Mitochondrial Injury and Impaired Liver Function in Aflatoxin Toxicity

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Mitochondrialverletzung und geschädigte Leberfunktion durch Aflatoxin-Toxicität


Summary. The effect of a single dose of aflatoxin B₁ on the activities of chick liver mitochondrial enzymes, viz., succinate dehydrogenase, malate dehydrogenase and isocitrate dehydrogenase was studied, 24 hours after the administration of the toxin. A drastic reduction in the activities of the above enzymes was observed indicating mitochondrial injury due to aflatoxin toxicity. A significant decrease in the activities of transaminases was noted. A rise in the activities of serum alkaline phosphatase and serum aldolase of chicks together with a decrease in the activities of the liver enzymes are suggestive of impaired liver function in aflatoxin B₁ poisoning.

Introduction

Aflatoxins are a group of secondary metabolites produced by Aspergillus flavus. Aflatoxin B₁ is the most potent hepatocarcinogen and toxic to many species of animals. A large number of biochemical changes in animals treated with aflatoxin have been documented in the literature. Reports on the biological effects elicited by aflatoxin in chicks are rather scanty. In the present study an attempt was made to study the effects of aflatoxin B₁ on chicks, which are the most common poultry birds in India, severely affected by aflatoxin toxicity arising from the contamination of poultry feed by aflatoxin. The results prompted that aflatoxin B₁ caused extensive liver damage and mitochondria dysfunction.

Materials and Methods

One day old, male chicks of Mexican White leghorn strain of 30—40 g weight were used in the present study. Aflatoxin B₁ was isolated in pure form from the culture extracts of A. flavus. The birds were injected intraperitoneally at a dose level of 2.7 mg/kg body weight in 0.1 ml of propylene glycol. The control birds were administered with propylene glycol. The chicks were fasted for 24 h before sacrifice.

Method of Sacrifice and Processing of Tissue. The birds were stunned by a sharp blow on the head and immediately decapitated. The tissue was immediately removed and processed according to the nature of the experiment. In general, the tissue blotted free of blood, was kept in an ice-cold chilled beaker. Blood was collected in heparinized tubes containing sodium fluoride to prevent coagulation and glycolysis.

Analysis

Assay of Pyridine Nucleotide Linked Dehydrogenases. The particulate and soluble enzyme preparations used in this study were obtained by centrifuging the liver homogenate in 0.25 M sucrose at 700 × g for 15 min and the resulting supernatant liquid was centrifuged at 10000 × g for 30 min. 5 min were allowed for temperature equilibration (25 °C) and for endogenous enzyme activity to subside after mixing the reactants and enzyme preparations. The reaction was started by the addition of the cofactor. In the case of malate dehydrogenase, however, oxalacetate was added to start the reaction. Readings were recorded at every 15 or 30 sec, when enzyme activity varied rectilinearly with time. Enzyme activity was calculated from the change in optical density at 340 nm. The activity was expressed in terms of μmoles of pyridine nucleotide reduced or oxidized per minute.

Isocitrate Dehydrogenase (three-D₃ L-isocitrate: NADP oxidoreductase (decarboxylating) (E. C. 1. 1. 1. 42): The method of Ochoa [1] was used. The reaction mixture consisted of 75 μmoles of Tris HCl (pH 7.4), 0.4 μmole of NADP⁺, 1.8 μmoles of MnCl₂, 0.8 μmole of Mn-trisodium isocitrate and enzyme in a final volume of 3.0 ml.
**Malate Dehydrogenase** (l-malate: NAD oxidoreductase, E.C.1.1.1.37). The activity of this enzyme was assayed by the method of Ochoa [2]. The assay system comprised of 0.3 ml of 0.25 M-Tris buffer (pH 7.4), 0.1 ml 1,5 mM-NADH, 0.1 ml of 7,6 mMoxalacetate, enzyme and enough water to make up to 3 ml.

**Succinate Dehydrogenase** (Succinate: (Aeceptor) oxidoreductase, E. C. 1.3.99.1). The method of Slater and Bonner [3] was used for the assay of this enzyme. The reaction mixture in a volume of 3 ml contained (final concentration) 0.1 M-phosphate buffer (pH 7.3), 0.1 M-sodium succinate, 0.01 M-KCN, 0.01 M-K₄Fe(CN)₆, and the enzyme preparation (10000 × g particulate fraction). The reduction of K₃Fe(CN)₆ was followed at 400 nm as a function of time. One unit of the enzyme causes a decrease in optical density of 0.01 per minute at 400 nm.

**Adenosine Triphosphatase** (ATP phosphohydrolase E. C. 3.6.1.3). The enzyme was assayed with Mg ++ as the activating cation, based on the procedure of Masoro et al. [4] excluding cysteine from the reaction mixture. The assay system contained 5 μmoles of MgCl₂, 50 μmoles of Tris HCl (pH 7.4), 5 μmoles of ATP (pH 7.4) and enzyme preparation (a 10% homogenate in 0.25 M sucrose). Activity was expressed as μmoles of inorganic phosphate liberated for 15 min under the conditions of assay.

