

MODIFICATION OF AFLATOXIN B₁-INDUCED CHANGES IN CERTAIN MITOCHONDRIAL ENZYMES AND LIPIDS BY MEDROXYPROGESTERONE ACETATE

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Summary : The effect of a single dose of 5 mg/kg body weight of aflatoxin B₁ on rat liver mitochondrial enzymes, succinate dehydrogenase (SDH) and Mg⁺⁺ adenosine triphosphatase (Mg⁺⁺-ATPase) and on certain lipids were studied at various intervals of time from 3 to 24 hours. A significant decrease in the specific activity of SDH was observed after 6, 12, 18 and 24 hr treatment. The Mg⁺⁺-ATPase activity remained unaffected up to 12 hr but appreciably decreased after 18 and 24 hr of the treatment. The level of phospholipids and cholesterol were not altered after 3, 6 and 12 hr treatment, thereafter (18 and 24 hr) an increase was observed in both the lipids following the aflatoxin treatment. Medroxyprogesterone acetate (MPA) did not cause any alteration in the specific activities of these enzymes as well as levels of cholesterol and phospholipids. The treatment with MPA caused significant increase in contents of cytochromes P-450, E₅ and activities of Aryhydrocarbon hydroxylase (AHH), UDP-glucuronyl transferase (UDP-GT) and NADPH-cytochrome C-reductase of hepatic microsomes.

It was observed that pretreatment with medroxy-progesterone acetate (MPA) could significantly minimize the depression caused in mitochondrial SDH and Mg⁺⁺-ATPase activities by aflatoxin B₁.

Key words : liver aflatoxin toxicity medroxyprogesterone acetate
succinate dehydrogenase ATPase

INTRODUCTION

Aflatoxins, which are hepatocarcinogens produced by *Aspergillus*, have been shown to be metabolised by mammalian hepatic mixed function oxidase (MFO) system to a number of oxidized products which are less carcinogenic and less toxic than the parent compound (3).

Aflatoxin B₁ (AFB₁) has been shown to exert disruptive effect on cellular membrane by altering their structural integrity and to elicit toxic effects in mitochondria (24). It has also been reported that AFB₁ caused decrease in mitochondrial enzymes (11).

Medroxyprogesterone acetate (MPA), a synthetic long acting contraceptive is also known to induce hepatic drug metabolizing enzymes including MFO system (22) and thus the drug has got the potentiality to promote the metabolism of xenobiotics. Therefore, there is a strong possibility that MPA may alter the biochemical effects of xenobiotics.

The present study was carried out with a view to find out the time at which maximum toxic effects are exhibited by AFB₁ on mitochondrial enzymes and lipids. Further experiments were planned to investigate the extent of modification of the toxicity of aflatoxin B₁ on these parameters by MPA.

MATERIAL AND METHODS

Female albino rats of Wistar strain weighing 100-125 g were used. The animals were fed *ad libitum* pellet diet (Hindustan Lever) and had free access to water. For examining the effects of Aflatoxin B₁ on mitochondrial enzymes, the animals were divided into two groups : (I) Control (II) Experimental. Each group consist of 30 rats.

The control group received the vehicle (0.1 ml) dimethyl sulfoxide (DMSO) only and experimental were administered intraperitoneally a single dose of AFB₁ 5 mg/kg, body weight. The animals were sacrificed at various intervals of time 3, 6, 12, 18, and 24 hr after administration of aflatoxin B₁. A batch of 5 animals were killed in each time interval under light anaesthetic ether. The liver was removed quickly, blotted and weighed. 10% homogenate of this tissue was prepared in cold 0.25 M sucrose and mitochondria was prepared according to the method of Johnson and Lardy (12), using MSE refrigerated centrifuge.

