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Effect of a single subtoxic dose of aflatoxin B_1 (AFB_1) on glucose-6-phosphate dehydrogenase in mouse liver

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A large body of data links aflatoxin B_1 (AFB_1*) to hepatocarcinogenicity and non-specific degeneration of the liver [1–4]. The toxic effects, however, have only been observed with high levels of AFB_1 that are not normally attained in food. *In vitro* studies have indicated that, in human liver, conversion of AFB_1 to several monohydroxylated aflatoxins B_1 by NADPH-dependent microsomal enzymes represents a major detoxification process [5, 6]. Alternatively, AFB_1 may be activated to form highly reactive 8,9-epoxy AFB_1, a metabolite thought to be the ultimate toxic agent, which in turn may be converted to either 8,9-dihydroxy AFB_1, another probable toxic metabolite, or to glutathione–AFB_1 conjugate, whose formation is believed to be another major detoxification process [7]. Glutathione reductase, a widely distributed enzyme which catalyzes the conversion of oxidized glutathione to reduced glutathione (GSH) at the expense of NADPH, maintains the intracellular concentrations of GSH [8]. Hence, it is conceivable that the rate of detoxification reactions depends on the availability of NADPH, most of which is derived from the hexose monophosphate shunt pathway. We have therefore explored the possibility of induction of the rate-limiting enzyme glucose-6-phosphate dehydrogenase by a subtoxic level of AFB_1.

Materials and methods

Chemicals. AFB_1, mercaptoethanol, glucose-6-phosphate, NADP, $MgCl_2$, Tris–HCl, KH_2PO_4 and EDTA were purchased from the Sigma Chemical Co., St. Louis, MO, U.S.A. The AFB_1 was dissolved in dimethyl sulfoxide (DMSO) (Fisher Scientific Chemical Co., Fair Lawn, NJ, U.S.A.) (5.56 mg/ml).

Treatment of animals. Male CFW Swiss mice (6–8 weeks) were used. Experimental mice were given a single intraperitoneal injection of AFB_1 (3.0 mg AFB_1/kg body weight in 0.02 ml DMSO). Control mice were injected with either 0.02 ml DMSO or phosphate-buffered saline (PBS), pH 7.4. All animals were fed normal mouse chow with free access to water. Body weights of the animals were recorded daily. At days 0, 7, 14, and 21, mice from each group were killed by cervical dislocation, and the liver glucose-6-phosphate dehydrogenate (G6PD) was partially purified.

Partial purification of liver G6PD. The liver from each mouse was homogenized in ice-cold KH_2PO_4–1.0 mM EDTA–5 mM mercaptoethanol–5% glycerol buffer (pH 7.4) (3 ml/g liver tissue) with a Potter–Elvehjem homogenizer for 60 sec. The homogenate was centrifuged in the cold at 400 *g* for 10 min. The supernatant fraction was recentrifuged at 10,000 *g* for 20 min, and aliquots of the resulting crude supernatant fraction (S-10) were analyzed for G6PD activity as described in the legend for Table 1. Pooled fractions of the supernatant fraction (S-10) were used for the partial purification of G6PD as previously described [9]. Briefly, the pooled S-10 fraction was filtered through several layers of cheesecloth. To the filtrate $(NH_4)_2SO_4$ (50% saturation) was added dropwise while stirring at 0–4°. The mixture was centrifuged at 5000 *g* for 10 min, and the precipitate was redissolved in the homogenizing buffer. The precipitation step was repeated and dialyzed against the homogenizing buffer minus EDTA for 17–20 hr in the cold. The partially purified G6PD was kept at −20° until use. Enzyme activities were determined as previously described [10], and protein was measured by a modification of the Lowry method [11].

