Similarity of Immunomodulating, Phagocytosis-Modulating, and Antitoxic Properties of Dipeptides and Their Constituent Amino Acids

G. A. Belokrylov, O. Ya. Popova, and E. I. Sorochinskaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 6, pp. 674-676, June, 1999
Original article submitted June 6, 1999

Immunomodulating, phagocytosis-modulating, and antitoxic properties of dipeptides depend on activities of their constituent amino acids. Some amino acids have considerable promise for efficient prevention of occupational diseases caused by chronic benzene intoxication.

Key Words: *amino acids; peptides; immune response; phagocytosis; detoxication*

Our previous experiments showed that immunomodulating activities of low doses of dipeptides Glu-Trp (thymogen) and Lys-Asp are determined by immune activities of their constituent amino acids [1,3]. The use of immunomodulators affecting specific and nonspecific resistances is of particular importance under conditions of ecological instability often accompanied by immunodeficiency in humans. Therefore, we studied immunomodulating, phagocytosis-modulating, and antitoxic properties of low doses of various dipeptides and their amino acids.

MATERIALS AND METHODS

In vivo experiments were performed on 5-week-old male CBA mice weighing 14-16 g (n=293). In *in vitro* experiments, mouse peritoneal granulocytes and splenocytes of mice were used. All experiments were conducted in two replications. Synthetic dipeptides Glu-Trp, Lys-Asp, Lys-Val, Asp-Glu, Glu-Asp, and Leu-Gly, individual amino acids (Sigma), and their combinations were studied. Benzene (Reanal) was used as a toxicant. Dry dipeptides and amino acids were weighted and the doses were calculated per 1 kg body weight.

All preparations were dissolved in apyrogenic physiological saline (Polfa) immediately before use and administered subcutaneously or *per os* (through a tube) for 10 days (6.5×10^{-2} mg/kg). Control animals received apyrogenic physiological saline. The mice were then intravenously (through the caudal vein) immunized with sheep erythrocytes (2×10^6). On the 4th day after immunization, experimental animals were decapitated and IgM antibody-forming cells (AFC) were counted in the spleen by a modified local hemolysis method [5] in 0.7% agarose (Sigma) [2]. The number of AFC was calculated per 10^6 nuclear cells.

In nonimmunized mice receiving test preparations, phagocytosis-modulating activity of peritoneal granulocytes was determined *in vitro* [1,3,4]. Granulocytes were obtained by washing the abdominal cavity with sterile Hanks' solution 2.5 h after the intraperitoneal injection of 10% sterile peptone (Nutritional Biochemical Corporation). The content of neutrophils in the abdominal cavity was not less than 97%. A one-day culture of *St. aureus* served as the test microbe. The phagocytic index (percent of phagocytizing neutrophils) and phagocytic number (the mean number of microbial cells in one phagocyte) were estimated [1,3].

In vitro, antitoxic activity of test preparations was studied on splenocytes after erythrocyte lysis with 0.65% ammonium chloride. Splenocytes (2.5×10^7 cells/ml) were mixed with equal volumes of test preparations (1.3×10^{-3} mg/ml), incubated at 37°C for 30 min, and washed 3 times with cold Hanks' solution. Benzene in a concentration of 8.8×10^{-2} mg/ml was added,

Institute of Experimental Medicine, Russian Academy of Medical Sciences, St. Petersburg

0007-4888/99/0006-0615$22.00

and the cells were incubated under similar conditions for 30 min and washed 5 times with Hanks' solution. Cell viability was determined using 0.2% Trypan blue (Sigma). Splenocytes in Hanks' solution and benzene-treated splenocytes served as the controls. The results were expressed as the index of cytotoxicity (in %) [1,3].

RESULTS

Dipeptides Glu-Trp and Lys-Asp, their constituent amino acids, and their mixtures displayed considerable immunomodulating, phagocytosis-modulating, and antitoxic properties (Table 1). Glu-Trp, Glu and Trp alone and in combination possess similar immunostimulating activity. Immunostimulating activity of dipeptides Glu-Asp and Asp-Glu, amino acids Glu and Asp, and their mixtures was also similar: the numbers of IgM AFC were 17.1±2.3, 19.4±3.5, 17.5±2.6, 18.8±1.9, and 19.4±3.5, respectively (8.6±0.8, 8.4±0.9, 7.9±0.6, 7.9±0.6, and 9.0±1.0, respectively, in the control; $p<0.01$). Each group comprised 10-20 mice (data not shown).

Dipeptide Leu-Gly, individual amino acids, and their mixture were inactive. The number of IgM AFC did not change after application of dipeptides and the mixture of amino acids (8.5±0.7÷9.6±1.5 *vs.* 8.6±0.8÷9.0±1.0 in the control) and after application of Leu and Gly solely (9.5±1.4 and 9.0±1.5, respectively, *vs.* 9.0±1.0 in the control). Each group consisted of 10-20 mice (data not shown).

