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Influence of Selenium on Hepatic Mitochondrial Antioxidant Capacity in Ducklings Intoxicated with Aflatoxin B_1

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Abstract The aim of the study was to investigate the effect of selenium on hepatic mitochondrial antioxidant capacity in ducklings administrated with aflatoxin B_1 (AFB_1). Ninety 7-day-old ducklings were randomly divided into three groups (groups I–III). Group I was used as a blank control. Group II was administered with AFB_1 (0.1 mg/kg body weight). Group III was administered with AFB_1 (0.1 mg/kg body weight) plus selenium (sodium selenite, 1 mg/kg body weight). All treatments were given once daily for 21 days. The results showed that the activities of mitochondrial superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and glutathione reductase (GR) in group II ducklings significantly decreased when compared with group I ($P<0.01$). Furthermore, the content of hepatic mitochondrial malondialdehyde (MDA) significantly increased ($P<0.01$). However, the activities of hepatic mitochondrial SOD, CAT, GSH-Px, and GR in group III ducklings significantly increased when compared with group II ($P<0.05$). In addition, the content of hepatic mitochondrial MDA significantly decreased ($P<0.01$). These results revealed that AFB_1 significantly induced hepatic mitochondrial antioxidant dysfunction. However, sodium selenite could significantly ameliorate the negative effect induced by AFB_1.

Keywords Sodium selenite · Hepatic mitochondrial · Antioxidant capacity · Aflatoxin B_1

Introduction

Aflatoxins, a group of extremely toxic and biologically active substances, are produced by the fungi *Aspergillus flavus* and *Aspergillus parasiticus*. Among them, aflatoxin B_1 (AFB_1) is a matter of concern due to their widespread contamination of cereal grain commodities, corn in particular, and their adverse effects on human and animal health. Aflatoxin B_1 is produced by toxigenic fungi belonging to the genus *Aspergillus* and has a long history of association with illness in domesticated animals and in humans [1, 2]. The liver is especially sensitive to AFB_1. The AFB_1 is firstly a hepatotoxin, causing an excessive buildup of hepatic lipids, with enlargement of the liver, proliferation of the biliary ducts [3], and hepatocellular carcinoma [4].

To date, the mechanisms underlying the AFB_1-induced cytotoxicity have not been fully clarified. It was considered that AFB_1-mediated cell injury may be due to the release of free radicals, which initiate lipid peroxidation [5]. Recently, the direct evidence of the involvement of free radicals in AFB_1 toxicity was demonstrated by Towner [6], who identified free radicals in vivo in rat bile following AFB_1 administration. It is known that oxidative stress can play an important role in the initiation of carcinogenesis through DNA damage [7]. This may be one of the mechanisms responsible for AFB_1-induced carcinogenesis. In a number of studies, the ability of antioxidants to defend chemical

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carcinogenesis when administered prior to or concomitantly with the carcinogen was demonstrated [8]. AFB_1-mediated hepatic lipid peroxidation and serum activity of transaminases were reduced by the pretreatment of rats with antioxidants, selenium, and vitamin E [5]. The present study was designed to assess the effect of selenium on hepatic mitochondrial antioxidant capacity in ducklings administered with AFB_1.

Materials and Methods

Drugs and Chemicals

Aflatoxin B_1, D-Mannitol, hydroxyethyl piperazine ethanesulfonic acid (HEPES), ethylene glycol tetraacetate (EGTA), sucrose, bovine serum albumin (BSA), dimethyl sulfoxide, coomassie brilliant blue, and rotenone were purchased from Sigma. Detection kit which includes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and malondialdehyde were purchased from Nanjing Jiancheng Bioengineering Institute. All other chemicals were of analytical grade.

Animals and Treatments

All experimental protocols were approved by the Ethics Committee of South China Agricultural University. This study was carried out on 90 Guangdong white ducklings weighing 180–200 g body weight. Seven-day-old ducklings were obtained from the Research Center of Experimental Animals at South China Agricultural University. The animals were randomly divided into three equal groups containing 30 ducklings with an equal number of male and female ducklings. Group I was used as control and intragastrically administered with dimethyl sulfoxide (DMSO). Group II was intragastrically administered with AFB_1 (0.1 mg/kg body weight). Group III was intragastrically administered with AFB_1 (0.1 mg/kg body weight) plus selenium (sodium selenite, 1 mg/kg body weight). These treatments were administrated once daily for a period of 21 days under the same condition. AFB_1 was diluted with DMSO for experimental groups. All ducklings had free access to water and food at room temperature during the study.