In order to find out the effect of pretreatment of MPA on the toxicity of Aflatoxin B₁ on mitochondrial enzymes and lipids, female rats (100-125 g) were divided into two groups : (I) Vehicle treated, (II) MPA treated. Each group consisted of 40 rats. The animals of group (I) were administered 0.1 ml of vehicle only (polysorbate-80, 0.237 mg; methylparaben, 0.134 mg; propylparaben, 0.0147 mg; polyethylene glycol 4000, 2.852 mg and sodium chloride 0.8567 mg per 0.1 ml) every week at 0, 7, 14 and 21 days while group (II) received MPA i/m 3.5 mg/100 body weight for the same period. On 22nd day, the animals of group I and II were further divided : (a) Vehicle treated (b) Vehicle treated + DMSO, (c) Vehicle treated + aflatoxin (d) MPA treated, (e) MPA treated DMSO (f) MPA treated + Aflatoxin B₁. Animals belonging to b and e groups were injected 0.1 ml of DMSO and c and f were administered intraperitoneally 5 mg/kg body weight of aflatoxin B₁. The animals were sacrificed at 24 hrs after various treatments. Mitochondrial preparations of the liver were prepared by Johnson and Lardy method (12). Succinic dehydrogenase (SDH) activity was determined by the method of Ernest and Abood (5)

and Mg^{++} -Adenosinetriphosphatase (Mg^{++} -ATPase) was assayed by the method of Quigley and Gotterer (19). Mitochondrial protein was estimated by the procedure of Gornall *et al.* (7).

The mitochondrial lipids were extracted according to the method of Folch *et al.* (6). The phospholipid phosphorus was estimated by Bartlett's method (1) and cholesterol by the method of Zak (25). Hepatic drug-metabolizing enzymes were determined in MPA treated group only and for this purpose, liver was perfused *in situ* through portal vein with 30-40 ml cold 0.15M NaCl. The tissue was homogenised in KCl-Tris-buffer, pH 7.4 (150 mM KCl/50 Tris-HCl) and diluted to concentration of 1 g wet weight/4 ml with KCl/Tris-buffer. A small portion of the homogenate was kept for glutathione estimation and rest was centrifuged in (Sorvall RC-5B) at 10,000 x g and then at 105,000 x g for 60 min in Beckman ultracentrifuge Model L5-50B.

Glutathione concentration was determined according to the method of Moron *et al.* (16) and activity of glutathione-S-transferase assayed following the procedure of Habig *et al.* (9). The contents of Cytochrome P-450 and b_5 were determined by the method of Omura and Sato (18). The activities of NADPH-cytochrome C-reductase, AHH and UDP-glucuronyl transferase were determined by the methods of Mazel (15), Nebert Gelboin (17) and Gorski and Kasper (8) respectively. Student's t-test was applied for the statistical analysis of data.

Chemicals : Aflatoxin B_1 and chemicals of vehicle were purchased from Sigma Chemical Company, USA and MPA was the generous gift from Upjohn Co., Belgium.

RESULTS

Time dependent effect of Aflatoxin : The effect of a single dose of Aflatoxin on the activities of liver mitochondrial SDH, Mg^{++} -ATPase and levels of phospholipids and cholesterol was studied at different intervals of time to find out the optimum time for maximum effect (Fig. 1). Maximum decrease in SDH and Mg^{++} -ATPase enzyme activity was observed at 2nd hr time intervals. Similarly the membrane lipid were altered after 18 hrs but maximum effect was observed at 24 hr. Thus, 24 hr period was chosen for further studies.

Microsomal enzyme induction and AFB toxicity : The effect of MPA on mitochondrial enzymes and lipids is presented in Table I. The specific activities of SDH and Mg^{++} -ATPase and contents of phospholipid and cholesterol content remained unaffected. A significant increase in hepatic MFO enzyme system namely cytochromes P-450, b_5 , Aryl-hydrocarbon hydroxylase, NADPH-Cytochrome C-reductase was observed in MPA treated rats. The activity of UDP-GT was also significantly augmented (Table II).

