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EFFECTS OF AFLATOXIN B1 ON LIPID PEROXIDATION AND ACTIVITIES OF ANTIOXIDANT ENZYMES IN RAT ORGANS AND ERYTHROCYTES

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The objective of this study was to evaluate the effects of aflatoxin B1 (AFB1) on the process of lipid peroxidation and activities of superoxide dismutase (SOD) and glutathione peroxidase in rat liver, kidney, brain and erythrocytes in the conditions of acute intoxication. The results of investigation show, that TBARS (thiobarbituric acid reactive substances) concentrations increased significantly during the 14-day period after intraperitoneal injection of AFB1 (in a dose 0.5 mg/kg body weight) in all analyzed cells. The activities of SOD and glutathione peroxidase decreased in rat organs and in erythrocytes dependently on cell type and time after administration of AFB1. It can be concluded that AFB1 is capable to induce the development of oxidative stress due to the stimulation of lipid peroxidation and inhibition of antioxidant enzyme activities in rat liver, kidney, brain and erythrocytes.

Keywords: aflatoxin B1, mycotoxin, lipid peroxidation, TBARS, antioxidant system.

Aflatoxins are the secondary metabolites, produced by some fungal species of the genus *Aspergillus* (primarily by *A. flavus* and *A. parasiticus*) [8]. Humans and farm animals can be exposed to aflatoxins mainly through consumption of contaminated foods and feeds. Inhalation of grain dusts, or skin contact with mold-infested substrates are another possible routes of aflatoxins entry into the organism [11, 30]. Aflatoxins are highly toxic, immunosuppressive, mutagenic, carcinogenic and teratogenic compounds [1, 11, 30]. Aflatoxin B1 (AFB1) is the most common and hazardous substance in this group, having the liver as its primary target organ [15, 25]. Apart to the liver, AFB1 also affects other organs in animal and human body [1, 29, 30].

Intoxication of both animals and humans by aflatoxins can be acute or chronic depending on the dose and duration of exposure. Prolonged low-level exposure to aflatoxins, in particular to AFB1, is associated with impaired growth, reduced feed intake and poor assimilation of nutrients, immunologic suppression, and increased risk of liver cancer incidence [14, 24, 30]. Acute aflatoxin poisoning can also occur in humans and domestic animals [11, 22]. Acute intoxication can result in aflatoxicosis, which manifests as severe hepatotoxicity [22]. While the liver is the principal organ affected, kidney, brain and other organs can also be impacted in animals suffering of aflatoxicosis [9, 18, 19].

Aflatoxins were shown to induce formation of reactive oxygen species (ROS) in a variety of cells [15, 29]. It is known that under oxidative stress conditions, excessive ROS can interact

with biomolecules (lipids, proteins and DNA), leading to metabolic disturbances and damages of cellular components [27]. Oxidative stress may contribute to aflatoxin-induced cytotoxicity and other pathological lesions observed in aflatoxicosis [15, 29]. However, susceptibility of cellular antioxidant defense system to aflatoxin poisoning was not investigated completely. The aim of this study was to research an influence of AFB1 on the processes of lipid peroxidation and activities of the enzymes of antioxidant system in the cells of liver, kidney and brain, as well as in the erythrocytes of albino rats in the conditions of acute intoxication.

Materials and methods

Adult male albino rats (150–170 g body weight) were used in the experiments. Animals were maintained at the vivarium conditions at 23ºC under 12:12 h light : dark cycle. The rats were fed by a standard laboratory diet with free access to drinking water. Animals were randomly divided into two groups: control (n=10) and experimental (n=10). The rats of experimental group were exposed to aflatoxin by a single intraperitoneal injection of AFB1 (dissolved in olive oil) in a dose 0.5 mg/kg body weight (1/12 LD_{50}). Animals of control group were injected with an equivalent volume of olive oil.

