Aflatoxin B_1 hepatotoxicity in rats pretreated with ethanol

T. Glinsukon, S. Taycharpipranai and C. Toskulkao

Department of Physiology, Faculty of Science, Mahidol University, Bangkok 4 (Thailand), 12 December 1977

Summary. Ethanol pretreatment has the potentiation of the aflatoxin B_1-induced hepatotoxicity was indicated by an increase in the activities of plasma GPT, plasma GOT and in the severity of liver necrosis. The effect of ethanol pretreatment on an increase in the accumulation of liver triglycerides is additive in nature.

Aflatoxin B_1 is a potent hepatocarcinogen in many animal species[1] and possible man[2]. It is produced by certain strains of *Aspergillus flavus*[1]. Recently, it was reported that peanuts were highly contaminated with *A.flavus* whereby the natural occurrence of aflatoxins was found[3,4]. In addition, peanuts are commonly used as a food by people in many parts of the world. In some places, peanuts are taken as a snack with beer and whisky. It is likely that the hepatotoxicity of aflatoxin B_1 contained in peanuts might be modified in persons who have been drinking alcohol. It is considered of interest to investigate whether the effects of ethanol are additive to the hepatotoxicity of aflatoxin B_1.

Methods. Adult female rats (150–200 g) of Fischer derived strain (Animal Production Center, Faculty of Science, Mahidol University, Bangkok 4) were maintained on commercial rat chow (Gold Coin Ltd, Singapore) and water ad libitum. The rats were pretreated with 4 oral doses of 1.0, 2.0 or 4.0 g/kg ethanol as a 50% solution in water at 48, 42, 24 and 18 h prior to i.p. administration of 1.0, 2.0 or 4.0 mg/kg aflatoxin B_1 (Makor Chemical Ltd, Israel) in 0.5 mg/kg dimethylsulfoxide. The rats were anesthetized with ether and bled in a heparinized syringe at 48 h after aflatoxin B_1 administration. Aliquots of the liver were frozen for analyses of triglycerides by a modified method of Fletcher[5]. Plasma activities of glutamic-pyruvic and glutamic-oxaloacetic transaminases were measured spectrometrically by a method of Reitman and Frankel[6]. In some experiments, sections of the liver were quickly removed and fixed in 10% buffered neutral formalin for histopathologic studies.

Results. Ethanol pretreatment significantly increased the aflatoxin B_1-induced hepatotoxicity at all doses of aflatoxin B_1 studied by 2–4 times in the activities of plasma GPT, plasma GOT and approximate 2 times in the levels of liver triglycerides (table). The potentiation was also observed in a similar manner by pretreatment the rats with lower doses of ethanol. In addition, the potentiation was evident when the liver was examined microscopically. The hepatic lesions revealed the periportal zone necrosis with mild fatty infiltration in the hepatocytes adjacent to the zone of necrosis in the rats treated with aflatoxin B_1 (4.0 mg/kg), whereas most severe necrosis and fatty infiltration was also observed in the rats pretreated with 4 oral doses of ethanol (4.0 g/kg) prior to aflatoxin B_1 (4.0 mg/kg) administration.

Discussion. During pretreatment of 4 oral doses of ethanol (4.0 g/kg), blood ethanol concentrations are similar to those reported in man during drinking, although the doses administered to these rats seem rather high[7]. Ethanol pretreatment produced a small but not significant increase in the activities of plasma GPT and plasma GOT. However, ethanol pretreatment prior to i.p. administration of aflatoxin B_1 produced a significant increase (approximate 4 times) in the activities of plasma GPT ($p < 0.05$) and plasma GOT ($p < 0.001$). These findings suggest that ethanol pretreatment potentiates the aflatoxin B_1-induced hepatotoxicity, and it is in contrast to additive effect on an increase in the accumulation of the liver triglycerides. The mechanisms responsible for the potentiation of aflatoxin B_1-induced hepatotoxicity by ethanol pretreatment are not yet known. It may be due to enhancement of the activities of certain drug-metabolizing enzymes by ethanol pretreatment[8,9] leading to an increase in the conversion of aflatoxin B_1 to active metabolite (2, 3-epoxide derivative)[10,11]. It is possible that binding of an increased active metabolite to the macromolecules of the hepatocytes may also be increased and it supposedly initiates the processes leading to hepatotoxicity. However, further research on this possible mechanism is under investigation in this laboratory.