**Alkaline Phosphatase** (Orthophosphoric monoester phosphohydrolase, E.C. 3.1.3.1). The enzyme was assayed by the method of King [5]. The assay system contained 2 ml of 0.1 M carbonate — bicarbonate buffer (pH 10), 2 ml of 0.01 M disodiumphenyl phosphate and enzyme. The activity was expressed in terms of μmoles of phenol liberated per hour under the conditions of the assay.

**Fruco8osediphosphate Aldolase** (Fructose-1,6-diphosphate D-glyceraldehyde-3-phosphatelyase, E.C. A.1.2.13). The procedure of Sibley and Lehninger [6] was used for the assay of this enzyme. The reaction mixture was made up of 1 ml of 0.1 M-Tris buffer (pH 8.6), 0.25 ml of 0.05 M-fructose 1,6-diphosphate (pH 8.6), 0.25 ml of 0.56 M-hydrazine, enzyme (1% liver homogenate in chilled distilled water) and water to make up to 2.5 ml.

One unit of aldolase is defined as the amount of enzyme required to split one μmole of fructose 1,6-diphosphate per minute at 37° C and pH 8.6.

**Glutamic Aspartic Transaminase** (l-aspartate: 2-oxoglutarate aminotransferase, E.C. 2.6.1.1), and **Glutamic-alanine tran£aminase** (l-alanine: 2-oxoglutarate aminotransferase). The procedure described by Wootton [7] was followed for the assay of these enzymes. The reaction mixture consisted of 100 μmoles of DL-aspartic acid (pH 7.4) or 100 μmoles of alanine, 1 μmole of a, 2-ketoglutarate and enzyme. The unit of activity was expressed in terms of μmoles of keto acid measured as pyruvate/h.

**Results**

The activities of isocitrate dehydrogenase (NADP-dependent) in 10000 × g particulate and supernatant fractions of control and aflatoxin B₁-treated chick liver are presented in Table 1. A significant decrease is observed in the activity of the enzyme of particulate fraction in the livers of aflatoxin B₁-treated chicks as compared with the control. A similar decrease is observed in the supernatant enzyme also. The data on the effect of aflatoxin B₁ on succinate dehydrogenase and malate dehydrogenase (10000 × g particulate fraction) in control and aflatoxin B₁-treated chick liver has been statistically evaluated and presented in Table 2. Succinate dehydrogenase activity is substantially lowered in the aflatoxin B₁-treated chick liver. Also malate dehydrogenase activity is inhibited by aflatoxin B₁. The activities of adenosine triphosphatase, and transaminases in the livers of control and aflatoxin B₁ treated chicks have been presented in Table 3. There is a significant decrease in the activity of adenosine triphosphatase in the livers of aflatoxin B₁-administered chicks as compared to controls. Also aflatoxin B₁ inhibits the transaminases. The activities of serum aldolase and serum alkaline phosphatase are elevated in aflatoxin B₁-treated chicks (Table 4).

**Discussion**

The choice of the dosage of the toxin used and the period of killing of the birds after administration of the toxin has been discussed in our earlier communications [8, 9]. Liver mitochondrial injury is one of the dominant features of aflatoxin toxicity. Reports in the extent of mitochondrial damage as a result of aflatoxin administration are, however, inconsistent. Theron et al. [10] demonstrated lysis of mitochondrial
Table 1. Activity of isocitrate dehydrogenase in the liver of control and aflatoxin B$_1$-treated chicks

<table>
<thead>
<tr>
<th></th>
<th>Isoeitrate dehydrogenase (10000 x g supernatant)</th>
<th>Isoeitrate dehydrogenase (10000 x g particulate fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Units/g fresh liver)</td>
<td>(Units/g protein)</td>
</tr>
<tr>
<td>Control</td>
<td>41.70 ± 4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Treated</td>
<td>19.70 ± 4.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Values expressed are the mean ± S. E for 6 birds.

$^a$ p < 0.01.

$^b$ p < 0.001.

$^c$ p < 0.05.

Table 2. Activities of succinate dehydrogenase and malate dehydrogenase in the liver of control and aflatoxin B$_1$-treated chicks

<table>
<thead>
<tr>
<th></th>
<th>Succinate dehydrogenase</th>
<th>Malate dehydrogenase</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Units/g fresh liver</td>
<td>Units/g protein</td>
</tr>
<tr>
<td>Control</td>
<td>5281 ± 362</td>
<td>362</td>
</tr>
<tr>
<td>Treated</td>
<td>1394 ± 73$^b$</td>
<td>73</td>
</tr>
</tbody>
</table>

Values expressed are the mean ± S. E. for 6 birds.