Five rats of experimental group and five control rats were sacrificed on the 7th day after injection of AFB1, and the remaining ten (5 experimental and 5 control animals) were sacrificed on the 14th day after AFB1 administration. Animals were killed by rapid decapitation after inhalation anesthesia accordingly to the regulations for euthanasia of animals used for scientific purposes [10].

At the time of sacrifice, blood samples were collected into heparinized test tubes, plasma was separated by centrifugation at 3 000 × g for 15 min. Buffy coat was removed by aspiration. Erythrocyte pellet was washed three times with 10 volumes of 0.85% NaCl by centrifuging suspension every time at 2 500 × g for 5 min. The erythrocytes were then lysed by two freezing-thawing cycles [7]. After diluting the suspension by the addition of three volumes of ice-cold double distilled water, it was centrifuged at 6 000 × g for 30 min on a refrigerated centrifuge to remove the stroma, and the supernatant (hemolysate) was collected.

Fresh organs (liver, kidney, and brain) were removed from euthanatized animals, cooled to the temperature 1–4°C in physiological saline, and dried with filter paper. The organs were cut into small pieces and homogenized using an MPW-324 homogenizer in 0.05 M tris-HCl buffer (pH 7.5) with addition of 0.25 M sucrose. The ratio of tissue mass to the volume of buffer solution was 1:9. The samples were then centrifuged in a refrigerated centrifuge at 10 000 × g for 30 min, and supernatant was used for analysis.

The intensity of lipid peroxidation (LPO) was estimated spectrophotometrically by thiobarbituric acid test, measuring the production of thiobarbituric acid reactive substances (TBARS) [4]. Superoxide dismutase (SOD, EC 1.15.1.1) activity was determined using the method, elaborated by Nishikimi et al. with modifications [2, 20]. Glutathione peroxidase (EC 1.11.1.9) activity was determined by measuring the rate of reduced glutathione (GSH) oxidation by *tert*-butyl hydroperoxide [3]. Protein concentrations in cell homogenates and red blood cell hemolysates were determined by the method of Lowry el al. [17].

The results, processed as means ± S.D. were analyzed using Student's test to determine the significance level.

Results and discussion

According to experimental data, exposure to AFB1 leads to intensification of the lipid peroxidation processes in rat liver, kidney and brain, as wells as in rats erythrocytes. As shown in Figure 1, the levels of LPO products, measured as TBARS, increased significantly in liver, kidney

and brain homogenates of experimental group animals during the 14-day period after AFB1 injection. The most considerable differences ($P<0.001$) in the concentrations of TBARS in mentioned organs (2.2-fold in liver and kidney, and 2.4-fold in brain) were observed on the 14th day of experiment compared with the levels seen in control group. Similar changes in TBARS concentrations were revealed in the erythrocytes of AFB1-injected rats. This index gradually increased ($P<0.05$) from 13.50±0.80 nmol/g hemoglobin (in control group) up to the level of 18.09±1.27 nmol/g hemoglobin on 14th day after toxin exposure (not shown in Fig. 1).

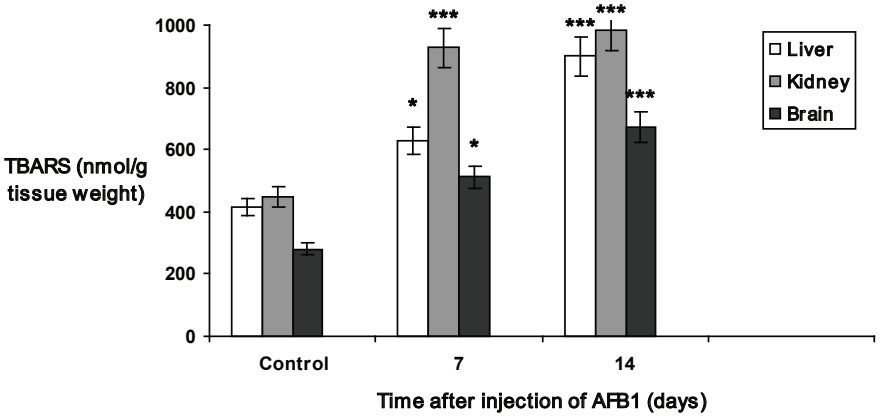

Fig. 1. Effects of aflatoxin B1 on TBARS concentrations in liver, kidney and brain homogenates (*, *** – statistically significant differences between control and experimental groups of animals: *– $P<0.05$; *** – $P<0.001$).

