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INCIDENCE OF TWO ORAL MYCOTOXICOSIS ON LIVER DRUG METABOLIZING ACTIVITIES IN THE RAT

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Aflatoxin B1 has been already described to induce change in hepatic drug metabolizing enzymes of rats receiving intraperitoneal administrations of the mycotoxins (1, 2). In rats given high oral doses of ochratoxin A, the hepatic alteration consisted of focal periportal areas of hepatic necrosis with the pyknosis and karyorrhexis of nuclei (3).

The present paper deals with the influence of ochratoxicosis and its comparison with aflatoxicosis on liver drug metabolizing enzymes and also characterizes the time required for enzymatic level disturbances in the course of these two oral toxicosis.

MATERIALS AND METHODS

Experiments have been conducted on Sprague Dawley male rats weighing 200-240 g. Animals were randomly distributed into control and treated groups of 8 rats. In a first study, a 1 mg/kg dose of aflatoxin B1 (Sigma) was administered each morning for 8 days by oesophagal intubation in a gummy solution (5 %). In a second study, rats received a daily 1.5 mg/kg oral dose of ochratoxin dissolved in an isotonic solution of sodium bicarbonate. 10 animals (2 control and 8 treated rats) were sacrificed after 1, 2, 4 and 8 dosages of aflatoxin B1 and after 4, 8, 11 and 15 administrations of ochratoxin A. After blood puncture through the abdominal aorta, the liver was removed immediately and used for the preparation of microsomes and cytosol.

The plasma glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and microsomal and cytosolic protein contents were determined (4). The hepatic content of cytochrome P450 and b5 was measured using the method of Omura and Sato (5). The activity of NADPH-cytochrome c reductase, aniline hydroxylase and aminopyrine demethylase microsomal activities were determined as previously described (6). UDP-glucuronosyl transferase and ethoxycoumarin O-deethylase were determined by using the method of Frei (7) and Aitio (8). Cytosolic glutathione transferase and acetyltransferase were measured as previously described (9, 10).

RESULTS AND DISCUSSION

8 days of aflatoxin B1 treatment caused a significant 12 % decrease in liver/body weight ratio. One, 2, 4 and 8 dosings led to significant increases in plasma levels of both GOT (210 to 535 %, respectively) and GPT (124 to 286 %).

No influence of aflatoxicosis on microsomal proteins, cytochrome b5 contents, aniline hydroxylase and glucuronosyl transferase activities was detected. Significant decreases in cytochrome P450, aminopyrine demethylase, ethoxycoumarin deethylase activities were obtained from 2 to 8 daily mycotoxin treatment whereas NADPH-cytochrome c reductase was increased in the same animals (table 1). Concerning cytosolic data, the proteins, the acetyl- and glutathione transferases appeared unchanged whatever the duration of intoxication was.

Chronic oral administration of aflatoxin B1 led to some different results in comparison with data already obtained following intraperitonal administration of the toxin, however decreases in cytochrome P450 contents or in aminopyrine demethylase activities are in agreement with data reported by other workers using parenteral administration (1, 2).

TABLE 1. Significant effects of aflatoxin B1 (1 mg/kg/day) and ochratoxin A (1.5 mg/kg/day) treatment on in vitro hepatic parameters in rats.

	Treatment period (days)				
Oral aflatoxicosis	Control	1	2	4	8
Cytochrome P450 (nmol/mg)	0.229 ± 0.030	0.238 ± 0.048	0.189 ± 0.020*	0.143 ± 0.041*	0.412 ± 0.023*
NADPH-cytochrome c reductase (nmol/mg x min)	37.3 ± 5.3	35.4 ± 4.4	36.3 ± 4.8	33.8 ± 5.9	51.7 ± 4.4*
Aminopyrine N-demethylase (nmol/mg x min)	0.33 ± 0.05	0.33 ± 0.05	0.26 ± 0.04*	0.03 ± 0.03*	0.06 ± 0.06*
Ethoxycoumarin O-deethylase (nmol/mg x min)	0.081 ± 0.025	0.102 ± 0.025	0.109 ± 0.015	0.061 ± 0.020	0.044 ± 0.015*

Oral ochratoxicosis	Control	4	8	11	15
Microsomal proteins (mg/g)	25.3 ± 2.2	24.6 ± 3.0	20.1 ± 2.8*	20.5 ± 2.6*	17.3 ± 1.9*
Cytochrome P450 (nmol/mg)	0.224 ± 0.024	0.240 ± 0.012	0.186 ± 0.050	0.196 ± 0.038	0.165 ± 0.027*
Aminopyrine N-demethylase nmol/mg x min)	0.40 ± 0.08	0.37 ± 0.09	0.28 ± 0.08	0.30 ± 0.11	0.17 ± 0.10*
Aniline hydroxylase (nmol/mg x min)	1.94 ± 0.14	1.66 ± 0.17	1.79 ± 0.11	1.61 ± 0.14	1.12 ± 0.19*

Results corresponded to means ± S.E.M. (n = 8), *significantly different from controls ($P < 0.05$).

The treament of animals with ochratoxin A did not affect the liver to body weight ratio. Increases in the levels of plasma GOT were significant following 11 and 15 administrations of this mycotoxin.

In case of ochratoxicosis, ethoxycoumarin deethylase tended to decrease but this decrease was not statistically significant. As indicated in table 1, microsomal proteins were significantly lowered in rats receiving at least 8 daily administrations. On the other hand, significant decreases in cytochrome P450 level, aminopyrine demethylase and aniline hydroxylase were obtained following 15 daily oral doses of ochratoxin A demonstrating the inhibitory effect of the toxin on mixed function oxidases.

This study demonstrate the more severe hepatotoxicity provoked by aflatoxin B1, since a similar decrease in microsomal cytochrome P450 (20-30 %) was induced respectively by 2 and 22.5 mg/kg cumulative dose of aflatoxin B1 and ochratoxin A. In each case, however, cytochrome P450 was significantly decreased whereas phase II liver biotransformation enzymes appeared unchanged probably because of the periportal necrosis localization induced by the two mycotoxicosis.

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