$^a$ Values expressed are the mean ± S. E. for 6 birds.

$^b$ p < 0.001.

$^c$ p < 0.01.

$^d$ p < 0.05.

Table 3. Activities of adenosine triphosphatase, glutamic aspartic transaminase and glutamic alaninetransaminase in the liver of control and aflatoxin B$_1$-treated chicks

<table>
<thead>
<tr>
<th></th>
<th>Adenosine triphosphatase</th>
<th>Glutamic aspartic transaminase</th>
<th>Glutamic alaninetransaminase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Units/g fresh liver</td>
<td>Units/g protein</td>
<td>Units/g fresh liver</td>
</tr>
<tr>
<td>Control</td>
<td>65.0 ± 1.6</td>
<td>11.87 ± 0.04</td>
<td>118.0 ± 4.16</td>
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<tr>
<td>Treated</td>
<td>38.4 ± 0.4$^b$</td>
<td>7.84 ± 1.08$^b$</td>
<td>83.2 ± 2.50$^b$</td>
</tr>
</tbody>
</table>

Values expressed are the mean ± S. E. for 6 chicks.

$^a$ Values expressed are the mean ± S. E. for 6 chicks.

$^b$ p < 0.05.

$^c$ p < 0.001.

Table 4. Activities of serum aldolase and serum alkaline phosphatase of control and aflatoxin B$_1$-treated chicks

<table>
<thead>
<tr>
<th></th>
<th>Serum aldolase (Units/100 ml)</th>
<th>Serum alkaline phosphatase (Units/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.4 ± 0.13</td>
<td>21.6 ± 1.1</td>
</tr>
<tr>
<td>Treated</td>
<td>8.6 ± 0.60$^b$</td>
<td>49.8 ± 0.7$^b$</td>
</tr>
</tbody>
</table>

Values expressed are the mean ± S. E. for 6 chicks.

$^a$ Values expressed are the mean ± S. E. for 6 chicks.

$^b$ p < 0.05.
membranes in ducklings, 4 hr after administration of aflatoxin. He reported that the toxin was transported by erythrocytes and, hence, all membrane structures in close contact with toxin-bearing red cells were found to be injured. Such intracellular effects on red cells have not been demonstrated in rat or monkey liver (Svoboda et al. [11]). Mitochondrial swelling and disfunction have been described in toxic injury to liver due to chemical poisons or endotoxins from bacteria and fungi (Rouiller [13]). Mitochondrial metabolism has therefore been investigated in the liver of aflatoxin B$_1$-treated chicks in the present study by assaying the activities of malate dehydrogenase, isocitrate dehydrogenase and succinate dehydrogenase. The administration of aflatoxin B$_1$ to chicks resulted in significant reduction in the activities of malate dehydrogenase and isocitrate dehydrogenase. This finding is in agreement with the reports of Brown [13] and Gumbmann and Williams [14]. A decrease in the activities of NAD-linked dehydrogenases has been reported in the liver of cord factor-treated mice (Kato et al. [15]). Mitochondrial injury due to toxins has been attributed by many investigators to physical injury that augments permeability of the mitochondrial membrane (Judah [16], Calvert and Brody [17], Recknagel et al. [18] and Rees and Sinha [19]). This would explain the diffusion of cofactors (Judah and Rees [20]) and the escape into the blood stream of certain enzymes like glutamate dehydrogenase and isocitrate dehydrogenase (Rees et al. [21]). The significant decrease in the activity of the dehydrogenases in the 10000 × g particulate fraction of aflatoxin B$_1$-treated chick liver could be due to changes in permeability. The diminished activities of NAD-dependent dehydrogenases in cord factor-treated mice have been ascribed to enhanced NADase activity and loss of pyridine nucleotide (Artman et al. [22, 23]). Tulpule [24] has reported enhanced NADase activity and reduced nicotinamide adenine dinucleotide levels in the liver of aflatoxin-treated ducklings. Thus the decrease observed in the NAD-dependent dehydrogenases of livers of aflatoxin B$_1$-administered chicks in the present study, could also be due to increased NADase activity and reduction in coenzyme content.

A significant reduction in the liver alkaline phosphatase activity is observed as a result of aflatoxin administration to chicks (Raj et al. [8]). The marked rise in serum alkaline phosphatase activity observed in the present study is compatible with the reduction in liver alkaline phosphatase activity. A significant rise in the activity of serum aldolase is noted (Table 4). This is in agreement with the report of Brown and Abrams [25]. Another effect of aflatoxin B$_1$ related to liver disfunction is the reduction in the activity of hepatic glutamic aspartic transaminase and glutamic alanine transaminase. The above mentioned effects of aflatoxin B$_1$ on alkaline phosphatase and aldolase in serum, and transaminase in liver of chicks, are correlated with the extensive liver damage caused by aflatoxin B$_1$.

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References

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