Aflatoxin B$_1$-induced changes of glutathione-S-transferase activity in the plasma and liver of the rat

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Background. The influence of low doses of aflatoxin B$_1$ (AFB$_1$) and partial hepatectomy (PH) on glutathione-S-transferase (GST) activity was studied in the plasma and liver of the rat. Methods. The animals were divided into four groups. The first (I) and the second (II) group were treated with AFB$_1$ freshly dissolved in dimethyl sulphoxide (DMSO), and administered as a single intraperitoneal dose of 50 µg/rat 24 hrs after the rats had undergone either sham operation or, 40% PH, respectively. The third group (III) of animals was treated with a total dose of 1 mg AFB$_1$ – 5 days per week during a period of 8 weeks. The non-treated animals were used as controls (C). Results. We observed a significant increase of GST activity in the plasma of all experimental groups compared to the controls (C), (p<0.02 to p<0.005). In the liver, the GST activity of all experimental groups was also significantly increased, compared to the controls (from p<0.02 to p<0.005). Conclusion. The administration of both single and multiple doses of AFB$_1$ led to long term increase of GST activity in the rat plasma and liver, and partial hepatectomy had no significant effect on this phenomenon.

Key words: aflatoxins; carcinogens; glutathione transferase; liver; plasma; rats.

Introduction

Aflatoxins are widely distributed in the nature and the most of these closely related mycotoxins is aflatoxin B$_1$ (AFB$_1$), which is a metabolite of certain Aspergillus species (A. flavus and A. parasiticus). These are widespread fungi, which can contaminate animal and human food (1). It is known that AFB$_1$ is hepatotoxic and hepatocarcinogenic in many animal species, including rats (2, 3), and also humans (4). The liver is an important target of the toxicity of drugs, xenobiotics and oxidative stress (5). Many chemical compounds are modified by liver extracts to yield active compounds (among these are epoxides), and their actions are usually directed to macromolecules such as DNA (6, 7). In order to exert its biological effects (acute toxicity, teratogenicity, mutagenicity and carcinogenicity), AFB$_1$ must be converted to its reactive epoxide (Figure 1) by cytochrome P450 enzyme system of liver and several other tissues of different animals (8, 9). The epoxide is highly reactive, and can form derivatives with several cellular macromolecules, including DNA, RNA, and proteins. It is believed that covalent interaction of epoxide with DNA is responsible for the initiation of carcinogenesis followed by the subsequent step of promotion and progression, and that it leads to cancer formation (7).

Hepatic antioxidants represent the major defence against toxic liver injury, and they act as anti-apoptotic agents (10).

With respect to the capacity for AFB$_1$ oxidation, striking differences exist among microsomes prepared from dif-
different species. Human liver microsomes are approximately one-fourth as efficient in activating \( \text{AFB}_1 \) as rat microsomes. For many years it has been perceived that the mouse and rat, though closely related, respond in a completely different way to hepatocarcinogenic effects of aflatoxin \( \text{B}_1 \). (11). Mouse microsomes have higher specific activity for \( \text{AFB}_1 \)-8,9-epoxide (AFBO) production than rat microsomes, but are resistant to the hepatocarcinogenic effects of \( \text{AFB}_1 \) because of the efficient conjugation of AFBO with glutathione (GSH) (12, 13). Glutathione-S-transferase (GST) is involved in detoxification of epoxides through conjugation between GSH and \( \text{AFB}_1 \), (14). GST is found in cytosol of cells in vertebrates, plants, insects, nematodes, yeasts, and aerobic bacteria. In mammalian organisms GST is present in all tissues, but the highest activity of this enzyme was found in the liver, representing up to 10% of total protein amount.

GST activity might be a key factor in determining individual or species susceptibility to \( \text{AFB}_1 \), and the major route of detoxification of \( \text{AFB}_1 \) via conjugation of AFBO with GSH is in rodents, noticeably.

Satoh et al. showed that hepatectomy enhanced tumor development in rats given chemical carcinogenes (15).