Mitochondrial Preparation

Mitochondria were isolated from the duckling liver by differential centrifugation as described by Tang [9], with modifications. On 7th, 14th, and 21st day after treatment, five ducklings were randomly taken out to obtain respectively about 15 g of liver in every experimental group. The liver was trimmed of fat and washed in ice-cold initial liver mitochondria isolation medium A (220 mM D-Mannitol, 70 mM sucrose, 2 mM HEPES, 1 mM EGTA, and 0.5 mg/mL BSA, adjusted to pH 7.0 with Tris). The liver was then weighed, minced, and transferred to a 50-mL capacity Potter homogenizer run at 2,000 rpm (0.15 mm radial pestle clearance) for 3 min. The homogenate was diluted to 300 mL using medium A (6×50 mL) and centrifuged for 10 min at 1,000×*g*. The supernatant was filtered through a four-layer cheesecloth and centrifuged again at 1,000×*g* for 10 min. After the supernatant was centrifuged at 10,000×*g* for 14 min, the pellet was suspended in 100 mL (2×50 mL) medium B, a second liver mitochondria isolation medium (220 mM D-Mannitol, 70 mM sucrose, 2 mM HEPES, and 0.5 mg/mL BSA, adjusted to pH 7.0 with Tris), and centrifuged for 10 min at 10,000×*g*. The resulting pellet was resuspended in liver mitochondria incubation medium C (220 mM D-Mannitol, 70 mM sucrose, and 2 mM HEPES, adjusted to pH 7.0 with Tris). All the steps are strictly operated on ice to guarantee the isolation of high-quality mitochondrial preparation. The protein concentration of the final mitochondrial pellet was determined with the Bradford's assay using bovine serum albumin as a protein standard [10].

Assessment of Mitochondrial Antioxidant Capacity

The activities of mitochondrial superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase were detected, according to instruction in the detection kit. Similarly, the content of mitochondrial malondialdehyde was detected. The activities of mitochondrial superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase are expressed as international units per milligram mitochondrial protein. The content of mitochondrial malondialdehyde is expressed as nanomol per milligram mitochondrial protein.

Statistical Analysis

The statistical significance of differences between groups in these studies was determined using a one-way analysis of variance and the results were presented as the mean±S.E. The significance level was $P<0.05$.

Results and Analysis

Hepatic Mitochondrial Superoxide Dismutase Activity

As shown in Table 1, the activity of hepatic mitochondria superoxide dismutase (SOD) was significantly affected after the ducklings were intragastrically administered with

Table 1 The activity of hepatic mitochondria superoxide dismutase (SOD) for 7, 14, and 21 days after the ducklings were administrated with AFB_1

Groups	Three different times after treatment (days)		
	7	14	21
I	202.04±11.34	195.16±10.46	180.78±9.19
II	159.67±5.15**	141.13±7.52**	117.73±8.99**
III	188.19±7.66*	170.65±10.75**	154.75±9.72**

It showed that the activity of hepatic mitochondrial SOD in group II ducklings significantly decreased, compared with group I ($P<0.01$). However, the activity of hepatic mitochondrial SOD in group III ducklings significantly increased, compared with group II ($P<0.01$). Data were expressed as the means±S.E. They were expressed as international units per milligram mitochondrial protein

Group I the control, *Group II* administered with aflatoxin B_1 (AFB_1), *Group III* administered with AFB_1 plus selenium (sodium selenite)

* $P<0.05$, ** $P<0.01$, significantly different, group II compared with group I; significantly different, group III compared with group II

AFB_1 for 7, 14, and 21 days ($P<0.01$). The activity of SOD decreased by 20.97%, 27.38%, and 34.89%, respectively. However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the activity of SOD increased by 17.86%, 20.92%, and 31.44% respectively, compared with group II ($P<0.01$).

Hepatic Mitochondrial Catalase Activity

As summarized in Table 2, the activity of hepatic mitochondria catalase (CAT) was significantly affected after ducklings were intragastrically administered with AFB_1 for 7, 14, and 21 days ($P<0.01$). The activity of CAT decreased by 26.16%, 31.91%, and 40.28%, respectively. However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the activity of CAT increased by 21.96%, 25.99%, and 30.50% respectively, compared with group II ($P<0.01$).