The stimulatory effects of AFB1 on the processes of lipid peroxidation in the analyzed organs and cells in this experiment could be due to AFB1-induced intracellular formation of reactive oxygen species [15, 29].

As known, under physiological conditions ROS are usually neutralized by cellular defense systems, which include antioxidant enzymes and non-enzymatic antioxidants [12, 27]. The enzymes of antioxidant system such as superoxide dismutase and glutathione peroxidase are known to scavenge ROS and protect the cells against deleterious consequences of lipid peroxidation [5, 6]. In order to evaluate the dynamics of mentioned enzymes activity changes in the conditions of acute intoxication by AFB1 the following stage of our experiment was aimed to evaluate the activities of SOD and glutathione peroxidase in the liver, kidney, brain and erythrocytes of the rats of experimental group.

The study results show different effects of AFB1 on the antioxidant system enzymes activities, largely depending on the investigated organ or cell type. The activity of superoxide dismutase in liver and brain homogenates, as well as in erythrocytes decreased on the 7th day after injection of AFB1 ($P<0.05–0.01$), while SOD activity in the kidney was unchanged on this stage of experiment (Fig. 2). On the 14th day after AFB1 administration SOD activity in all investigated cells of animals of experimental group decreased of 53–67% compared to control values ($P<0.05–0.01$).

The changes in glutathione peroxidase activity in the rats erythrocytes of experimental group resembled the dynamics of superoxide dismutase, with significant decline ($P<0.001$) on the 7th and 14th day after exposure to AFB1 (Fig. 3). The enzyme activities in liver and brain homogenates decreased on the 7th day ($P<0.05$), but returned to the control level on the 14th day after AFB1 injection. At the same time, glutathione peroxidase activity was unaltered in rats kidney during the whole experimental period.

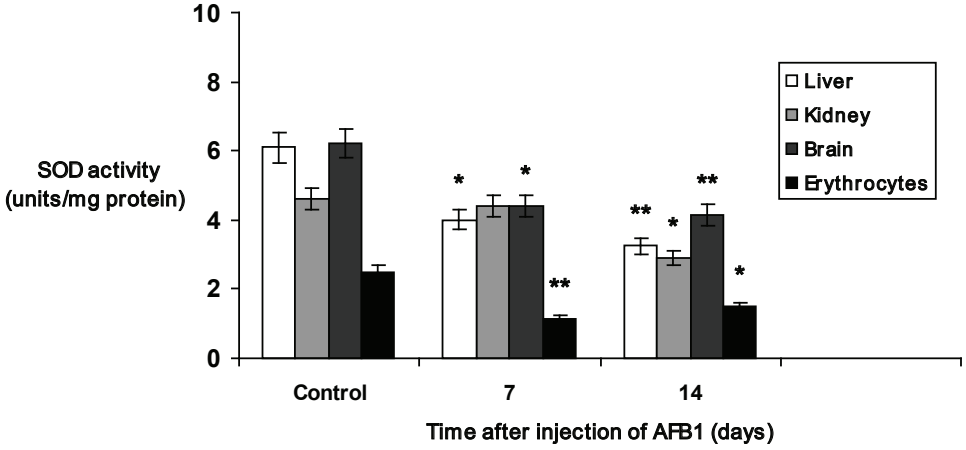

Fig. 2. Superoxide dismutase activity in liver, kidney and brain homogenates, and erythrocytes of AFB1-injected and control rats (*, ** – statistically significant differences between control and experimental groups of animals: *– P<0.05; ** – P<0.01).

