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THE INFLUENCE OF AFLATOXIN B1 ON THE CONCENTRATION OF NUCLEAR FACTOR ^{K}B IN RATS' LIVERS

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ABSTRACT

Introduction. Aflatoxins are metabolites produced by Aspergillus flavus and Aspergillus parasiticus. Due to the high prevalence of aflatoxin-containing products they are common issue of the observational studies. Observational studies have demonstrated the hepatotoxic effects of aflatoxins in humans. However, the exact pathogenetic mechanisms of the effect of aflatoxin B1 on the above-mentioned hepatotoxicity have not yet been known.
Aim of the study. The aim of the study was to assess the toxic effects of different doses of aflatoxin B1. The analyze was performed using assessment of concentration of NF-κB in liver tissue homogenates after a 7-day intoxication with this mycotoxin.
Material and methods. The studies were carried out on Wistar male rats which were selected randomly, according to the principle of simultaneity for the control group and the study groups. The concentration of NK-κB was determined by immunoenzymatic ELISA in the obtained supernatants of liver taken from decapitated animals. The statistical analysis was performed with Statistica 13.3 (Statsoft, USA).
Results. The statistical significance of the difference between the concentrations in the control and study group receiving 1.0 mg/kg of aflatoxin B1 and between the control and study group who received aflatoxin B1 at a dose of 2.0 mg/kg body weight ($p < 0,05$) were demonstrated. A significant relationship was also found between the level of dose of aflatoxin B1 administered to the rats and the concentration of NF-κB. Negative correlations were obtained. The higher dose administered to rats - the lower level of measured concentration of NF-κB.
Conclusions. The study of the influence of aflatoxin B1 on the level of NF-κB transcription factor may significantly contribute to understand the mechanism of its action, influence on inflammatory, apoptotic and carcinogenic processes in the liver and determine its safe level in food intended for humans and animals.
Keywords: aflatoxin B1, nuclear factor-κB, liver

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INTRODUCTION

Aflatoxins are secondary metabolites mainly produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The main source of exposure to aflatoxins is oral. Infection is mostly often caused by consumed grains of cereals, nuts, dried fruits, spices, but also meat and dairy products from animals consuming contaminated feed. Due to the high prevalence of aflatoxin-containing products, the legal standards have been introduced to specify the maximum level of these mycotoxins in food. Aflatoxin B1 is the most toxic one of all aflatoxins. It is due to the presence of a lactone ring and two furan rings, the outermost of which has a double bond. Because of the presence of this double bond the aflatoxin B1 molecule can associate more closely with the protein or DNA molecule, which disturbs the functioning of the cell. The International Agency for Research on Cancer (IARC) qualified aflatoxin B1 (AFB1) to group I - carcinogenic to humans [1]. In animal model studies using aflatoxin B1, the effects of toxic influence affected liver cells most commonly.

Observational studies have also demonstrated the hepatotoxic effects of aflatoxins in humans. However, the exact pathogenetic mechanisms of the effect of aflatoxin B1 on the above-mentioned hepatotoxicity have not yet been known.

The aim of the study was to assess the toxic effects of different doses of AFB1 by measuring the concentration of NF-κB in rats livers tissue homogenates. The nuclear factor κB (NF-κB) represents a group of inducible transcription factors that regulate many genes involved in various processes of the immune and inflammatory response. By performing these functions, it plays a main role in regulating inflammatory signaling pathways in many organs, including the liver.

AIM

The aim of the study was to assess the toxic effects of different doses of aflatoxin B1. The analyze was performed using assessment of concentration of NF-κB in liver tissue homogenates after a 7-day intoxication with this mycotoxin

MATERIALS AND METHODS

The studies were carried out on Wistar male rats with a body weight of 190-200g, which were selected randomly, according to the principle of simultaneity for the control group and the study groups. The tested animals were divided into 4 groups, 9 in rats in each:

- Group No. 1 - Animals administered with aflatoxin B1 intragastrically at a dose of 0.5 mg/kg body weight for 7 days.
- Group No. 2 - Animals administered with aflatoxin B1 intragastrically at a dose of 1.0 mg/kg body weight for 7 days.
- Group No. 3 - Animals administered with aflatoxin B1 intragastrically at a dose of 2.0 mg/kg body weight for 7 days.
- Group No. 4 - Control group - animals administered with redistilled water intragastrically for 7 days.