The effect of pretreatment of various doses of ethanol on the activities of plasma glutamic-pyruvic and plasma glutamic-oxaloacetic transaminases and liver triglycerides measured 48 h after administration of various doses of aflatoxin B_1 in rats

Treatment[a] (mg/kg)	Pretreatment[b] (g/kg)	Enzyme activity (IU/ml)[c] PGPT	PGOT	Liver[c] triglycerides (mg/g liver)
Experiment A (4–8)				
DMSO 0.5	H_2O 4.0	25.3 ± 0.7	50.3 ± 3.2	5.4 ± 0.7
DMSO 0.5	EtOH 4.0	38.3 ± 4.2[h]	57.3 ± 4.6[h]	16.6 ± 0.9[g]
AFB_1 1.0	H_2O 4.0	42.3 ± 11.3	85.3 ± 7.5	7.1 ± 0.6
AFB_1 1.0	EtOH 4.0	78.5 ± 11.7[d]	105.6 ± 4.4[f]	16.8 ± 2.8[g]
AFB_1 2.0	H_2O 4.0	171.0 ± 40.8	240.4 ± 40.3	11.2 ± 1.4
AFB_1 2.0	EtOH 4.0	660.0 ± 229.1[e]	1190.0 ± 391.4[d]	25.9 ± 3.7[g]
AFB_1 4.0	H_2O 4.0	181.0 ± 56.3	274.4 ± 82.3	17.4 ± 1.8
AFB_1 4.0	EtOH 4.0	860.0 ± 293.9[d]	1240.0 ± 330.8[g]	34.7 ± 4.6[f]
Experiment B (4–6)				
AFB_1 4.0	H_2O 1.0	209.6 ± 130.4	342.4 ± 120.6	14.4 ± 1.2
AFB_1 4.0	EtOH 1.0	540.2 ± 94.6[d]	953.3 ± 115.7[e]	33.5 ± 5.9[g]
AFB_1 4.0	H_2O 2.0	288.0 ± 124.3	384.8 ± 102.1	15.6 ± 2.1
AFB_1 4.0	EtOH 2.0	710.3 ± 48.9[e]	1006.7 ± 213.7[e]	31.5 ± 6.7[g]

[a] All rats were killed 48 h after the i.p. administration of aflatoxin B_1 in 0.5 mg/kg dimethylsulfoxide (DMSO). Number of rats is given in parentheses. [b] 4 oral doses of ethanol (as a 50% solution in water) or water were given 48, 42, 24 and 18 h prior to i.p. administration of aflatoxin B_1. [c] Each value is the mean of ± SE. Significant difference was greater than corresponding group given water. [d] $p < 0.05$, [e] $p < 0.025$, [f] $p < 0.01$, [g] $p < 0.001$ and [h] not significant (Student's t-test).

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The influence of simultaneously administered mexamine on the distribution of cystamine-^{35}S

A. Stoklasová, J. Křížala and V. Chmelař

Department of Biochemistry and Department of Radiobiology, Faculty of Medicine, Charles University, Hradec Králové (Czechoslovakia), 2 December 1977

Summary. The ^{35}S-distribution after simultaneous administration of a mixture of cystamine-^{35}S and mexamine (20 mg/kg + 10 mg/kg) has changed as compared with the groups treated with cystamine-^{35}S only.

