BIOPHYSICS AND BIOCHEMISTRY

Immune Response, Phagocytosis, and Detoxication Ability under the Unfluence of Peptide and Amino Acid Preparations

G. A. Belokrylov, O. N. Derevnina, O. Ya. Popova, I. V. Molchanova, and E. I. Sorochinskaya

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 121, No. 5, pp. 509-512, May, 1996
Original article submitted March 21, 1995

Dipeptides Glu-Trp and Lys-Asp and peptide mixtures of the 5th thymosine fraction, as well as thymaline, stimulate the immune response when applied subcutaneously during 5 days and intensify the activity of neutrophils and protect splenocytes from the toxic effect of benzene and aflatoxin B_1 *in vitro*. Amino acids (Glu, Trp, Lys, Asp, and Arg) and amino acid mixtures (levamine-70, cerebrolysine, and aviamine) differently affect the indexes of specific and nonspecific resistance under the same conditions and at the same dose.

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

Chronic microbic and nonmicrobic intoxications are known to inhibit the factors of specific and nonspecific resistance, which promotes intercurrent infections [2,7]. For this reason the immunocorrection of such pathological states should include drugs with combined action of specific and nonspecific parameters of immune defense. Some peptides [3,9], amino acids, and their mixtures [1,4,8,9] are able to act upon certain chains of specific and nonspecific resistance, but the combination of these effects has not been studied.

The aim of the present investigation was to examine the combined effects of some peptide and amino acid preparations on such indexes of specific and nonspecific resistance as the immune response, phagocytosis, and detoxication *in vitro* and *in vivo*.

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

MATERIALS AND METHODS

Experiments *in vivo* were carried out on 325 male mice weighing 14-16 g and on 25 chicks weighing 120-125 g. Mouse and chick splenocytes were used in the experiments *in vitro*. The following substances were studied: levamine-70 (Leiras, an artificial mixture of 13 amino acids), cerebrolysine (Ebave, hydrolysate of brain tissue containing 18 amino acids), aviamine, (Drug Manufacturing Plant, St. Petersburg), hen blood hydrolysate consisting of 18 amino acids, thymaline (Drug Manufacturing Plant, St. Petersburg), a polypeptide mixture of fresh calf thymus glands with a molecular weight of 1000-10,000 D, prepared by acetic acid extraction [6], the 5th fraction of thymosine (a polypeptide mixture of calf thymus glands with a molecular weight of 1000-12,000 D, prepared by a described method [10]), dipeptides Glu-Trp (thymogene) and Lys-Asp, synthesized by the method of classic synthesis in solu-

0007-4888/96/0005-0462$15.00

tion, individual amino acids Lys, Asp, Glu, Trp, and Arg (Sigma), and their mixtures. Benzene (Reanal) and aflatoxin B_1 in benzene (Nutrition Institute, Russian Academy of Medical Sciences) with a toxin content of 10.5 μg/kg were used to exert a toxic effect on cells.

Cerebrolysine, aviamine, thymaline, and the 5th fraction of thymosine were dosed according to protein, the content of which was 1.0, 0.032, 0.64, and 0.91 g/ml, respectively, and levamine was dosed by the total content of amino acids, 0.53 g/ml, and converted to 1 kg of weight. The preparations were administered subcutaneously for 5 days on apyrogenic physiological saline (Polfa) at 6.5×10^{-2} mg/kg. The mice were then immunized i.v. once with sheep erythrocytes (2×10^6), and 4 days later the number of antibody-producing cells (APC) was determined in each spleen using a method described elsewhere [11]. The number of APC was converted to 10^6 karyocytes. The phagocytosis-stimulating activity of the preparations was assessed *in vitro* using our own method [9]. *St. aureus* was used as the test microbe. The phagocytosis index was determined as the percent of neutrophils participating in phagocytosis and the phagocytosis number as the mean number of microbial cells in one leukocyte [9]. The detoxicating ability of the preparations was assessed *in vitro* using mouse and chick splenocytes liberated from erythrocytes by treatment with 0.65 and 0.7% solutions of ammonium chloride, respectively. Splenocytes (2.5×10^7 cells/ml) were mixed with the preparations in equal volumes (1.3×10^{-3} mg/ml), incubated at 37°C for 30 min, washed three times in cold Hanks solution, added to a 10^{-4} dilution of benzene or aflatoxin B_1 in benzene, incubated for 30 min under the same conditions, and washed five times with Hanks solution, after which cell viability was determined using a 0.2% aqueous solution of trypan blue (Sigma). Splenocytes in Hanks solution and splenocytes treated with benzene or aflatoxin B_1 in benzene at a 10^{-4} dilution served as the control. The results were expressed in cytotoxicity indexes in percent [5].

