Difco) containing 4 mg/ml killed *Mycobacterium tuberculosis* was injected (100 μg) to 5-6-week-old C57Bl/6 females in the tail base on days 1 and 8 of the experiment. In addition, each animal was intraperitoneally injected with 200 μl saline with 300 ng Pertussis toxin (Sigma) on day 1 and 48 h later. Compound GB-115 in a dose of 1 mg/kg was injected intraperitoneally on days 9-15 of the experiment. Neurological disorders were evaluated on days 11-16 by a common 5-point neurological deficiency score [11]: 0: no signs; 1: limp tail; 2: partial paralysis of one or two hind limbs; 3: complete paralysis of hind limbs; 4: hind limb paralysis and fore limb paraparesis; and 5: death. Spontaneous motor activity was evaluated on day 16 over 5 min using Ugo Basile actometer and the thymus and spleen weights were measured [8].

Fragments of the brain for histological study were fixed in 96% ethanol. After standard processing and embedding in paraffin, sections (3-5 μ) were sliced and stained by gallocyanin chromium alum and post-stained with eosin. The sections were embedded in balm and examined in transmitting light under a Leitz microscope.

The effects of GB-115 on ROS production by neutrophils were studied in the chemiluminescent test after 3 intraperitoneal injections of 1 mg/kg of the compound (the last injection was made 20 min before blood collection). The cells were isolated from heparin-treated blood of F_1(CBA×C57Bl/6) mice by the method developed by Sigma. Luminol-dependent opsonized zymosan-stimulated chemiluminescence (Sigma; 100 μg/ml concentration in the sample) was recorded on a Bioorbit 1251 chemiluminometer. Chemiluminescent evaluation procedure was described previously [3].

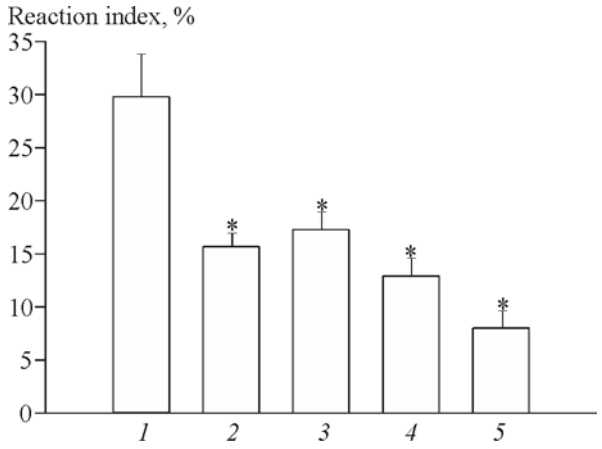

Fig. 1. Anti-inflammatory effect of GB-115 in acute exudative inflammation in response to ConA in mice. *1*) control; *2*) GB-115, 0.1 mg/kg; *3*) GB-115, 1 mg/kg; *4*) GB-115, 10 mg/kg; *5*) claritin, 1.3 mg/kg. *$p<0.01$ compared to the control.

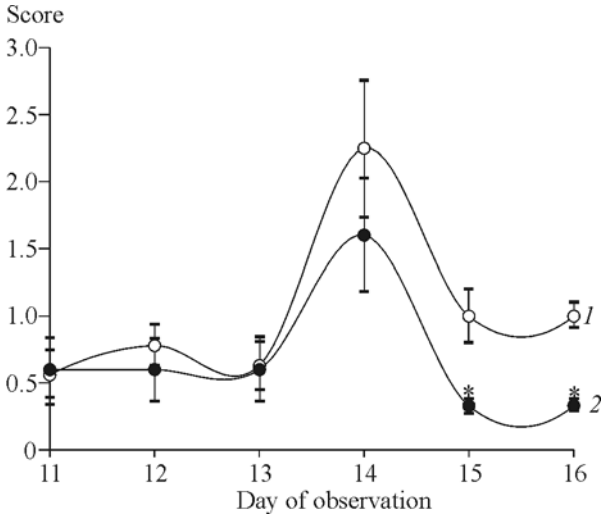

Fig. 2. Effect of GB-115 on severity of neurological disorders in EAE in C57Bl/6 mice. *1*) EAE; *2*) EAE+GB-115, 1 mg/kg. *$p<0.05$ compared to EAE.