The increased and prolonged radioprotective effect of a mixture of aminodisulfides or aminothiols with indolalkylamines has been demonstrated by many authors[1-4]. Some investigators suppose that aminodisulfides or aminothiols protect the gastrointestinal tract against ionizing radiation, whereas indolalkylamines protect bone marrow[1,2]. The distribution of cystamine in the organism of rats and mice was studied several times while ^{35}S-labelled cystamine was used. The irregular distribution of cystamine has been confirmed, the highest level being found in the radiosensitive tissues and kidney[5-7]. Only a few reports have been published concerning the influence of mexamine (5-MOT) on the distribution of cystamine under the simultaneous administration of these 2 agents in mice. Titov and Mordukhovitch[8] found an increased ^{35}S-activity in the small intestine, lungs, liver and brain and a fall in the activity in the kidney and muscles of mice treated with a mixture of cystamine-^{35}S and 5-MOT (comparing with the group treated with cystamine-^{35}S only). In rats, the concentrations of nonprotein SH-groups were followed after the administration of cystamine and 5-MOT, but ^{35}S-distribution has not been studied under these conditions[9]. The aim of our work was to ascertain, if mexamine influenced the ^{35}S-distribution in rats treated with an optimal protective mixture of cystamine-^{35}S and 5-MOT during the period of maximal radioprotective effect (1st h after administration of radioprotective agents).

Material and methods. Adult Wistar rats (males, weight 200 g) were used for the experiments. They were fed standard Larsen diet and water ad libitum. 1 group of rats was treated with ^{35}S-labelled cystamine × 2 HCl (20 mg/kg of b.wt referred to the base content) by an i.p. injection. The 2nd group was treated with a mixture of cystamine-^{35}S × 2 HCl and 5-methoxytryptamine × HCl (20 mg/kg + 10 mg/kg referred to the base content). These amounts were dissolved in an isotonic NaCl solution in such a dilution as to give 0.2 ml of the solution per 20 g b.wt. The total radioactivity administered per rat was 70 μCi. Cystamine-^{35}S × 2 HCl was synthesized by Dr. Kozák from the Biophysical Institute in Prague. 5-Methoxytryptamine (mexamine) was obtained from Koch-Light, Ltd. The number of rats in the experimental groups varied between 6 and 9. The rats were sacrificed by decapitation 10, 20, 30 and 60 min after the administration of radioprotective agents. The determinations were carried out in 10% homogenates of the liver, spleen, kidney, small intestine, bone marrow and in the blood. The blood was collected into the tubes with heparine. The bone marrow was obtained from the femur and tibia. A 10 cm section next to the pylorus was dissected from the small intestine. For experiments, 0.5 ml of the homogenates or 0.1 ml of the blood were used. The samples were solubilized in 1 ml of hyamine (Koch-Light, Ltd) and decoloured with H_2O_2, if necessary. After being solubilized, the samples were neutralized with HCl and 10 ml of Bray's

The concentrations of labelled sulfur in various tissues of rats after the administration of cystamine-^{35}S or a mixture of cystamine-^{35}S with 5-methoxytryptamine (5-MOT) expressed in percent of activity administered

Tissue	10 min		20 min		30 min		60 min	
	Cystamine	Cystamine + 5-MOT	Cystamine	Cystamine + 5-MOT	Cystamine	Cystamine + 5-MOT	Cystamine	Cystamine + 5-MOT
Liver	12.60 ± 1.30	12.20 ± 1.41	10.54 ± 0.81	12.33* ± 0.74	10.76 ± 0.88	12.71* ± 1.42	10.29 ± 1.25	9.67 ± 1.61
Spleen	0.99 ± 0.23	1.10 ± 0.29	0.62 ± 0.04	0.77 ± 0.19	0.72 ± 0.05	0.75 ± 0.13	0.53 ± 0.06	0.48 ± 0.07
Kidney	3.08 ± 0.50	1.89* ± 0.38	2.54 ± 0.27	2.08* ± 0.44	2.16 ± 0.25	2.40 ± 0.22	1.61 ± 0.30	2.04 ± 0.42
Small intestine	3.73 ± 0.71	3.75 ± 0.39	2.19 ± 0.26	3.57* ± 0.63	3.30 ± 0.39	3.32 ± 0.46	3.05 ± 0.41	3.48 ± 0.69
Bone marrow	0.42 ± 0.09	0.12* ± 0.03	0.58 ± 0.06	0.37* ± 0.06	0.56 ± 0.04	0.58 ± 0.07	0.47 ± 0.05	0.55* ± 0.05
Blood	0.28 ± 0.05	0.30 ± 0.02	0.28 ± 0.04	0.39* ± 0.04	0.27 ± 0.05	0.37* ± 0.03	0.23 ± 0.06	0.31 ± 0.08

* Significant difference ($p < 0.05$).