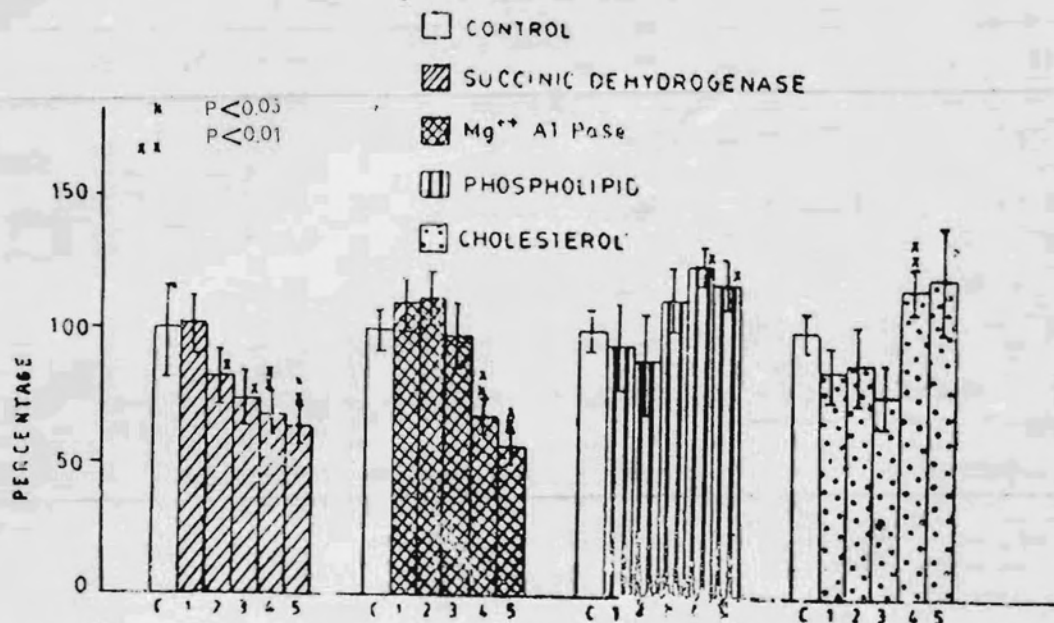


Fig. 1 : Effect of Aflatoxin B₁ on liver mitochondrial SDH and Mg⁺⁺-ATPase activities and levels of phospholipids and cholesterol. 1, 2, 3, 4, 5 represent 3, 6, 12, 18, 24 time interval in hrs at which the animals were sacrificed after administration of Aflatoxin B₁. Values are expressed as percentage taking control as 100. Each value is a mean \pm S.D. of five individual mitochondrial preparations.

TABLE I : Effect of Aflatoxin B₁ on rat liver mitochondrial SDH, Mg⁺⁺-ATPase, total cholesterol and phospholipid in rats pretreated with MPA.

Treatment	Protein (mg/g of liver)	SDH (Sp. activity)	Mg ⁺⁺ -ATPase (Sp. activity)	Cholesterol ^Z	Phospholipid ^Z
(a) Vehicle	18.5 \pm 2.5	48.5 \pm 4.2	0.205 \pm 0.020	0.280 \pm 0.014	1.1 \pm 0.32
(b) Vehicle+DMSO	17.7 \pm 3.1	48.1 \pm 4.0	0.24 \pm 0.014	0.242 \pm 0.065	1.00 \pm 0.19
(c) Vehicle +Aflatoxin	16.2 \pm 3.1	30.6 \pm 5.0 ^d	0.136 \pm 0.015 ^{***d}	0.365 \pm 0.035	1.48 \pm 0.23 ^{ad}
(d) MPA	18.7 \pm 2.9	52.8 \pm 9.8	0.203 \pm 0.017	0.252 \pm 0.018	1.32 \pm 0.24
(e) MPA+DMSO	17.8 \pm 4.2	50.0 \pm 8.2 ^e	0.245 \pm 0.019 ^{***e}	0.272 \pm 0.015 ^{**e}	1.10 \pm 0.11 [*]
(f) MPA+Aflatoxin	16.9 \pm 3.3	47.8 \pm 5.3 ^{oe}	0.185 \pm 0.018 ^{***f}	0.345 \pm 0.26 ^{***f}	1.22 \pm 0.185

Value are mean \pm S.D. of 5 individual mitochondrial preparations.

X = μ moles of triphenyltetrazolium chloride (TTC) reduced/hr/mg protein.

Y = μ moles of Pi liberated/hr/mg protein

Z = mg/g wet tissue

d = Significant as compared to vehicle+DMSO group.

e = Significant as compared to vehicle+aflatoxin.

f = Significant as compared to MPA+DMSO.

