Differences in the Phagocytic Activity of Granulocytes and Their Immunocorrection in Chronic Aflatoxicosis B_1 and Benzene Poisoning

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After 60 days of intoxication, low doses of aflatoxin B_1 decreased the total protein concentration, an indicator of phagocytosis completeness, and had no effect on the level of lysosomal cationic proteins. Benzene decreases both parameters, but does not affect the total protein content. The amino acid preparations cerebrolysin and aviamine and glutamic acid normalize the phagocytic activity of granulocytes decreased by benzene, but not by aflatoxin.

Key Words: *aflatoxin B_1; benzene; amino acid preparations; phagocytosis*

The functional activity of monocytes is suppressed in chronic aflatoxin and benzene intoxication [4,5]. We found no published reports about the effects of aflatoxin B_1 (AFB_1) and benzene on the phagocytic activity of granulocytes and phagocytosis-correcting ability of amino acid preparations.

Our aim was to study the phagocytic activity of granulocytes and to evaluate the phagocytosis-correcting potentialities of amino acid preparations at different stages of chronic aflatoxicosis B_1 and benzene intoxication.

MATERIALS AND METHODS

Experiments were carried out on 100 male CBA mice starting from the age of 4 weeks (body weight 14-16 g) and 260 broiler chickens starting from the age of 7 days (body weight 50-60 g). AFB_1 (Institute of Nutrition, Russian Academy of Medical Sciences) in benzene with AFB_1 content 10^{-2} mg/ml, benzene (Reanal), glutamic acid (GA, Sigma), aviamine (chicken blood hydrolysate, Pharmacy Factory, St. Petersburg), and cerebrolysin (cerebral tissue hydrolysate, Abave) were used.

Cerebrolysin and aviamine were dosed by protein, whose content was 1000 and 32 mg/ml, respectively, and GA was dosed by dry substance and calculated per kg body weight. The amino acid preparations were dissolved and toxins emulgated in normal saline. They were administered to mice through a tube and to chicken with fodder for various periods in doses: AFB_1 and benzene 2.5×10^{-9} and 2.2×10^{-4} mg/kg for mice and 6.5×10^{-4} and 88 mg/kg for chicken, respectively, aviamine and cerebrolysin 6.5×10^{-2} and 65 mg/kg, and GA 5×10^{-9} mg/kg for both. Control animals received normal saline according to a similar protocol.

To obtaine peritoneal granulocytes, control and experimental animals were injected intraperitoneally with sterile 10% peptone solution [1,3] and the following parameters of phagocytic activity of peritoneal granulocytes were determined *in vitro:* phagocytic index (PI) (percentage of granulocytes participating in phagocytosis), phagocytic number (mean arithmetic number of bacterial cells per phagocyte) [3], and phagocytosis completeness index (the percent ratio of the number of granulocytes with the signs of complete phagocytosis to the total number

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of granulocytes containing phagocytosed bacteria) [1]. For studies of phagocytosis completeness, chicken previously administered 10% peptone were intraperitoneally injected with $15\text{-}18\times10^9$ one-day culture of test bacterium, and phagocytosis completeness was evaluated at different periods. The phagocytosis completeness was assessed by the ability of bacteria to grow and divide after a 2-h culturing of the leukocyte-bacterial mixture in agar at 37°C. The test bacterium was *St. albus* strain 9198 which changes dark-violet color for pink when stained according to Romanowsky.

In other groups (chicken) administered the drugs, lysosomal cationic proteins (LCB) were measured in peripheral blood granulocytes as described previously [2] and the total protein concentration was measured in the serum by the Biuret method. The LCB level was expressed in arbitrary units which were calculated as described elsewhere [2].

RESULTS

AFB_1 increased the PI in mice at all intervals of the observation periods (15 or 60 days) (Table 1). In chicken AFB_1 exhibited such an activity only at the early period of poisoning. Benzene did not affect the PI in both species irrespective of the duration of application.