The ability of Glu-Trp and Glu+Trp mixture to protect splenocytes from *in vitro* toxic effects of benzene and their immunostimulating properties were similar. Antitoxic activities of Glu and Trp alone were lower than those of their mixture and Glu-Trp dipeptide. However, phagocytosis-stimulating activity of Glu, Trp, and Glu+Trp was higher ($p<0.01$) than activity of Glu-Trp dipeptide. Phagocytosis-stimulating and antitoxic activities of dipeptide Lys-Asp and amino acid mixture were similar, but the effects of individual amino acids on phagocytosis and detoxification were different. Lys had no effect on immune response, possessed no antitoxic activity, but stimulated phagocytosis. Asp increased the indexes of specific (immune response) and nonspecific (phagocytosis and detoxication) resistances (Table 1).

Antitoxic properties of Asp were also manifested when benzene (8.8×10^{-2} mg/ml) was filtered through gauze impregnated with the amino acid (1.3×10^{-3} mg/ml) followed by incubation at 37°C for 30 min. Desiccation of this gauze did not decrease the antitoxic activity of Asp. In a concentration of 1.3×10^{-9} mg/ml, Asp lost its activity. Glu, methionine, and tryptophan in concentrations of 1.3×10^{-3} mg/ml also displayed high activities. Glu was also active in a concentration of 1.3×10^{-7} mg/ml (Table 2).

Immunomodulating activities of dipeptides, individual amino acids, and their mixtures administered subcutaneously or through a tube were similar.

Our findings show that immunomodulating, phagocytosis-modulating, and antitoxic properties of various dipeptides depend on activities of their constituent amino acids. Therefore individual amino acids (rather than dipeptides) are appropriate for the correction of impaired specific and nonspecific resistances under conditions of ecological instability. High antitoxic activities of amino acids absorbed on a gauze allow us to recommend them (in low concentrations) for pre-

TABLE 1. Immunomodulating, Phagocytosis-Modulating, and Antitoxic Properties of Dipeptides and Their Amino Acids (*M±m*)

Preparation	Number of IgM AFC per 10^6 spleen karyocytes	Phagocytic index, %		Cytotoxic index for benzene, %
	in vivo	*in vivo*	*in vitro*	*in vitro*
Control	8.6±0.8 (20)	18.6±2.3 (10)	17.3±0.4	21.1±1.4
Glu-Trp (thymogen)	18.8±2.4* (10)	31.5±0.4* (8)	35.2±0.6*	0*
Glu+Trp	23.5±1.9* (18)	46.0±2.6* (9)	37.4±1.6*	0*
Glu	19.3±2.8* (10)	48.3±3.6* (8)	43.6±3.4*	8.9±1.9*
Trp	18.5±1.4* (10)	44.0±1.9* (10)	40.3±1.2*	11.8±2.3*
LysAsp	18.5±2.5* (11)	31.2±0.8* (9)	30.7±3.2*	15.1±1.3*
Lys+Asp	20.0±1.8* (10)	28.1±1.6* (10)	25.1±1.8*	12.9±2.4*
Lys	10.0±1.8 (10)	35.1±1.9* (10)	40.6±2.6*	21.5±2.0
Asp	38.4±1.0* (20)	31.2±1.9* (10)	25.9±1.5*	14.1±1.7*

Note. The number of animals is shown in parentheses. For *in vitro* experiments, granulocytes and splenocytes from 2-3 mice were pooled. Each value results from measurements of 900-1000 cells. Granulocyte and splenocyte viability in Hanks' solution was not less than 90-94%. Here and in Table 2: *$p<0.01$ compared with the control.

TABLE 2. Antitoxic Properties of Amino Acids Absorbed on Gauze (*M*±*m*)

Preparation	Concentration, mg/ml	Cytotoxic index for benzene (%) with amino acids absorbed on gauze	
		wet	dry
Control (cells in Hank's solution)	0	25.2±3.1	22.0±2.9
Asp	1.3×10^{-3}	12.6±2.3*	12.0±2.3*
	1.3×10^{-9}	26.0±3.1	25.8±2.8
Glu	1.3×10^{-3}	4.5±1.4*	8.5±1.7*
	1.3×10^{-7}	5.5±1.6*	8.9±2.0*
Trp	1.3×10^{-3}	6.3±1.7*	6.0±1.6*
Met	1.3×10^{-3}	11.2±2.2*	19.2±2.8

vention of occupational diseases caused by chronic benzene intoxication.

REFERENCES

1. G. A. Belokrylov, O. N. Derevnina, O. Ya. Popova, *et al.*, *Byull. Eksp. Biol. Med.*, **121**, No. 5, 509-512 (1996)
2. G. A. Belokrylov, I. V. Molchanova, and E. I. Sorochinskaya, *Int. J. Immunopharmacol.*, **12**, No. 8, 841-845 (1990).
3. G. A. Belokrylov, O. Ya. Popova, O. N. Derevnina, *et al.*, *Drug Dev.*, **24**, No. 2, 115-127 (1998).
4. G. A. Belokrylov, O. Ya. Popova, I. V. Molchanova, *et al.*, *Int. J. Immunopharmacol.*, **14**, No. 7, 1285-1292 (1992).
5. N. K. Jerne and A. A. Nordin, *Science*, **140**, No. 3565, 405 (1963).