RESULTS

Compound GB-115 in doses of 0.1, 1, and 10 mg/kg inhibited the inflammatory reaction by 47.3, 41.9, and 56.7%, respectively. The reference drug (claritin) 3.7 times reduced the inflammatory reaction (Fig. 1).

Carrageenan-induced paw edema test revealed a statistically significant 18% decrease in exudative edema 4 h after carrageenan injection in animals injected with GB-115 in a dose of 0.1 mg/kg (Table 1). Dipeptide in doses of 1 and 10 mg/kg suppressed exudative edema starting from the second hour of the experiment; the maximum difference in comparison with the control was recorded during the third (peak of reaction) and fourth hours of the experiments. GB-115 in a dose of 1 mg/kg significantly reduced edema by 24.8, 28.8, and 25.1% 2, 3, and 4 h after carrageenan injection, respectively ($p<0.05$). Diclofenac significantly reduced edema by 33.2, 45.2, and 42.7% during the same periods in comparison with the control. After 1 h, anti-inflammatory activity of GB-115 in a dose of 10 mg/kg was higher than that of diclofenac, after 2-4 h it was comparable to that of the reference drug.

Evaluation of the time course of neurological disorders in EAE showed that chronic GB-115 treatment on days 9-15 reduced the severity of pathological symptoms in comparison with the control group of mice with EAE ($p<0.01$; Fig. 2). On day 16 of the disease, spontaneous motor activity in animals with EAE decreased by 2.3 times (104.0±26.3 arb. units *vs.* 239.7±6.4 arb. units in intact mice). The dipeptide significantly increased motor activity (167.2±13.0 arb. units) in comparison with untreated mice with EAE. Measurements of the immune organ weights in con-