The aim of this study was to investigate the influence of low doses of \( \text{AFB}_1 \) and partial hepatectomy (PH) on GST activity in the plasma and liver of the rats.

**Methods**

Male rats of the inbred Albino Oxford (AO) strain (4–6 weeks old) were used in the experiment. Animals were kept in wire-bottomed cages under the standardized conditions of humidity, light and temperature at the Institute for Medical Research of the Military Medical Academy. Food and water were given *ad libitum*.

The animals were divided into four groups. The first (I) and the second (II) group were treated with \( \text{AFB}_1 \) freshly dissolved in dimethyl sulfoxide (DMSO), administered as a single intraperitoneal dose of 50 \( \mu \)g/rat 24 hrs after the rats underwent a sham operation, or 40% PH, respectively. The third group (III) of rats was treated with a total dose of 1 mg \( \text{AFB}_1 \) -5 days per week during 8 weeks. Non treated animals were used as the controls (C).

Partial hepatectomy was performed according to the technique of Higgins and Anderson (16). The animals were sacrificed 13–15 months after the administration of \( \text{AFB}_1 \). The livers were excissed within 3 minutes and prepared for further analysis. All chemicals were the products of Sigma (St. Louis, MO, USA). Fresh blood was immediately collected using heparin (1000 U/ml) as anticoagulant. Aliquots of blood were taken immediately after exsanguinations and centrifuged for the separation of plasma. For the determination of GST activity in the plasma and liver 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate (17). Protein content was determined by the method of Lowry *et al*, using bovine serum albumin as a referent value (18).

The obtained data were statistically analyzed and expressed as mean values \( \pm SE \), and differences between the experimental and the control group were estimated by Student’s *t*-test (19). The value of *p*<0.05 was considered statistically significant.

**Results**

In our experiments we observed a significant increase of GST activity in the plasma of all three experimental groups compared to controls, as presented in Figure 2.

**Fig. 1** – Conversion of aflatoxin \( \text{B}_1 \) to aflatoxin \( \text{B}_1 \) - 8,9 epoxide in the liver

![Aflatoxin B<sub>1</sub> to Aflatoxin B<sub>1</sub> - 8,9 Epoxide](image-url)

<table>
<thead>
<tr>
<th>Activity</th>
<th>C</th>
<th>I</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>nmol GSH/min/ml plasma</td>
<td>(5)</td>
<td>(4)</td>
<td>(6)</td>
<td>(7)</td>
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**Fig. 2** – Changes of glutathione-S-transferase (GST) activity in the plasma of rats sacrificed 13–15 months after \( \text{AFB}_1 \) treatment. AFB<sub>1</sub> was administered intraperitoneally to rats in the single dose of 50 \( \mu \)g/rat 24 hrs after the rats underwent a sham operation (I) or 40% partial hepatectomy (II) and in multiple doses 5 days/week (total doses of 1 mg \( \text{AFB}_1 \) in 8 weeks) (III). Nontreated animals represented controls (C). The number of animals is given in parentheses.

The sham operated rats 24 hrs before the treatment with \( \text{AFB}_1 \) showed lower than 2-fold increase in GST activity, compared to the control value (p<0.02). The most significant increase (near 2-fold, p<0.005) was in the group of rats II which underwent hepatectomy 24 hrs before the single treatment with \( \text{AFB}_1 \). The highest increase in GST activity in plasma was detected in the group of rats III, which were treated for two months with 1 mg of \( \text{AFB}_1 \) as a total dose (2.5-fold, p<0.01).

Changes of GST activity in the liver of the rats treated by \( \text{AFB}_1 \) are presented in Figure 3A and 3B. A significant...
increase (p<0.01) of specific activity of GST from group II was found in comparison to the control animals (Figure 3A). When the activity of GST was expressed in gram of the wet mass of tissue (Figure 3B) the values were more significant in all three experimental groups of animals in respect to the control rats (p<0.01 and p<0.005).