RESULTS

Individual peptides (Lys-Asp, Glu-Trp) and peptide mixtures (the 5th fraction of thymosine and thymaline) intensify the immune response, phagocytosis, and detoxication (Table 1). Individual amino acids (Glu, Asp, Trp, Lys, and Arg) affect the indexes of specific and nonspecific resistance differently. Glu-

TABLE 1. Immune- and Phagocytosis-Modulating and Detoxicating Properties of Amino acid and Peptide Preparations (*M±m*)

Preparation	Number of IgM-APC per 10^6		Phagocytosis index		Index of cytotoxicity after treatment of splenocytes with preparations *in vitro*	
	spleen karyocytes	spleen karyocytes (control)	with preparations *in vitro*	with Hanks solution (control) *in vitro*	with benzene	with aflatoxin
Aviamine	19.0±2.3*	11.0±1.6	30.3±0.7*	17.3±0.4	12.9±2.4**	21.2±2.9**
Cerebrolysine	34.0±3.8*	11.0±1.6	18.2±2.4	20.6±0.6	4.3±1.4*	23.5±2.1**
Levamine	24.5±3.5*	11.0±1.6	17.4±2.2	20.6±0.6	0*	20.0±2.8**
5th fraction of thymosine	22.9±1.6*	11.0±1.6	22.4±1.7**	7.3±0.4	0*	10.8±1.6*
Thymaline	15.4±2.5*	7.9±0.6	34.0±3.9**	17.3±0.4	0*	6.4±1.2*
Glu-Trp	18.8±2.4*	8.6±0.8	35.2±0.6*	17.3±0.4	0*	15.3±2.5**
Glu+Trp	23.5±1.9*	8.4±0.6	37.4±2.6*	17.3±0.4	0*	15.3±2.5**
Lys-Asp	18.5±2.5*	8.6±0.8	30.7±3.2*	17.3±0.4	15.1±1.3**	12.9±2.4**
Lys+Asp	20.0±1.8*	8.4±0.6	25.1±1.8**	17.3±0.4	12.9±2.4**	15.1±2.5**
Lys	10.0±1.8	11.1±1.0	44.7±2.9*	19.1±0.8	21.5±2.0	32.9±3.1
Arg	6.0±0.8*	10.7±0.7	30.5±2.2*	18.2±1.2	25.3±3.0	27.0±1.2
Asp	38.4±1.0*	12.0±1.0	27.2±1.6**	18.2±1.2	14.1±1.7**	9.1±1.4*
Glu	18.5±1.4*	12.0±1.0	45.8±3.6*	18.2±1.2	8.0±1.9*	12.0±2.3*
Trp	27.8±3.4**	11.8±1.0	48.3±1.4*	20.7±4.8	11.8±2.3**	18.8±2.8**

Note. Cells of peritoneal exudate (phagocytosis) with a 97-98% neutrophil content were obtained from 6-8 mice 2.5 h after intraperitoneal injection of sterile 10% peptone solution. Each number is a result of calculation of no less than 800-1000 neutrophils. The cytotoxicity index of benzene and aflatoxin B_1 in benzene for cells not treated with a preparation (control) was 21.1±1.4 and 31.0±2.3%, respectively. Cell viability in Hanks solution was 85-90%. Here and in Table 2: *$p<0.01$, **$p<0.05$ as compared to the control.

tamine and aspartic acids and tryptophan possess marked immune- and phagocytosis-stimulating and detoxicating properties. Lysine and arginine stimulate phagocytosis, but do not protect splenocytes from the toxic action of benzene or aflatoxin. These amino acids differ in their effect on the immune response: lysine does not alter it, while arginine depresses it. The amino acid mixtures aviamine, cerebrolysine, and levamine possess both immunostimulating and detoxicating properties, but differ in their effect on phagocytosis. Cerebrolysine and levamine do not affect the phagocytic activity of neutrophils, whereas aviamine stimulates it. Amino acid and peptide preparations protect not only mouse splenocytes (Table 1), but also chick splenocytes (Table 2) from the toxic action of benzene and aflatoxin, the protective properties of cerebrolysine, levamine, and aviamine differing from each other. While cerebrolysine and levamine protect chick cells from both benzene and aflatoxin B_1, aviamine protects them only from aflatoxin (Table 2). The detoxicating properties of aviamine are preserved *in vitro* when it is added directly to mouse and chick cells mixed with aflatoxin; the cytotoxicity indexes are 15.6±2.5 and 19.7±2.8% versus 33.5±3.5 and 31.5±2.3% in the control ($p<0.01$, data not given in the tables). The addition of aviamine to chick feed (6.5×10^{-2} mg/kg body weight) during 10 days raises the resistance of splenocytes *in vitro* to benzene and to aflatoxin B_1 in benzene, and the cytotoxicity indexes are lowered, respectively, from 26.6±3.1 and 47.7±3.5% in the control (splenocytes from intact chicks treated with benzene or aflatoxin B_1) to 11.3±2.3 and 25.0±3.0% in the experiment ($p<0.01$).

