IMMUNOLOGY AND MICROBIOLOGY

Tripeptide Gly-His-Lys is a Hepatotropic Immunosuppressor

M. Yu. Smakhtin, L. A. Sever'yanova, A. I. Konoplya, and I. A. Shveinov

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Ten intraperitoneal injections of tripeptide Gly-His-Lys in doses of 1.5, 5, 50, 150, and 450 mg/kg stimulated mitotic activity of hepatocytes and dose-dependently suppressed immune reactivity (number of antibody-producing cells and delayed-type hypersensitivity reaction).

Key Words: *tripeptide Gly-His-Lys; hepatocyte mitotic index; immune reactivity*

Some regulatory peptides affect cell growth and differentiation, modulate functional activity of the central nervous system, and regulate immune reactions [1,4]. One of these peptides is growth factor tripeptide (NH_2) Gly-L-His-L-Lys(COOH). This peptide stimulates hair growth, collagen synthesis by fibroblasts, and accumulation of the connective tissue matrix, which contributes to rapid healing of wounds and ulcers. It was hypothesized that this peptide acts as a physiological stimulator of wound healing [7,8,10]. Gly-His-Lys binds Cu^{2+}, thus playing a role in activation of copper-containing enzymes and maintenance of antioxidant processes, cell bioenergetics, and synthesis of elastin, collagen, and catecholamines [6], which improve regeneration and immune functions. Here we studied hepatotropic and immunomodulatory activities of this regulatory peptide.

MATERIALS AND METHODS

Experiments were performed on CBA mice and Wistar rats weighing 20-25 and 180-220 g, respectively. The preparation was synthesized at the St. Petersburg State University. Gly-His-Lys was dissolved in sterile isotonic NaCl and administered intraperitoneally (10 times) in doses of 0.5, 1.5, 5, 50, 150, and 450 mg/kg to mice (0.1 ml) and rats (0.2 ml) at 24-h intervals.

The animals were killed by exsanguination 1 day after the last injection. The liver was isolated, fixed with 10% neutral formalin in 0.1 M phosphate buffer (pH 7.2), and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin and examined morphologically. The mitotic index of hepatocytes was calculated. Sheep erythrocytes served as the antigen.

The peptide was administered for 10 days before, during, and after immunization. The intensity of the humoral immune response was estimated by the number of antibody-producing cells in the spleen 5 days after immunization [3]. The delayed-type hypersensitivity (DTH) reaction in rats was assayed by the difference between the weights of regional (site of antigen administration) and contralateral (popliteal) lymph nodes and counts of nucleated cells in these lymph nodes [5].

The arithmetic means and standard errors were calculated. The results were analyzed by Student's *t* test and Mann—Whitney test.

RESULTS

The peptide in a dose of 0.5 mg/kg had no effect on the mitotic index of hepatocytes. However, Gly-His-Lys in a dose of 1.5 mg/kg or higher markedly increased this parameter (Table 1). The peptide in doses of 1.5 and 150 mg/kg produced similar effect on mitotic activity of hepatocytes. After administration of the peptide in higher doses (150 and 450 mg/kg) signs of liver

Department of Pathophysiology, Kursk State Medical University. ***Address for correspondence:*** big@ksmu.kursknet.ru. Smakhtin M. Yu.

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TABLE 1. Effects of Peptide Gly-His-Lys on Immunomodulatory Activity and Mitotic Index of Hepatocytes ($M \pm m$, n=8-9)

Peptide dose, mg/kg	Antibody-producing cells	Difference in the weight, mg	Difference in the count of nucleated cells	Mitotic index, $^{o}/_{oo}$
Control (NaCl)	36.1±6.4	2.4±0.3	0.57±0.08	3.8±0.8
0.5	34.4±5.1	2.3±0.3	0.55±0.07	5.8±1.2^{+*}
1.5	22.3±3.8^{+*}	1.5±0.2^{+*}	0.33±0.05^{+*}	12.7±2.5^{+*}
5	21.1±3.2^{+*}	1.4±0.2^{+*}	0.34±0.05^{+*}	12.6±2.2^{+*}
50	11.7±2.0^{+*}	1.2±0.1^{+*}	0.24±0.04^{+*}	13.7±2.8^{+*}
150	10.2±1.7^{+***}	0.9±0.1^{+**}	0.20±0.03^{+***}	12.6±2.5^{+*}
450	9.3±1.5^{+***}	0.7±0.1^{+***}	0.17±0.02^{+***}	12.5±2.4^{+*}

Note. p<0.05: $^{+}$compared to the control; *compared to 0.5 mg/kg; **compared to 0.5 and 1.5 mg/kg; ***compared to 0.5, 1.5, and 5 mg/kg.

degeneration appeared. These changes were more pronounced in animals receiving 450 mg/kg Gly-His-Lys.

Gly-His-Lys in doses of 1.5, 5, 50, 150, and 450 mg/kg suppressed the humoral immune response. This effect was not observed after administration of 0.5 mg/kg Gly-His-Lys (Table 1). In contrast to hepatotropic regenerative activity, the immunosuppressive effect of Gly-His-Lys depended on its dose and was maximum after administration of peptide in a dose of 450 mg/kg. Hence, Gly-His-Lys produces a dose-dependent immunosuppressive effect and a dose-independent stimulatory effect on physiological regeneration in hepatocytes.

The effects of Gly-His-Lys on DTH and humoral immune response were similar. In *in vitro* cultures biological activity of Gly-His-Lys is manifested not only in the stimulation of cell growth and differentiation, but also in pronounced toxic influence [9]. These data are consistent with our *in vivo* experiments, when the peptide in high doses caused degenerative changes in the liver. Therefore, *in vivo* Gly-His-Lys should be used in low physiological doses producing hepatotropic and moderate immunosuppressive effects (1.5 and 5 mg/kg).

It should be emphasized that the immunosuppressive effect was observed after administration of the peptide in a dose, which enhanced mitotic activity of hepatocytes (1.5 μg/kg). These data suggests that the liver is involved in the immunosuppressive effects of Gly-His-Lys. Previous studies showed that regenerating liver cells stimulate proliferative activity of lymphocytes that play a role in the regulation of cell regeneration [2]. It can be hypothesized that proliferating (low differentiated) lymphocytes cannot adequately respond to foreign antigens. This explains the immunosuppressive effect of Gly-His-Lys. It cannot be excluded that Gly-His-Lys stimulates proliferation not only in hepatocytes, but also in splenocytes, thus reducing their ability to react to foreign antigens.

In our experiments tripeptide Gly-His-Lys stimulated mitotic activity of hepatocytes and dose-dependently suppressed the humoral and cellular immune responses. These results should be taken into account during therapy of liver diseases associated with high reactivity of the immune system (autoimmune reactions). Under these conditions peptide activators of cell immunity can aggravate symptoms of pathological processes [4].

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