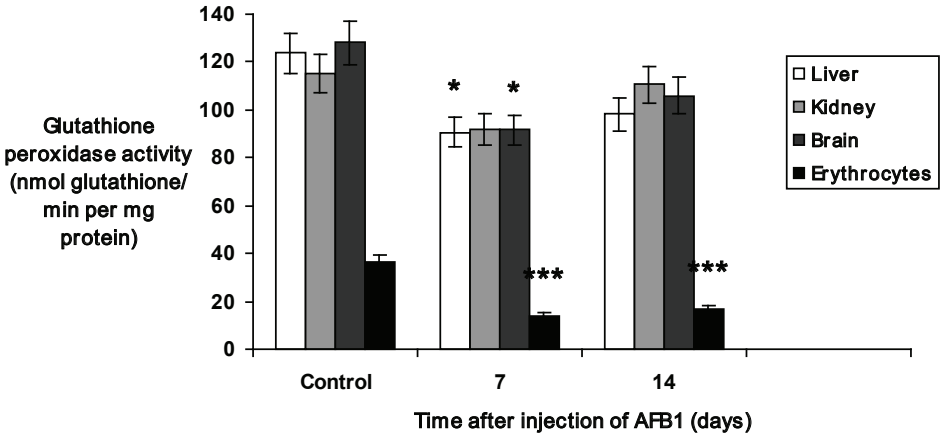

Fig. 3. Glutathione peroxidase activity in liver, kidney and brain homogenates, and erythrocytes of AFB1-injected and control rats (*, *** – statistically significant differences between control and experimental groups of animals: *– P<0.05; *** – P<0.001).

Considering the results of this study, we can conclude, that acute exposure of animals to aflatoxin B1 results in stimulation of lipid peroxidation in the liver, kidney, brain and erythrocytes. The data concerning the elevated levels of LPO products in the cells of AFB1-administered rats are in agreement with the findings regarding to the stimulatory effects of AFB1 on intracellular formation of reactive oxygen species [15, 29].

It has been shown that lipid peroxidation induces disturbance of cellular membrane organization and leads to alteration of integrity and functional failure of membrane constituents [12]. Further, the end products of LPO are cytotoxic and mutagenic, capable to modify biologically essential molecules such as proteins and DNA bases, and to affect the affinity of hemoglobin for oxygen [21]. Thus activation of lipid peroxidation processes as well as excessive formation of ROS and LPO products can be accompanied by cell damage and can have harmful consequences to many cellular functions depending on the cell type [12, 27].

Under such circumstances, the activities of superoxide dismutase and glutathione peroxidase are important indices, which show the defensive potencies of the cells. Superoxide dismutase protects the cells from oxidative damage by breaking down a potentially hazardous free radical superoxide ($O_2^{-\bullet}$) to hydrogen peroxide (H_2O_2) and molecular oxygen [6]. Glutathione peroxidase is known to be involved in detoxication of the produced hydrogen peroxide, and can interact with lipid peroxidation by decomposing the lipid hydroperoxides to corresponding lipid alcohols [5].

However, according to results of this study, the activities of SOD and glutathione peroxidase were inhibited at different stages of the experiment in the cells of AFB1-administered rats. The most marked changes were revealed in erythrocytes, in which significant decrease in the activities of mentioned enzymes was observed during the 14-day period after injection of AFB1. Because of considerable inhibition of key antioxidant enzymes and incapability of these cells to synthesize enzyme molecules, the erythrocytes could be especially susceptible to AFB1-induced oxidative stress. Therefore metabolic damage in erythrocytes, caused by intensified lipid peroxidation, could be associated with membrane destruction and impairment of oxygen transport function. In particular, consumption of aflatoxin-contaminated feed cause hemolytic anemia in chickens [26]. The mammalian species (dogs, rabbits, pigs, cattle) often suffer from anemia, caused by aflatoxin intake [28].

The adverse consequences of inhibition of antioxidant enzymes, particularly in the initial period of intoxication (7 days after injection of AFB1) could be also evident in the liver and brain of experimental group rats. The available data suggest that ROS formation and stimulation of LPO processes are implicated in the mechanisms of AFB1-induced hepatotoxicity [15] and in the neurological signs associated with aflatoxicosis in animals [9, 13].

