The pretreatment effects of pentoxifylline on aflatoxin B1-induced oxidative damage in perfused rat liver


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Introduction

Aflatoxins are toxic secondary metabolites produced by the fungi Aspergillus flavus and A. parasiticus (Choi et al; Preetha, Kanniappan et al. 2006). They are the most highly toxic form of aflatoxins B1, B2, G1 and G2. Aflatoxins induce many forms of oxidative damage, including hepatotoxicity, hepatocarcinogenesis and mutagenic effects. Aflatoxin B1 (AFB1) is the most potent of these compounds. AFB1 is a potent toxicant and is capable of inducing oxidative stress in the liver. Oxidative stress is a key factor in the pathogenesis of aflatoxin-induced liver injury (Towner et al., 2002, 2003; Preetha et al., 2006). Oxidative stress is produced by the production of reactive oxygen species (ROS) including the superoxide anion, hydrogen peroxide (H2O2), and the hydroxyl radical via cytochrome P450 metabolism and an iron-mediated mechanism (Choi et al., 2010; Shen et al., 1995; Preetha et al., 2006). ROS cause oxidative stress by damaging cellular membranes and components. Evidence has accumulated that AFB1-induced hepatotoxicity and hepatocarcinogenesis via oxidative stress are inhibited by non-natural and natural antioxidants such as silymarin (Rastogi et al., 2001), crocin (a natural carotenoid) (Wang et al., 1991), green tea (Qin et al., 1997) and butylated hydroxyanisole (Choietal., 1991).

Pentoxifylline (PTX) is a derivative of methyIxanthine that has hematologic and immunomodulating properties. It also has anti-inflammatory and antioxidant effects (Abdin et al., 2010; Abdel-Salam et al., 2003; Radfar et al., 2005). PTX has an inhibitory effect on xanthine oxidase, which is involved in the formation of oxygen free radicals, and down-regulates tumor necrosis factor alpha (TNF-α) production. This cytokine causes increased H2O2 production from mitochondria. PTX is a nonselective inhibitor of phosphodiesterase (PDE), which plays a role in nitric oxide production, and decreases lipid peroxidation via inhibition of PDE (Aabdin et al., 2010;...
The present study was designed to investigate the protective effect of against AFB1-induced oxidative damage in perfused rat liver.

**Materials and Methods**

**Chemicals**

AFB1 and glutathione (GSH) were obtained from Sigma (St. Louis, MO, USA). Perfusion fluids comprised Krebs-Henseleit bicarbonate buffer (KHBB; 118 mM NaCl, 6 mM KCl, 1.1 mM MgSO4, 24 mM NaHCO3, and 1.25 mM CaCl2). These materials were purchased from Merck.

**Animals**

Male Wistar rats (200–300 g) were obtained from the vivarium section of the Department of Pharmacology, Tehran University of Medical Sciences, Tehran, Iran. The animals were housed in cages at room temperature. They had free access to standard diet and tap water until surgery, when they were deprived of food.

Sixteen rats were divided into four groups of four rats each: control (A), PTX (B), AFB1 (C) and AFB1 + PTX (D). Group B and D rats were injected with PTX (100 mg/kg intraperitoneally) (Leist et al., 1996; Barton et al., 2001) 24 h before being anesthetized. All rats were anesthetized by an intraperitoneal injection of a mixture of ketamine and xylazine (60 mg/ml and 8 mg/ml, respectively) (Mikoloff et al., 1987; Mehrvar and Zhang, 2002). The anterior abdomen was cleaned with alcohol and a ventral longitudinal midline incision was made extending from the pubis to the upper chest. The animals were hepatized by injecting into the inferior vena cava anterior to the renal vein in the liver and ligating the inferior vena cava. The hepatic portal vein (inlet) and the thoracic inferior vena cava (outlet) were then cannulated. The liver was perfused with KHBB (pH 7.4 ± 2) saturated with 95% O2/5% CO2, through a catheter cannulated into the portal vein. The perfusate was collected from a catheter in placed in the superior vena cava via the right atrium. The flow rate of perfusion was 20 ml/min at 15–20 cm H2O. The flow rate was measured by fractional the effluent. In the rats of group C and D, 1 ppm AFB1 was infused (about 3 μl/g live weight) into the portal vein. This concentration was selected based on our preliminary study of different doses of AFB1 (0.01, 0.1 and 1 ppm). Group A rats were infused with KHBB.

Sample collection

Samples (1.5 ml) were taken from the outlet at 0, 15, 30, 45, 60, 75, 90, 105 and 120 min in and stored at −20 °C for measurement of alanine aminotransferase (ALT), aspartate transaminase (AST) and lactate dehydrogenase (LDH) levels. The middle lobe of the perfused livers was used for measurement of malondialdehyde (MDA), GSH and total protein.