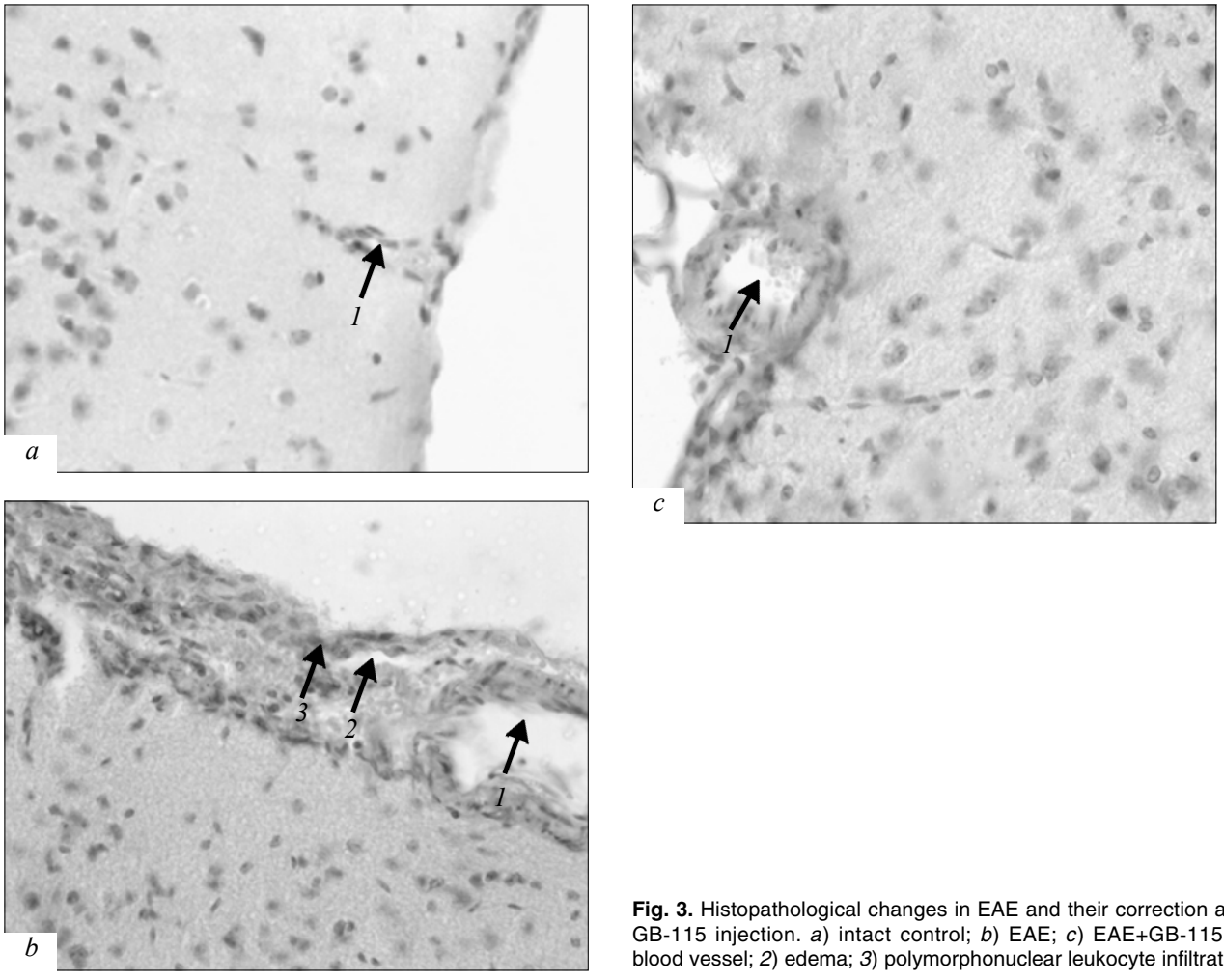

Fig. 3. Histopathological changes in EAE and their correction after GB-115 injection. *a*) intact control; *b*) EAE; *c*) EAE+GB-115. *1*) blood vessel; *2*) edema; *3*) polymorphonuclear leukocyte infiltration.

trol animals with EAE showed that the spleen weight significantly increased by 1.8 times and thymus weight decreased 3-fold in comparison with the corresponding parameters in intact animals (Table 2). Treatment with GB-115 restored thymus weight and caused virtually no changes in the spleen weight. Histological studies showed that EAE was associated with edema and infiltration of the perivascular space in the brain tissue by polymorphonuclear leukocytes; these phenomena were arrested by GB-115 injection (Fig. 3).

Studies of GB-115 effects on ROS generation by neutrophilic granulocytes in the chemiluminescence

TABLE 1. Anti-Inflammatory Effect of GB-115 on Carrageenan-Induced Paw Edema in Rats

Time, h	Control	GB-115			Diclofenac, 6.75 mg/kg
		0.1 mg/kg	1 mg/kg	10 mg/kg	
0	29.7±0.7	32.3±1.2	32.7±1.7	28.3±0.4	27.8±0.8
1	38.1±1.6	35.7±1.2	36.7±1.2	30.6±0.5^{**++}	40.1±1.7
2	44.3±2.3	38.1±1.8	37.4±0.8*	33.3±1.2**	36.8±1.8*
3	45.1±2.3	38.9±1.9^{++}	35.9±1.8^{**++}	32.1±1.4**	29.4±0.6**
4	41.0±2.9	33.6±1.6^{*+}	33.6±1.6^{*+}	30.7±0.8**	28.8±0.5**

Note. *p<0.05, **p<0.01 compared to the control; ^{+}p<0.05, ^{++}p<0.01 compared to diclofenac.