* = P < 0.05 ; ** P < 0.01 ; *** P < 0.001

TABLE II : Effect of MPA on hepatic drug-metabolizing enzymes system

Parameter	Control	MPA treated
Cytochrome p-450 (nmol/mg/min)	0.82±0.08	0.99±0.1*
Cytochrome b ₅ (nmol/mg/min)	0.50±0.05	0.66±0.08**
Arylhydrocarbon hydroxylase (AHH) (p-mol/mg/min)	225.0±32.0	362.0±40.0***
UDP-gucuronyl transferase (nmol/mg/min)	36.4±4.5	43.6±6.6
NADPH-Cytochrome C-reductase (nmol/mg/min)	38.8±4.4	46.2±5.8*
Glutathione (µg/g liver)	3.2±0.51	3.74±0.6
Glutathione-5-transferase n mole CDNB conjugated/min/mg soluble protein	705±61.0	718±80.0

Values are mean ± S.D. of 5 individual observations. Values of Cytochrome P-450, b₅, AHH, UDP-glucuronyl transferase and NADPH-Cytochrome C-reductase are expressed as per mg microsomal protein.

P < 0.05 ; **P < 0.01 ; ***P < 0.001, significant as compared to control.

The results of AFB₁ treatment (24 hrs) on liver mitochondrial enzymes, SDH and Mg⁺⁺-ATPase as well as on lipids of rats pretreated with MPA for 30 days are shown in Table I. The decrease in specific activities of SDH and Mg⁺⁺-ATPase was prevented to a great extent by MPA, but not the AFB₁ induced accumulation of mitochondrial phospholipid and cholesterol contents.

DISCUSSION

The results obtained on the depression of SDH activity following AFB₁ administration are in accordance with those of Theron (24) who observed a progressive decrease in staining intensity in tissue sections incubated for demonstration of SDH and ATPase activities in animals killed between 8 to 24 hr. Brown and Abrams (2) had reported that AFB₁ markedly decreased the activity of certain mitochondrial dehydrogenases in livers. Similar results were observed by Raj and Venkitesubramanian (20) in chicks treated with AFB₁. They observed inhibition of SDH activity in chick liver 24 hr after a single dose of AFB₁. The results of present study showed that action of the toxin on SDH activity was time dependent and maximum decrease in its activity was observed after 24 hr.

There are conflicting reports regarding the effects of aflatoxin B₁ on Mg⁺⁺-ATPase activity. Clifford and Rees (4) as well as Ramachandra Pai *et al.* (21) observed that aflatoxin did not alter Mg⁺⁺-ATPase activity. However, Shankaran *et al.* (23) observed decrease in Mg⁺⁺-ATPase after 2 hrs and then increase after 8 hrs of aflatoxin administration but

in the present study a significant decrease in Mg⁺⁺-ATPase activity was noted after 18 and 24 hr treatment with aflatoxin B₁. The alteration in Mg⁺⁺-ATPase activity might be due to uncoupling of oxidative phosphorylation by aflatoxin as observed by Ramachandra Pai *et al.* (21).

The elevation in mitochondrial phospholipid and cholesterol content might be due to accumulation of lipids in the liver as effect on lipid metabolism is the primary lesion during aflatoxicosis (10).

There has been great deal of controversy on the role of metabolic activation of AFB₁ in the acute toxicity of the compound. It has been reported that there is reduction in cytotoxicity after metabolic activation of AFB₁ (14). But others have contradicted this view and have reported that the acute toxicity is dependent on the conversion of AFB₁ into a toxic metabolite (13). In the present study aflatoxin toxicity was diminished in MPA treated rats.

The mechanism of decrease in aflatoxin B₁ toxicity by MPA could be due to the stimulatory effect of this drug on hepatic MFO system leading to greater conversion of it to its hydroxylated derivatives which may be less toxic than the parent compound. It is, therefore, reasonable to conclude that acute mitochondrial membrane damage as seen with the decrease in enzyme activity is due to the native compound which possibly gets converted to less toxic metabolites by microsomal enzymes.

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