Table 2 The activity of hepatic mitochondria catalase (CAT) for 7, 14, and 21 days after ducklings administrated with AFB_1

Groups	Three different times after treatment (days)		
	7	14	21
I	111.19±8.51	104.61±7.72	96.08±5.13
II	82.10±6.03aa	71.23±6.36aa	57.38±6.90aa
III	100.13±7.33b	89.74±5.03bb	74.88±6.38bb

It showed that the activity of hepatic mitochondrial CAT in group II ducklings significantly decreased, compared with group I ($P<0.01$). However, the activity of hepatic mitochondrial CAT in group III ducklings significantly increased, compared with group II ($P<0.01$). Data were expressed as the means±S.E. They were expressed as international units per milligram mitochondrial protein

aa $P<0.01$ (significantly different when group II compared with group I); b $P<0.05$ (significantly different when group III compared with group II); bb $P<0.01$ (significantly different when group III compared with group II)

Hepatic Mitochondrial Glutathione Peroxidase Activity

As shown in Table 3, the activity of hepatic mitochondria glutathione peroxidase (GSH-Px) was significantly affected after ducklings were intragastrically administered with AFB_1 for 7, 14, and 21 days ($P<0.01$). The activity of GSH-Px decreased by 25.81%, 29.08%, and 33.25%, respectively. However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the activity of GSH-Px increased by 16.79%, 19.29%, and 20.36% respectively, compared with group II ($P<0.05$).

Hepatic Mitochondrial Glutathione Reductase Activity

As summarized in Table 4, the activity of hepatic mitochondria glutathione reductase (GR) was significantly affected after ducklings were intragastrically administered with AFB_1 for 7, 14, and 21 days ($P<0.01$). The activity of GR decreased by 20.92%, 24.49%, and 33.02%, respectively. However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the activity of GR increased by 13.52%, 20.24%, and 23.66% respectively, compared with group II ($P<0.05$).

Hepatic Mitochondrial Malondialdehyde Content

As shown in Table 5, the content of hepatic mitochondria malondialdehyde (MDA) was significantly affected after

Table 3 The activity of hepatic mitochondria glutathione peroxidase (GSH-Px) for 7, 14, and 21 days after ducklings administrated with AFB_1

Groups	Three different times after treatment (days)		
	7	14	21
I	109.58±6.40	99.84±5.72	88.01±7.29
II	81.30±5.59aa	70.81±5.35aa	58.75±4.21aa
III	94.95±6.49b	84.47±5.89b	70.71±4.10bb

It showed that the activity of hepatic mitochondrial GSH-Px in group II ducklings significantly decreased, compared with group I ($P<0.01$). However, the activity of hepatic mitochondrial GSH-Px in group III ducklings significantly increased, compared with group II ($P<0.05$). Data were expressed as the means±S.E. They were expressed as international units per milligram mitochondrial protein

aa $P<0.01$ (significantly different when group II compared with group I); b $P<0.05$ (significantly different when group III compared with group II); bb $P<0.01$ (significantly different when group III compared with group II)

Table 4 The activity of hepatic mitochondria glutathione reductase (GR) for 7, 14, and 21 days after ducklings administrated with AFB_1

Groups	Three different times after treatment (days)		
	7	14	21
I	76.49±3.91	70.14±5.52	65.75±5.73
II	60.49±2.05[aa]	52.96±3.28[aa]	44.04±3.65[aa]
III	68.67±5.99[b]	63.68±4.42[b]	54.46±6.09[bb]

It showed that the activity of hepatic mitochondrial GR in group II ducklings significantly decreased, compared with group I ($P<0.01$). However, the activity of hepatic mitochondrial GR in group III ducklings significantly increased, compared with group II ($P<0.05$). Data were expressed as the means±S.E. They were expressed as international units per milligram mitochondrial protein

[aa] $P<0.01$ (significantly different when group II compared with group I); [b] $P<0.05$ (significantly different when group III compared with group II); [bb] $P<0.01$ (significantly different when group III compared with group II)

ducklings were intragastrically administered with AFB_1 for 7, 14, and 21 days ($P<0.01$). The content of MDA increased by 42.31%, 210.00% and 250.91% respectively. However, in group III ducklings intragastrically administered with AFB_1 plus selenium, the content of MDA decreased by 25.64%, 45.81%, and 42.49% respectively, compared with group II ($P<0.01$).