**Discussion**

Different studies in rats have demonstrated the potent toxic and carcinogenic effects of aflatoxins (2). Despite the differences, (different sources of aflatoxin - food contaminated with AFB; or purified AFB; different routes of administration, different periods of administration and observation, different basic diets and different strains of rats), all of these studies found that aflatoxin was a very potent hepatic carcinogen in rats fed for the period of 20 weeks, or longer. A few studies found that even single, relatively high doses were capable of producing hepatocellular carcinoma (12). The selected doses of AFB; that were used in our study were potentially carcinogenic (20, 21). Sublethal doses of aflatoxins led to chronic toxicity that caused carcinogenic changes. Lethal dose of AFB; varied and depended on strain difference, animal's age and other factors (22). Many of the earlier studies showed that multiple doses of AFB; led to the development of liver tumors in rats between the 1st and the 2nd year after poisoning (23). Other studies showed that PH combined with AFB; enhanced tumor development (20).

An important detoxification pathway in animals – the conjugation of AFB; to GSH (mediated by GST) and its subsequent excretion is related to AFB; toxicity resistance (24). Differences in AFB; susceptibility of various species depended on their hepatic cytosol capability to inactivate AFBO by conjugation with GSH (25, 26). Mice occurred highly resistant to the hepatocarcinogenic effects of aflatoxin (27). Although mouse microsomes produced relatively more AFBO than rat microsomes under similar incubation conditions, mice were more resistant to the hepatocarcinogenic effects of AFB; because of the efficient conjugation of AFBO with GSH (28). Liver cytosolic fractions from the mouse had 50- to 100-fold greater AFBO conjugating activity than those from the rat (12).

Individuals with high activity of oxidative enzymes and/or low activity of detoxifying enzymes might be at the increased risk for certain types of cancers. For AFB; cancer risk estimation, the rates of cytochrome P450 (CYP)-mediated AFB; activation, as well as GST-catalyzed AFBO -de toxification must be considered (29).

Stone et al. (28) analyzed hepatic cytosolic fractions prepared from 14 human donors for GST activity. Human liver cytosolic GST exhibited low activity towards AFBO (0.17·1.46 μmol/min/mg). Hepatic GST-AFBO activities of the rat, hamster and mouse were 70-, 465-, and 3545-fold greater, respectively, than the ones observed in human liver using microsomal-generated AFBO.

In our experiment, total hepatic GST activities towards AFBO measured one year after AFB; poisoning, were increased 10–40%, compared to the controls.

Earlier studies (15) with purified GST from the rat liver indicated different activity of certain class of this enzyme towards AFBO. In the direct contrast to a large number of other drug-metabohizing enzymes, GST-P (subunit of neutral GST purified from placenta) was found not to be inducible in the livers of rats by short-term administration of drugs or carcinogens such as phenobarbital, a butylated hydroxyanisole, N-2-fluorenylacacetamide, 3-methylcholanthrene, 3-methyl-4-dimethylaminobenzene. However, two chemical carcinogens diethylanisamine and N-2-fluorenylacacetamide, plus partial hecatopexy induced preneoplastic foci and hyperplastic nodules in rat liver and elevated the amounts of GST-P 30–50 times, compared to normal liver (15). On the basis of their results, Satoh et al. concluded that GST-P might be expected to have a crucial role in relation to the resistance mechanism or, more directly, to the growth of preneoplastic cells.

Liu et al. (30) found persistent expression of GST-P in all the livers of AFB; treated rats during three time intervals (1, 3, and 12 months) after AFB; poisoning. Since there were no liver tumors induced within one year after AFB;
treatment, these data suggested that all AFB1-treated animals were at the preneoplastic stage of hepatocarcinogenesis. The GST-P positive foci thought represented the populations of the "initiated" cells with altered gene expression (15). AFB1 induction resulted in GST-P overexpression, but not in the expression of p53 tumor-suppressor gene that played an important role in hepatocarcinogenesis (30). These results suggested that the p53 gene mutation might not have occurred at this early stage of AFB1 - induced hepatocarcinogenesis.