In order to find out whether the different effects of AFB_1 and benzene on PI were due to reactions of toxins with different granulocyte subpopulations, we studied the cytotoxicity of toxins in 10^{-4} dilutions *in vitro*. In a population of murine and chicken granulocytes *in vitro* pretreated with benzene, the number of dead cells doubled after additional treatment with AFB_1 and was 34.9±1.7 and 37.3±1.7% in comparison with 17.2±1.3 and 15.9±1.3% cells dead in benzene.

In contrast to toxins, amino acid drugs affected the phagocytic activity of granulocytes similarly. Aviamine increased the PI in both mice and chicken, irrespective of the dose and duration of treatment. The effects of GA on mice and chicken were similar at both periods. Aviamine in any dose and GA in a dose of 5×10^{-9} mg/kg used together with AFB_1 did not modify the ability of the toxin to increase PI, and increased it in combination with benzene ($p<0.05$).

Even after a 60-day application, the studied toxins did not decrease but even increased PI; therefore, their effects on the processes occurring inside phagocytes were studied: the ability of granulocytes to disintegrate bacterial cells and alteration of intracellular LCP concentration. In order to find out whether the toxins immediately affect the biochemical processes inside phagocytes or their effects, as the effects of hepatotropic toxins, are mediated by changes in the functional activity of hepatocytes, we analyzed changes inside phagocytes and changes in the total protein concentration which reflects the status of protein metabolism in the liver. During early (15 days) intoxication, AFP_1 did not affect the total protein concentration, index of phagocytosis completeness, and LCP content. The latter value

TABLE 1. The Phagocytic Activity of Granulocytes and Phagocytosis-Correcting Activity of Amino Acid Drugs at Different Terms of Aflatoxin and Benzene Intoxication ($M\pm m$)

Preparation	PI, %				Index of completeness, %		Total protein, g/liter	
	mice		chicken		chicken		chicken	
	15 days	60 days	15 days	60 days	15 days	60 days	15 days	60 days
Normal saline (control)	25.9±0.8	22.0±1.9	25.5±1.6	23.5±1.7	87.0±1.7	92.0±1.3	35.3±1.3	41.3±2.3
Benzene	27.1±4.1	24.5±1.7	27.0±0.7	27.8±2.2	80.3±1.6**	37.0±2.4*	38.8±1.2	44.4±0.3
AFB_1 in benzene	39.5±3.1*	33.6±2.4*	43.6±2.8*	24.4±2.1	83.3±1.5	61.0±2.0*	37.1±1.3	30.8±1.0*
Benzene+aviamine, 6.5×10^{-2} mg/kg	29.7±0.8*	28.5±2.3**	37.5±2.3*	28.4±1.9**	92.0±1.9	86.0±2.4	35.3±1.3	44.4±0.3
AFB_1+aviamine, 6.5×10^{-2} mg/kg	36.6±3.3*	35.6±2.4*	37.5±3.0*	36.7±2.1*	30.5±2.3*	58.0±1.9*	54.2±3.8*	31.4±0.3*
AFB_1+aviamine, 65 mg/kg	33.9±2.5**	33.3±2.3*	41.8±2.6*	40.2±2.2*	36.5±2.5*	55.5±2.5*	33.2±2.4	35.3±0.4*
Aviamine, 6.5×10^{-2} mg/kg	30.3±1.3**	29.5±2.2**	40.5±2.5*	33.9±2.2*	85.0±1.9	88.0±1.5	34.1±1.3	38.7±1.8
Aviamine, 65 mg/kg	31.2±0.8*	30.5±1.9**	44.5±2.8*	42.8±2.7*	88.1±1.6	89.0±1.6	35.3±1.3	39.0±1.7
GA	45.8±3.5*	42.2±2.6*	46.2±2.8*	48.8±2.5*	85.6±2.0	80.0±2.0*	35.3±1.3	42.1±0.3
Benzene+GA	42.6±3.6*	—	42.6±2.7*	—	85.0±2.1	96.0±1.0	34.2±1.5	44.7±0.3
AFB_1+GA	38.5±3.2*	—	45.5±2.9*	—	97.0±1.0*	14.0±1.7*	38.8±0.6	35.3±0.4*