Results and discussion

To test the possibility that a subtoxic dose of AFB_1 induces the synthesis of glucose-6-phosphate dehydrogenase (G6PD) in the mouse liver, 3.0 mg/kg AFB_1 was injected intraperitoneally (LD_{50} i.p. in adult mouse: 60 mg/kg). The G6PD was prepared from the liver, and the enzyme activities were measured both in the crude fraction and the partially purified fraction. As shown in Table 1, in the PBS control and DMSO-treated mice, the mean G6PD value in the crude fraction on day 7 were 0.46 ± 0.07 and 0.44 ± 0.15 nmol/mg protein respectively. The corresponding value in the mice injected with AFB_1 was 1.26 ± 0.44 nmol/mg. Thus, the intraperitoneal injection of AFB_1 significantly stimulated ($P \leq 0.05$) the G6PD. A subsequent decline in the G6PD levels may have coincided with metabolic inactivation of AFB_1. The results with the partially purified G6PD also indicate the stimulatory effect of AFB_1 although the increase was about twice that of the PBS control. With exception of the samples derived from the DMSO-treated mice, there was noted a substantial loss of the enzyme activities, particularly in the 7-day sample from the AFB_1-treated animal. A possible explanation for the loss of the enzyme activity is that the G6PD isolated from the animal sources including mouse liver is labile [12]. Attempts to minimize the loss of the enzyme activity by addition of 5% glycerol/5 mM mercaptoethanol/1 mM EDTA in the assay solution, however, were unsuccessful.

* Abbreviations: AFB_1, aflatoxin B_1; Tris, Tris(hydroxymethyl)methylamine; GSH, reduced glutathione; DMSO, dimethyl sulfoxide; and NADPH, reduced nicotinamide adenine dinucleotide phosphate.

Table 1. Effect of a single subtoxic dose of AFB_1 on G6PD

Treatment	G6PD activities* (nmoles/mg)							
	Crude (S-10) fraction				Partially purified†			
	Day 0	Day 7	Day 14	Day 21	Day 0	Day 7	Day 14	Day 21
PBS	0.45 ± 0.11 (2)	0.46 ± 0.07 (2)	0.45 ± 0.04 (2)	0.40 ± 0.09 (2)	0.32	0.35	0.37	0.30
DMSO		0.44 ± 0.15 (2)	0.50 ± 0.09 (2)	ND‡		0.49	0.52	ND
AFB_1		1.26 ± 0.44§ (3)	0.71 ± 0.18§ (3)	0.54 ± 0.09 (3)		0.76	0.65	0.42

* The assay mixture consisted of 2.5 ml of 50 mM Tris–HCl, pH 7.4, 0.2 ml of 0.1 M $MgCl_2$, 0.1 ml of 10 mg/ml glucose-6-phosphate, 0.01 ml of 10 mg/ml NADP, and 0.01 ml of enzyme preparation. At the end of a 30-min incubation at ambient temperature, absorbance changes were measured at 340 nm. Values are means ± standard errors of three separate determinations performed in duplicate. The number of animals in each group is given in parentheses.

† G6PD was partially purified from the pooled crude fractions.

‡ ND, not determined.

§ Signicantly different from the PBS control by analysis of variance ($P \leq 0.05$).

Nevertheless, the AFB_1 effect on the induction of G6PD is quite apparent in both cases. It should also be mentioned that there were no differences between the group with respect to body weight and physical appearance. Further, the AFB_1-treated animals showed no overt sign of lesion or tumorous changes of the liver.

Maintenance of GSH levels in red blood cells largely depends on the availability of NADPH [13], and it is possible that a similar metabolic relationship prevails in the hepatocytes. GSH has been shown to be important not only in the detoxification of many electrophilic metabolites including epoxy AFB_1 [14] but also in the protection of tissue macromolecules, e.g. peroxidation of membrane lipids by superoxide anion. NADPH thus participates directly or via maintenance of GSH level in the detoxification of AFB_1.

While many of the studies on the metabolism of a number of carcinogens including AFB_1 and benzopyrene have focused on the induction of mixed-function oxidases as pivotal enzymes, based on the present observation, together with the documented role of GSH, we suggest that an induction of G6PD occurs as an overriding event in the inactivation of AFB_1. Phenobarbital, for instance, has been shown to lower the plasma level of AFB_1 [15] and is found to be related to induction of mixed-function oxidases, but the nonspecific induction of G6PD by phenobarbital may be the primary response causing lowering of the AFB_1 level.

Further studies are necessary to ascertain the role of AFB_1 in the induction of G6PD under the conditions of repeated administration of, and various amounts of, AFB_1.

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