In our experiments neoplastic nodules and hepatocellular carcinoma were not detected in the group of rats II which underwent 40% PH 24 hrs before the single treatment with AFB1 (results not presented). It occurred, that the strain difference and the timing of AFB1 administration in relation to PH might have had a critical role in tumor development (31). The experiments in which rats underwent two thirds PH, and 24 hrs later received 0.25 mg/kg body weight of AFB1, showed a significantly higher incidence of hepatocellular carcinoma, compared to non-hepatectomized rats sacrificed between the 55th and the 65th week (20). Mortality during AFB1 administration was much lower when the rats underwent a one third hepatectomy (40%), than in the experiment in which more extensive hepatectomy was performed (70%) (31). In our experiments the mortality from hepatectomy was 70% in AFB1-treated rats.

In almost all the experimental systems, multiple dosing AFB1 was required for carcinogenicity (13). A single administration of AFB1 to the rats resulted in maximum liver AFB1-DNA adduct levels 2 hrs after poisoning. The rapid removal of these adducts (88% after 24 hrs) might be related to the requirement for multiple exposures to AFB1 for the induction of tumors in the Fischer rats (21).

The effect of chronic administration of AFB1 on GST (hepatic phase II metabolizing enzyme) was measured one year after poisoning in the group III of animals treated with total dose of 1 mg AFB1 - 5 days/week in the period of 8 weeks. GST activity was significantly increased after multiple doses of AFB1.

Guerre et al. (32) reported a significant decrease in cytosolic GST in a rabbits sacrificed 24 hrs after the last oral administration (0.10 mg/kg AFB1 for 5 days). In rats GST activity measured with CDNB as a substrate occurred unaffected by such a treatment, suggesting differences in the rat GST sensitivity to AFB1 compared to the rabbit (33).

A variety of dietary factors have been shown to influence the carcinogenicity of aflatoxin. Wang et al. (34) have found that crocetin (a natural carotenoid) enhanced GST activity in rat liver and protected against the AFB1 hepatotoxicity. The incidence of liver lesions in male Wistar rats treated with AFB1 (total dose of 1.125 mg/rat) and crocetin was significantly reduced (for 40%) in respect to the animals treated with AFB1 alone.

Conclusion

Consistent with the obtained data we concluded that the administration of both single and multiple doses of AFB1 led to a long time increase of GST activity in the rat plasma and liver, and that partial hepatectomy had no significant effect on this phenomenon.

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Apstrakt

PROMENE U AKTIVNOSTI GLUTATION-S-TRANSFERAZE U PLAZMI I JETRI PACOVA INDUKOVANE AFLATOKSINOM B1

Uvod. Ispitivan je uticaj niskih doza aflatoksina B1 (AFB1) i parcijalne hepatektomije (PH) na aktivnost glutation-S-transferaze (GST) u plazmi i jetri pacova.

Metode. Životinje su podeljene u četiri grupe. Prva (I) i druga (II) grupa su tretirane AFB1 prethodno sveže rastvaranim u dimetilsulfoksidu (DMSO) i davanim u jednoj intraperitonealnoj dozi od 50 μg po pacovu (24 časa posle izvršene lažne operacije ili 40% PH). Treća grupa (III) bila je tretirana ukupnom dozom od 1 mg AFB1, pet puta nedeljno tokom osam nedelja. Životinje koje nisu tretirane predstavljale su kontrolnu grupu (C).

Rezultati. Značajno povećane GST aktivnosti (p<0,02 – p<0,005) u poređenju sa kontrolnom grupom, utvrđeno je i u plazmi i u jetri svih ispitivanih grupa pacova. Zaključak. Dobijeni rezultati pokazuju da jednokratna, kao i višekratna primena niskih doza AFB1 dovodi do dugotrajne indukcije aktivnosti GST u plazmi i jetri pacova, te da parcijalna hepatektomija nema veći uticaj na ovaj fenomen.

Ključne reči: aflatoksin; karcinogeni; glutation transferaze; jetra; plazma; pacovi.

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