At the same time, the activities of antioxidant enzymes in rats kidney were less susceptible to the inhibitory influence of AFB1 in comparison to other organs analyzed in this study. Unaltered activities of SOD and glutathione peroxidase in rat kidney on the 7th day after injection of AFB1 could be one of the features that might determine the resistance of kidney cells to the toxic effects of AFB1 metabolites [16, 23].

The results of this investigation show that AFB1 exhibits stimulatory effects on the process of lipid peroxidation, and adversely affects the activities of SOD and glutathione peroxidase in the cells of rat organs as well as in erythrocytes. The revealed effects suggest the capability of aflatoxin B1 to induce the development of oxidative stress in animal cells. However the sensitivity of antioxidant enzymes to AFB1 in rat liver, kidney and brain is different, that probably depends on specificity of cellular metabolism and duration of period following the administration of AFB1.

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ВПЛИВ АФЛАТОКСИНУ B1 НА ПРОЦЕС ПЕРОКСИДНОГО ОКИСНЕННЯ ЛІПІДІВ І АКТИВНІСТЬ ЕНЗИМІВ АНТИОКСИДАНТНОГО ЗАХИСТУ В ОРГАНАХ ТА ЕРИТРОЦИТАХ ЩУРІВ

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Метою дослідження було вивчити вплив афлатоксину B1 (AFB1) на процес пероксидного окиснення ліпідів і активність супероксиддисмутази (СОД) та глутатіонпероксидази в печінці, нирках, мозку й еритроцитах щурів за умов гострої інтоксикації. Результати дослідження свідчать, що концентрація ТБК-реактивних продуктів значно підвищується впродовж 14-добового періоду після внутрішньочеревного введення AFB1 (в дозі 0,5 мг/кг маси тіла) у всіх аналізованих органах і еритроцитах. Активність СОД і глутатіонпероксидази зменшувалася в органах і еритроцитах щурів залежно від типу клітин та часу після введення AFB1. З отриманих результатів можна зробити висновок, що афлатоксин B1 здатний індукувати розвиток окисного стресу за рахунок стимуляції пероксидного окиснення ліпідів та інгібування активності антиоксидантних ферментів у клітинах печінки, нирки, мозку і в еритроцитах щурів.

Ключові слова: афлатоксин B1, мікотоксин, пероксидне окиснення ліпідів, ТБК-реактивні продукти, антиоксидантна система.

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ВЛИЯНИЕ АФЛАТОКСИНА B1 НА ПРОЦЕСС ПЕРЕКИСНОГО ОКИСЛЕНИЯ ЛИПИДОВ И АКТИВНОСТЬ ЭНЗИМОВ АНТИОКСИДАНТНОЙ ЗАЩИТЫ В ОРГАНАХ И ЭРИТРОЦИТАХ КРЫС

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Целью исследования было изучить влияние афлатоксина B1 (AFB1) на процесс перекисного окисления липидов и активность супероксиддисмутазы (СОД) и глутатионпероксидазы в печени, почках, мозге и эритроцитах крыс в условиях острой интоксикации. Результаты исследования показали, что концентрация ТБК-реактивных продуктов значительно увеличивается в течение 14-суточного периода после внутрибрюшного введения AFB1 (в дозе 0,5 мг/кг массы тела) во всех анализированных органах и эритроцитах. Активность СОД и глутатионпероксидазы уменьшалась в органах и эритроцитах крыс в зависимости от типа клеток и времени после введения AFB1. Из полученных результатов можно сделать вывод, что афлатоксин B1 способен индуцировать развитие окислительного стресса за счет стимуляции перекисного окисления липидов и ингибирования активности ферментов антиоксидантной системы в клетках печени, почки, мозга и в эритроцитах крыс.

Ключевые слова: афлатоксин B1, микотоксин, перекисное окисление липидов, ТБК-реактивные продукты, антиоксидантная система.