The findings attest to the different effects of the peptide mixtures (5th fraction of thymosine and thymaline) and of the amino acids (levamine and cerebrolysine) on the specific and nonspecific indexes of resistance. The peptide mixtures possess a combined effect on the indexes of specific and nonspecific defense, intensifying antibody production, phagocytosis, and detoxication. Both the amino acid mixtures and the peptide mixtures stimulate the immune response and have detoxicating properties, but they affect phagocytosis nonuniformly. Levamine and cerebrolysine do not affect the phagocytosis process, while aviamine enhances it. Individual peptides, Glu-Trp and Lys-Asp, like the peptide mixtures, exert a combined effect on the specific and nonspecific indexes. In this case the combination of their effect is determined by the joint action of the amino acids, namely, of one amino acid (Asp) in Lys-Asp peptide and of both amino acids (Glu and Trp) in the Glu-Trp peptide. The combined effect on the specific and nonspecific indexes

TABLE 2. Detoxicating Activity of Amino acid and Peptide Preparations *in vitro* for Chick Splenocytes (*M±m*)

Preparation	Cytotoxicity index of benzene and aflatoxin after treatment of splenocytes with preparations, %	
	benzene	aflatoxin
Aviamine	22.8±2.9	17.5±2.7*
Cerebrolysine	3.5±1.3*	16.5±2.6*
Levamine	10.3±2.2**	23.6±2.8**
Glu-Trp	3.5±1.3*	23.0±3.0*
Glu+Trp	8.7±2.0*	15.9±2.6*
Glu	1.6±0.9*	7.4±1.8*
Trp	3.5±1.3*	23.0±3.0*
Control (untreated splenocytes)	18.3±2.7	34.8±3.4

of peptides with a large number of amino acids probably depends not only on the presence of an amino acid with combined action but also on the spatial structure of the peptides. Thus, tuftsin intensifies both the immune response and the phagocytic activity of neutrophils [5]. Pentagastrin and cholecystokinin stimulate the immune response, but do not affect phagocytosis, whereas neurotensin, conversely, intensifies phagocytosis but does not alter the immune response [5]. The difference in the effect on specific and nonspecific indexes of resistance produced by peptide and amino acid preparations confirms our previous assumption [8] that two independent peptide and amino acid systems of immune regulation exist.

REFERENCES

1. G. A. Belokrylov, in: *Immunophysiology*, E. A. Korneva (Ed.) [in Russian], St. Petersburg (1993), pp. 320-367.
2. S. M. Gusman, in: *Chronic Occupational Benzene Poisoning* [in Russian], Baku (1970), pp. 120-121.
3. V. N. Zakharov, A. V. Karaulov, V. V. Sokolov, and V. N. Frash, in: *Changes in the Blood System Exposed to Radiation and Benzene* [in Russian], Novosibirsk (1990), pp. 199-205.
4. P. N. Kiselev, in: *Toxicology of Infectious Processes* [in Russian], Leningrad (1971), pp. 284-289.
5. I. V. Molchanova, "*Immunomodulatory properties of gastrointestinal peptides*," Abstract of Dissertation [in Russian], Leningrad (1990).
6. V. G. Morozov, V. Kh. Khavinson, and O. A. Pisarev, *Dokl. Akad. Nauk SSSR*, **233**, No. 3, 491-494 (1977).
7. V. A. Tutel'yan and L. V. Kravchenko, *Mycotoxins (Medical and Biological Aspects)* [in Russian], Moscow (1985).
8. G. A. Belokrylov, I. V. Molchanova, and E. I. Sorochinskaya, *Int. J. Immunopharmacol.*, **12**, No. 8, 841-845 (1990).
9. G. A. Belokrylov, O. Ya. Popova, I. V. Molchanova, *et al.*, *Ibid.*, **14**, No. 7, 1285-1292 (1992).
10. A. L. Goldstein, A. Guha, M. M. Zatz, *et al.*, *Proc. Natl. Acad. Sci. USA.*, **69**, No. 7, 1800-1803 (1972).
11. N. K. Jerne and A. A. Nordin, *Science*, **140**, 405-406 (1963).