Detoxication of Benzene and Aflatoxin B_1 with Amino Acid and Peptide Preparations in Mice and Chicken

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Incubation of mouse and chicken splenocytes with amino acid or peptide preparations *in vitro* increases cell resistance to benzene and aflatoxin B_1. Short-term (15 days) treatment of chicken with an amino acid mixture (aviamine) in combination with benzene also increased splenocyte resistance to toxin *in vitro*. By contrast, aviamine in combination with aflatoxin B_1 sharply decreased cell resistance to toxin. Glutamic acid possessed no such properties.

Key Words: *benzene; aflatoxin B_1; detoxication*

Detoxication after exposure of mammals and birds to benzene and aflatoxin B_1 (AFB_1) is a pressing problem. The data on antitoxic activity of natural immunomodulators, in particular amino acids and peptides, against benzene or AFB_1 are scanty and obtained primarily in *in vitro* experiment [1,2,5].

We compared antibenzene and antiaflatoxin activities of some amino acid and peptide preparations *in vitro* and the antitoxic effects of amino acid preparations *in vitro* and *in vivo*.

MATERIALS AND METHODS

In vivo experiments were carried out on 5-week-old male CBA mice weighing 14-16 g and 10-day-old chicken weighing 50-60 g. *In vitro* experiments were carried out on splenocytes of these animals. The following amino acids (Sigma) were used: asparaginic and glutamic acids, tryptophan, methionine, glycine, isoleucine, arginine, lysine; levamine-70, a mixture of 13 amino acids (Leiras); cerebrolysin and cerebral tissue hydrolysate (Ebeve), consisting of 18 amino acids; aviamine, chicken blood protein hydrolysate (St. Petersburg Drug Plant); dipeptides GluTrp (thymogen), LysAsp and their amino acid mixtures; polypeptides: thymosin fraction 5 and thymalin, extract from fresh calf thymuses containing at least 50 peptides with molecular weights of 1-12 kD. Dipeptides were prepared by classical synthesis in solution.

Antitoxic activity of glutamic acid and aviamine, an amino acid mixture used in poultry factories, was studied *in vivo*.

In *in vitro* experiments chicken or mouse splenocytes (2.5×10^7 cells/ml) after erythrocytes elimination with 0.7 and 0.65% ammonium chloride, respectively, were incubated with equal volumes of test compounds (1.3×10^{-3} mg/ml) at 37°C for 30 min, washed 3 times with cold Hanks' solution, and then incubated with benzene (Reanal) diluted to 10^{-4} or AFB_1 (10^{-2} mg/ml, Institute of Nutrition, Russian Academy of Medical Sciences) in benzene under the same conditions. After incubation (30 min) the mixture was washed 5 times in Hanks' solution, and cell viability was evaluated by trypan blue exclusion (0.2%, Sigma). Viability of splenocytes treated with toxins was taken as the control. At least 200 nuclear cells were counted in each experiment. Results were expressed in cytotoxicity indexes (in %) calculated by the formula: [(percent of dead cells in experiment minus percent of dead cells in control)/(100 minus percent of dead cells in control)]×100 [5].

In *in vivo* experiments glutamic acid (5×10^{-9} mg/kg) or aviamine (6.5×10^{-2} mg/kg) alone or in combination with test toxins (diluted to 10^{-4}) were administered through a tube to mice and with fodder to

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TABLE 1. Antitoxic Activity of Amino Acid and Peptide Agents towards Mouse Splenocytes *In Vitro* (*M*±*m*)

Agent	Cytotoxicity index, %	
	benzene	AFB_1 in benzene
Levamine	0**	0**
Cerebrolysin	4.3±1.4**	21.2±2.9*
Aviamine	12.9±2.4**	21.1±2.9*
Asparaginic acid	14.1±1.7*	9.1±1.4**
Arginine	25.3±3.0	27.0±1.2
Lysine	21.5±2.0	32.9±3.1
Glycine	0**	0**
Methionine	0**	7.5±1.3**
Isoleucine	6.7±1.2**	8.4±1.3**
Glutamic acid	8.0±1.9**	12.0±2.3**
Tryptophan	11.8±2.3**	18.8±2.8*
Glu+Trp	0**	15.3±2.5*
GluTrp (thymogen)	0**	15.3±2.5*
LysAsp	15.1±1.3**	12.9±2.4**
Lys+Asp	12.9±2.4**	15.1±2.5*
Thymosin fraction 5	0**	10.8±1.6**
Thymaline	0**	6.4±1.2**
Control (cells in Hanks' solution)	21.1±1.4	31.0±2.3

Note. Here and in Table 2: *$p<0.05$, **$p<0.01$ *vs.* the control. Agents were tested in a concentration 1.3×10^{-3} mg/ml. Each value is a result of estimation of at least 400-600 cells. Cell viability in Hanks' solution was 90-95%.

chicken during 15 days (8-10 mice and 5-7 chickens per group were examined). The animals were decapitated, and splenocyte sensitivity to toxins was evaluated *in vitro* as described above and expressed in cytotoxicity index. *In vivo* and *in vitro* experiments were repeated at least 2-3 times.