Note. *$p<0.01$, **$p<0.05$ *vs.* the control. Dash shows that no experiments were made. Five animals per group were examined.

did not differ from the control and was 1.8±0.03 arb. U. At later terms AFB_1 decreased the total protein concentration and did not change LCP content, which was 1.6±0.13 *vs.* 1.8±0.03 arb U in the control (5 chicken were examined in each group).

Suppression of the phagocytic index by ABF_1 manifested itself in decreased ability of granulocytes to disintegrate bacterial cells and in the growth of staphylococci outside phagocytes. This phenomenon was observed as early as 10-30 min after intraperitoneal infection of chicken but not after 24 h.

In contrast to AFB_1, benzene did not affect the total protein concentration, and changed the index of phagocytosis completeness at both terms. In later period of intoxication, benzene decreased the content of LCB in phagocytes: 1.4±0.04 *vs.* 1.8±0.02 arb. U in the control ($p<0.01$). As AFB_1, benzene promoted the growth of unphagocytized staphylococci. Unlike in experiments with AFB_1, the growth of bacterial cells outside phagocytes was observed only 2 h after infection and was still observed after 24 h.

Aviamine in all the studied doses did not affect the index of phagocytosis completeness irrespective of the duration of treatment, while GA decreased this index and did not affect the content of LCP in phagocytes. The level of LCP was normal: 1.7±0.08-1.8±0.02 arb. I. Cerebrolysin in both doses did not modify the index of phagocytosis completeness at early terms (15 days), which varied from 82.0±2.2 to 87.0±+2.4%. In a low dose in combination with AFB_1 it decreased the index from 87.0±1.7% in the control to 24.5±1.8% and promoted the growth of staphylococci outside phagocytes (5 chicken per group were examined). Under similar conditions, aviamine in a low dose at the early term and GA at a later term decreased the index of phagocytosis completeness (Table 1) but did not lead to growth of unphagocytized bacterial cells. In the early period of intoxication, cerebrolysin, aviamine, and GA normalized the index of phagocytosis completeness, which was decreased by benzene. In late period of intoxication, aviamine and GA normalized the index of phagocytosis completeness decreased by benzene and the level of LCP.

The phagocytic number did not change in any experiment and varied from 1.7±0.6 to 1.9±0.7 in comparison with 2.3±0.7 in the control.

These results indicate that the index of phagocytosis completeness is the most informative parameter in the studies of the effects of various compounds on the phagocytic ability of granulocytes. The decrease in the LCP level correlates with the completeness index only in a grave suppression of the disintegrating ability of granulocytes.

Benzene and AFB_1 affect different subpopulations of granulocytes. This is confirmed by an increase in PI by AFB_1 (but not by benzene) *in vivo* and doubling of the number of dead cells in a population of granulocytes pretreated with benzene *in vitro*.

Suppression of disintegration of bacterial cells inside granulocytes by AFB_1 may be caused by inhibition of protein production in hepatocytes. This is confirmed by a decrease in the granulocyte activity caused by AFB_1 but not by benzene, which was paralleled by a decrease in the total protein concentration.

The phagocytosis-modulating effects of the amino acid preparations in chronic aflatoxicosis B_1 and benzene intoxication are different. Aviamine, cerebrolysin, and GA suppress the disintegrating ability of granulocytes, while in benzene intoxication these drugs normalize the index of phagocytosis completeness. A decrease in the total protein concentration by AFB_1 (but not by benzene) at the late stage of intoxication and different effects of amino acid drugs on the nonspecific resistance parameters decreased by toxins indicate different mechanisms of the development of chronic aflatoxicosis B_1 and benzene intoxication.

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