Discussion

Aflatoxins are secondary toxic fungal metabolites produced by *A. flavus* and *A. parasiticus*. There are four naturally occurring aflatoxins, the most hepatotoxic being AFB_1, and three structurally similar compounds, namely aflatoxin B_2, aflatoxin G_1, and aflatoxin G_2. It is well known that AFB_1 may induce the production of free radicals and/or the reduction of antioxidant defenses [11, 12]. In animal organism, reactive oxygen metabolites (ROM) include intracellular thiols (SH), MDA. The MDA production is recognized as an important factor in determining alteration of membrane fluidity [13, 14] and increase of membrane fragility accompanying final cell death [15]. The ROM content in animal cells may be increased by several factors, including metabolism of xenobiotics, such as the mycotoxins, with the onset of oxidative stress conditions as a result [16]. Several authors [17–19] reported that some mycotoxins can cause cell membrane damage through the increase of lipid peroxidation. Moreover, results of those studies indicate the important role of the ROM production in explaining the cytotoxic and, possibly, genotoxic potential of the mycotoxins. In continuation of these studies, we investigated the effect of AFB_1 on ducklings' hepatic mitochondrial antioxidant capacity in this research. For 7, 14, and 21 days after ducklings administrated with AFB_1 respectively, the results showed that the activities of mitochondrial SOD, CAT, GSH-Px, and GR in group II ducklings (administered with AFB_1) significantly decreased when compared with group I ($P<0.01$). Furthermore, the content of hepatic mitochondrial MDA in group II significantly increased ($P<0.01$).

Table 5 The content of hepatic mitochondria MDA for 7, 14, and 21 days after ducklings administrated with AFB_1

Groups	Three different times after treatment (days)		
	7	14	21
I	0.45±0.05	0.50±0.06	0.55±0.03
II	0.78±0.04[aa]	1.55±0.04[aa]	1.93±0.04[aa]
III	0.58±0.03[bb]	0.84±0.04[bb]	1.11±0.03[bb]

It showed that the content of hepatic mitochondrial MDA in group II ducklings significantly increased, compared with group I ($P<0.01$). However, the content of hepatic mitochondrial MDA in group III ducklings significantly decreased, compared with group II ($P<0.01$). Data were expressed as the means±S.E. They were expressed as nanomol per milligram mitochondrial protein

[aa] $P<0.01$ (significantly different when group II compared with group I); [bb] $P<0.01$ (significantly different when group III compared with group II)

Although the mechanism underlying the hepatotoxicity of aflatoxins is not fully understood, several reports suggest that toxicity may ensue through the generation of intracellular reactive oxygen species like superoxide anion, hydroxyl radical, and hydrogen peroxide during the metabolic processing of AFB_1 by cytochrome P450 in the liver [20]. These species may attack soluble cell compounds as well as membranes, eventually leading to the impairment of cell functioning and cytolysis [21, 22]. But peroxidative damages induced in the cell are encountered by elaborate defense mechanisms, including enzymic and nonenzymic antioxidants. AFB_1-mediated hepatic lipid peroxidation and serum activity of transaminases were reduced by the pretreatment of rats with antioxidants, selenium, and vitamin E. The defense mechanisms can be reinforced by increasing dietary intake of antioxidants and micronutrients such as vitamin E and selenium (Se). The importance of Se is linked to by its role as a constituent of several enzymes of the physiological antioxidative system, as well as enzymes such as thioredoxin reductase, whose chief function is to facilitate electron transport. The reduction of reactive oxygen metabolites by glutathione peroxidases helps to maintain membrane integrity [23]. In this study, we assessed the effect of selenium on hepatic mitochondrial antioxidant function in ducklings administrated with AFB_1.

For 7, 14, and 21 days after ducklings administrated with AFB_1 respectively, it showed that the activities of hepatic mitochondrial SOD, CAT, GSH-Px, and GR in group III ducklings (administered with AFB_1 plus sodium selenite) significantly increased when compared with group II ($P< 0.05$). In addition, the content of hepatic mitochondrial MDA in group III significantly decreased ($P<0.01$). The results of the present study demonstrate that sodium selenite significantly ameliorates the negative effect of AFB_1 on hepatic mitochondrial antioxidant capacity in ducklings.

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