RESULTS

Of all *in vitro* tested amino acid and peptide preparations only arginine and lysine did not protect mouse splenocytes (Table 1). Other agents were active and enhanced splenocyte resistance to toxins, especially to benzene, in both animal species (Tables 1 and 2). The most active amino acids were glycine, isoleucine, and glutamic acid and amino acid mixture cerebrolysin. GluTrp dipeptide was highly active.

In mouse splenocytes, *in vitro* antitoxic activity of peptide mixtures (thymosin fraction 5, thymaline) against benzene and AFB_1 was equally pronounced (Table 1). Amino acid mixture and aviamine protected mouse splenocytes from both toxins *in vitro* (Table 1) and chicken splenocytes mainly from AFB_1 (Table 2). Antitoxic activity of aviamine *in vitro* was observed when it was directly added to mouse or chicken cells with AFB_1, the cytotoxicity indexes being 15.6±2.5 and 19.7±2.8% *vs.* 33.5±3.5 and 31.5±2.3% in the control, respectively ($p<0.01$). Aviamine or glutamic acid also increased resistance of chicken splenocyte to AFB_1, the cytotoxicity index decreased to 25.0±3.0 and 16.6±2.3%, respectively, *vs.* 47.7±3.5% in the control (intact chicken splenocytes *in vitro* treated with AFB_1) ($p<0.01$). Similar effects were obtained with mouse splenocytes.

Addition of aviamine (but not glutamic acid) in the same dose to chicken ration together with AFB_1 drastically decreased splenocyte resistance to AFB_1 *in vitro* (AFB_1 cytotoxicity index 73.4±3.1 *vs.* 29.8± 3.2% in the control, $p<0.01$). Contrary to this, addition of aviamine together with benzene to fodder increased splenocyte resistance, benzene cytotoxicity index being 11.5±2.5 *vs.* 26.8±3.4% in the control (intact cells *in vitro* treated with benzene) ($p<0.01$).

The data indicate that amino acid and peptide preparations exert pronounced antitoxic effects *in vitro* and *in vivo*. It is a general biological phenomenon, because the antitoxic effects of the studied compounds manifest towards mouse and chicken cells.

Bearing in mind the pronounced decrease of benzene cytotoxicity against mouse and chicken cells in the presence of amino acid and peptide preparations, normalization of phagocytosis [4] and antibody production [3] suppressed by benzene, we recommend these agents for prevention and immunocorrection of chronic benzene poisoning in mammals and birds.

TABLE 2. Antitoxic Activity of Amino Acid and Peptide Agents towards Chicken Splenocytes *In Vitro* (*M*±*m*)

Agent	Cytotoxicity index, %	
	benzene	AFB_1 in benzene
Levamine	10.3±2.2*	23.6±2.8*
Cerebrolysin	3.5±1.3**	16.5±2.6**
Aviamine	18.3±2.3	17.5±2.7**
Glutamic acid	1.6±0.6**	7.4±1.8**
Tryptophan	3.5±1.3**	23.0±3.0*
Glu+Trp	8.7±2.0**	15.9±2.6**
GluTrp (thymogen)	3.5±1.3**	13.0±3.0*
Glycine	0**	11.5±1.6**
Isoleucine	10.6±1.5**	14.1±1.7**
Methionine	9.3±1.4**	24.5±2.1*
Control (cells in Hanks' solution)	19.3±2.3	34.3±3

However, in chronic aflatoxicosis B_1 amino acid preparations should be used with caution, because low doses of aviamine in combination with microdoses of AFB_1 can very rapidly (within 2 weeks) induce splenocyte hypersensitivity to the toxin, reduce phagocytosis completeness index [4], and cause death of up to 23% birds [3].

Differences in the immune effects of amino acid preparations during exposure to benzene and AFB_1 dictate the necessity of a differentiated approach to application of immunomodulator drugs in different abnormalities.

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