After 7 days of experiment, the animals were decapitated after intraperitoneal administration of a hypnotic agent. Liver was taken from them for further testing. The animal tissue taken for testing was homogenized in PBS buffer in the proportions: 0.5 g of tissue per 2 ml of buffer. The homogenate was centrifuged at 14,000 / min. for 15 minutes at 4°C. The concentration of NK-κB was determined by immunoenzymatic ELISA in the obtained supernatants. The statistical analysis of results of the subjected tests was performed in the Statistica 13.3 program using the Shapiro-Wilk test for normality analysis, Mann-Whitney U test (for variables not meeting the assumptions for parametric tests), student's t-test (for variables meeting the assumptions for parametric tests) and the test ANOVA Kruskal - Wallis by rank for more than two variables. The results were considered statistically significant with significance level $p < 0.05$.

RESULTS

The mean concentration of NF-κB (ng/ml) in the control group was 1951.68 ± 773.07 and in the study groups, respectively: for the group administered with aflatoxin B1 at a dose of 0.5 mg/kg – 2218.68 ± 603.35, aflatoxin B1 at a dose of 1.0 mg/kg – 350.53 ± 89.09 and aflatoxin B1 at a dose of 2.0 mg/kg – 224.54 ± 134.89. This data is shown in Table 1. No statistical significance of the difference between the average concentrations in the control group and the group receiving 0.5 mg / kg of aflatoxin B1 ($p = 0.426$) was demonstrated.

The statistical significance of the difference between the concentrations in the control and study group receiving 1.0 mg/kg of aflatoxin was demonstrated using the non-parametric U-Mann-Whitney test. It showed a significant difference in the mean concentration in this two groups ($p = 0.000412$).

To compare and check the statistical significance of difference between mean concentration of control and study group who received aflatoxin B1 at a dose of 2.0 mg/kg body weight the U-Mann-Whitney test was performed. It showed a significant difference in the mean concentration in this two groups ($p = 0.0004$).

Kruskal-Wallis test was used to compare the differences between the mean concentration in the control group and the two groups (study group receiving 1.0 mg/kg of aflatoxin, and 2.0 mg/kg of aflatoxin). The test result indicates the statistical significance of the studied data ($p = 0.0001$). Based on these, it can be concluded that the results obtained in the study indicate a significantly lower concentration of NF-κB in ng/ml in the livers of rats under the influence of aflatoxin B1 at doses higher than 0.5 mg / kg of the animal's body weight (Table 2).

In order to perform a more detailed analysis of the differences between the pairs of trials, the POST-HOC analysis of Dunn in the Bonferonni modification was performed. It indicates that significant differences were found between the control group and the group receiving aflatoxin B1 at a dose of 1.0 mg / kg body weight, and the control group and the group receiving aflatoxin B1 at a dose of 2.0 mg / kg body weight. It also showed no significant differences between the groups that received aflatoxin B1 at a dose of 1.0 mg/kg body weight and 2.0 mg/kg body weight. The results are presented in the table (Table 3).

In order to check the correlation between the concentrations of NF-κB in ng/ml and the dose of

aflatoxin B1 administered to the study groups, the Pearson r correlation was used due to the normal distribution of variables. There was a significant relationship between the dose of aflatoxin B1 administered and the level of NF-κB concentration in ng / ml. Negative correlations were obtained. The higher the dose administered to rats, the lower the level of measured NF-κB in ng/ml.

Statistical significance of differences between NF-κB concentrations in the control group and two study groups was also demonstrated (p = 0.0001). A significant relationship was also found between the level of dose of aflatoxin B1 administered to the rats and the concentration of NF-κB. Negative correlations were obtained - the higher dose administered to rats with the lower level of measured concentration of NF-κB.

The Kruskal-Wallis test was also performed to compare the differences between the average concentration in the control group and the two study groups. The test result indicates the statistical significance of the studied data (p = 0.0001).

Tab. 1

Basic statistics of the studied quantitative variables in the each group of animals included in the experiment examining the level of NF-κB in ng/ml in the liver.

Group	N	Medium	SD
Aflatoxin B1 0,5mg/kg	9	2218.682	603.3593
Aflatoxin B1 1mg/kg	9	350.533	89.0901
Aflatoxin B1 2mg/kg	9	224.549	134.8872
Control Group	9	1951.680	773.0677

Tab. 2

Kruskal-Wallis test comparing the measurements: the control group, the group receiving aflatoxin B1 1mg / kg, and aflatoxin B1 2mg / kg.

Group	Test Kruskala-Wallisa: H (2, N=27 =19.47443, p=0.0001		
	R:23.00	R:12.222	R: 6.7778
Control Group		0.011912	0.000043
Aflatoxin B1 1mg/kg	0.0119		0.43693
Aflatoxin B1 2mg/kg	0.00004	0.4369	

Tab. 3

Dunn's POST-HOC test modified by Bonferonni comparing the measurements from the control group, the group receiving aflatoxin B1 at a dose of 1 mg / kg, and aflatoxin B1 at a dose of 2 mg / kg.