TABLE 2. Effects of GB-115 on Thymus and Spleen Weights of Mice with EAE

Group	Thymus, mg/10 g body weight	Spleen, mg/10 g body weight
Intact control (*n*=12)	41.1±3.2	40.9±1.9
EAE control (*n*=6)	13.5±1.4*	72.3±4.1*
EAE+GB-115, 1 mg/kg (*n*=8)	36.1±3.2^{+}	67.2±14.3

Note. *n*: number of animals in the group. *p<0.01 compared to intact control, ^{+}p<0.05 compared to EAE group.

test showed that the dipeptide inhibited significantly (70.0±6.4 mV) the chemiluminescent response of mouse neutrophils to zymosan in comparison with the control level of stimulated chemiluminescence (123.3±44.7 mV).

The results are in line with published data on the anti-inflammatory and antiradical effects of glycine-containing peptides [1,3]. Presumably, one of the mechanisms of anti-inflammatory effect of the dipeptide is suppression of ROS generation by neutrophils.

It was hypothesized that oligodendroglia and lymphocytes have a common antigen [13], which leads to thymus atrophy in EAE. It was assumed that induction of lymphocyte apoptosis in EAE modulated the Th_1/Th_2 balance and stimulated the production of antibodies to MOG, leading to more intense demyelination and subsequent progress of the disease. It is therefore of principal importance that GB-115 induces recovery of atrophic thymus in mice with EAE.

Hence, the detected anti-inflammatory effects of GB-115 and its capacity to inhibit the production of ROS provide new data on its mechanisms of action and suggest its further development as a drug for the treatment of neuroimmune disorders associated with inflammatory processes.

REFERENCES

1. I. P. Ashmarin, *Neirokhimiya*, **24**, No. 1, 3-7 (2007).
2. T. A. Gudasheva, E. P. Kir'yanova, L. G. Kolik, *et al.*, *Bioorg. Khim.*, **33**, No. 4, 413-420 (2007).
3. L. P. Kovalenko, M. G. Miramedova, S. V. Alekseeva, *et al.*, *Eksp. Klin. Farmakol.*, No. 2, 53-55 (2002).
4. L. P. Kovalenko, E. V. Shipaeva, S. V. Alekseeva, *et al.*, *Byull. Eksp. Biol. Med.*, **144**, No. 7, 54-57 (2007).
5. L. G. Kolik, T. A. Gudasheva, and S. B. Seredenin, *Ibid.*, **135**, No. 5, 519-523 (2003).
6. L. G. Kolik, V. N. Zhukov, and S. B. Seredenin**,** *Eksp. Klin. Farmakol.*, **70**, No. 2, 8-10 (2007).
7. *Drug Register of Russia*, Ed. G. L. Vyshkovskii [in Russian], Issue No. 14, Moscow (2006), P. 264.
8. *Manual of Experimental (Preclinical) Studies of New Pharmacological Substances*, Ed. R. U. Khabriev [in Russian], Moscow (2005).
9. S. B. Seredenin, T. A. Gudasheva, N. I. Zaitseva, *et al.*, *Byull. Izobreten.*, No. 11, Patent of the Russian Federation No. 2227144 (2005).
10. E. V. Shipaeva, L. P. Kovalenko, S. V. Khaidukov, *et al.*, *Byull. Eksp. Biol. Med.*, **145**, No. 5, 548-551 (2008).
11. V. Brundula, N. B. Rewcastle, L. M. Metz, *et al.*, *Brain*, **125**, Pt. 6, 1297-1308 (2002).
12. E. J. Freireich, E. A. Gehan, D. P. Rall, *et al.*, *Cancer Chemother. Res.*, **50**, No. 4, 219-244 (1966).
13. I. Tsunoda, J. E. Libbey, L. Q. Kuang, *et al.*, *Am. J. Pathol.*, **167**, No. 61, 1631-1646 (2005).