Group – compaired pair	Test statistics	Significance	Bonferonni corrected significance
Aflatoxin B1 2mg/kg - Aflatoxin B1 1mg/kg	5.444	0.146	0.437
Aflatoxin B1 2mg/kg – control group	16.222	0.000015	0.000044
Aflatoxin B1 1mg/kg – control group	10.778	0.004	0.012

DISCUSSION

It is estimated that as many as 4.5 billion people worldwide are exposed to aflatoxins, including the most important of them aflatoxin B1 [2]. The main source of exposure to aflatoxins is the oral route [3]. Consumption of products contaminated with these mycotoxins is common due to the large variety of products potentially contaminated with mycotoxins. They are most often found in cereal grains, nuts, dried fruit, spices.

The aim of our study was to evaluate the toxic effect of aflatoxin B1 based on the ng/ml concentrations of the transcription factor NF-κB in rats' livers under the influence of various doses of this mycotoxin. The liver was considered to be the primary organ examined because it is the organ most toxic for aflatoxin B1. It is related to the metabolism of this toxin mainly in the liver. The hepatotoxic and hepatocarcinogenic effects of aflatoxin B1 have been well documented in various animal species (Wogan, 1999) [4]. As early as the 1960s, a study by Svobody et al. [5] on aflatoxin B1-induced ultrastructural and biochemical abnormalities in the liver of rats revealed abnormalities in the fine nuclear structure following exposure to this mycotoxin. They were accompanied by a decrease in cytoplasmic RNA and protein content, as well as a decrease in nuclear protein levels.

Rotimi et al [6] showed that acute exposure to high doses of aflatoxin B1 for 7 days induced liver damage with accompanying dyslipidemia.

The nuclear factor κB (NF-κB) was used as a marker of potential liver damage. The key role of NF-κB in the liver is also emphasized by the fact that genetic ablation of NF-κB regulators in murine models leads to spontaneous liver damage, fibrosis and hepatocellular carcinoma [7].

Meki et al. [8] showed that intoxication with aflatoxin B1 significantly increases the level of the apopotic marker - caspase-3. The results of the analyzed

studies showed a significant increase in the concentration of pro-apoptotic factors such as caspase-3 under the influence of aflatoxin B1 intoxication. In the authors' own research, however, a statistically significant decrease in the concentration of the nuclear factor κB (NF-κB), activated under the influence of pro-apoptotic factors [9]. This decrease was also dependent on the dose of aflatoxin B1 administered to rats and significantly higher at higher concentrations of the administered mycotoxin. Low doses of aflatoxin B1 did not cause significant changes in the mean concentration of nuclear factor κB (NF-κB) compared to the control group. However, Luedde et al. [10] define the role of the nuclear factor κB (NF-κB) as a potential key regulator of inflammatory processes in the liver.

It is required for hepatocyte survival and liver homeostasis. The functions of fibrogenesis-active hepatic cells and myofibroblasts are also regulated by NF-κB. κB. The key role of NF-κB in regulating cell death, inflammation and wound healing makes it an important modulator of the progression of NF-κB liver disease and a potential link between chronic liver damage, fibrosis and hepatocellular carcinoma, which may be important in planning therapy these conditions and targeting this transcription factor.

The key role of NF-κB in the liver is also emphasized by the fact that genetic ablation of NF-κB regulators in murine models leads to spontaneous liver damage, fibrosis and hepatocellular carcinoma [11].

Numerous studies also indicate the participation of aflatoxin B1 in the pathogenesis of the above-mentioned hepatocellular carcinoma.

The control of apoptosis is extremely important in the process of carcinogenesis. Pikarsky et al. [12] demonstrated a procarinogenic role of NF-κB in Mdr2 - mice that developed hepatocellular carcinoma in response to chronic biliary hepatitis.

The presented results of own research require continuation, which may contribute to a better understanding of the mechanisms of hepatotoxic activity of aflatoxin B1. The study of the influence of aflatoxin B1 on the level of the NF-κB transcription factor may significantly contribute to the understanding the influence on inflammatory, apoptotic and carcinogenic processes in the liver. It can help establish a safe level in food intended for humans and animals. The transcription factor NF-κB may also be a potential pharmacotherapeutic target in the prevention and treatment of liver diseases caused by the toxic effects of aflatoxin B1.

CONCLUSION

The study of the effect of aflatoxin B1 on the level of the NF-κB transcription factor may significantly contribute to the understanding of its mechanism of action, the influence on inflammatory, apoptotic and carcinogenic processes in the liver, and to establish its safe level in food for humans and animals. The transcription factor NF-κB may be a potential pharmacotherapeutic target in the prevention and treatment of liver disease caused by the toxic effects of aflatoxin B1.

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