Sample analysis

Enzymes levels were quantified based on ultraviolet light methods using a commercial kit from Tesh Gostaran Hayan (Tehran, Iran). Lipid peroxidation was determined in liver tissue homogenate according to the thiobarbituric acid method (Esterbauer and Cheeseman, 1990). GSH was estimated by the Kuo and Hook standard method (Kuo and Hook, 1982) and total protein was estimated by the Bradford method (Bradford, 1976).

Statistical analysis

Values are presented as mean ± SE. Data were analyzed by one-way analysis of variance, followed by Tukey's post hoc test for multiple comparisons. Differences were considered to be statistically significant at p < 0.05.

Results

Liver viability and damage parameters

Flow rate showed a time-dependent decrease but there were no significant differences among the groups (Figure 1). ALT is an enzyme in the hepatocyte cytoplasm that is released following minor liver damage (Bessems et al., 2006). The increase in ALT release in the AFB1 group was significant after 60 min in comparison with the control group (Figure 2). In the others groups, we also observed a statistically significant difference, particularly in the AFB1 + PTX group. AST is an enzyme in both hepatocyte cytoplasm and mitochondria and is released in response to major liver damage (Bessems et al., 2006). A statistically significant difference was observed between the AFB1 group and the controls, but not between AFB1 and AFB1 + PTX (Figure 3). LDH is a general tissue damage marker; it is present in hepatocytes and non-parenchymal cells but is not a liver-specific enzyme (Bessems et al., 2006). An increase in LDH release was observed from 30 min to 120 min with fluctuations in the AFB1 group. There were significant differences between the AFB1 and control groups and between AFB1 and AFB1 + PTX (Figure 4).

MDA and GSH concentration in liver

AFB1 reduced GSH concentrations in comparison with the control group. In the PTX + AFB1 group, the increase of GSH concentration was not significant.
The aim of this study was to investigate the effect of pretreatment with PTX on AFB1-induced hepatotoxicity in perfused rat liver. We used the perfused rat liver model because it is considered a suitable system for chemically induced hepatotoxicity (Lupo et al., 1986). Most studies on the effects of AFB1 in isolated perfused rat liver have focused on hepatic uptake and disposition, net synthesis of albumin, fibrinogen, and α-glycoprotein, and carcinogenicity (John and Miller, 1969; Unger et al., 1977; Essigmann et al., 1980). Furthermore, the first site of AFB1 damage is the liver. In our study, AFB1 at 1 ppm concentration affected ALT, AST, and LDH activity in rat liver. The increase of enzyme levels was partially time dependent. Elevations of AST levels were significant in comparison with the control group, showing that AFB1 caused major liver damage. Changes in ALT and LDH levels are a manifestation of minor liver damage. Rat liver pretreated with PTX before administration of AFB1 showed a decrease of ALT, AST, and LDH activity, but this was not statistically significant. The perfusion flow rate was approximately constant in all four groups. Only in the AFB1 group after 60 min of perfusion was a decrease of flow rate observed. Numerous studies have reported increased activity of ALT, AST, and LDH in liver during aflatoxin treatment in vivo. Most studies of perfused rat liver have demonstrated an increase of ALT, AST, and LDH levels (Determann et al., 1998; Yokoyama et al., 2006; Alexandrova et al., 2007). PTX showed the release of AST, ALT, and LDH induced by aflatoxin B1 but there were no significant differences among the groups.

Table 1: Concentrations of GSH, protein, and MDA in perfused rat liver at the end of the experiment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Concentration</th>
<th>MDA (nmol)</th>
<th>Protein (μg/ml)</th>
<th>GSH (μmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>0.405 ± 0.13</td>
<td>148.2 ± 16.3</td>
<td>669 ± 85.1</td>
</tr>
<tr>
<td>PTX</td>
<td></td>
<td>0.6 ± 0.12</td>
<td>193.7 ± 39.4</td>
<td>436 ± 112.5</td>
</tr>
<tr>
<td>AFB1</td>
<td></td>
<td>2.18 ± 0.17</td>
<td>123.3 ± 33.1</td>
<td>91.7 ± 2.2</td>
</tr>
<tr>
<td>AFB1 + PTX</td>
<td>1 ppm</td>
<td>0.95 ± 0.01</td>
<td>523.3 ± 76.2</td>
<td>238 ± 0.06</td>
</tr>
</tbody>
</table>

*Statistically significant difference at p < 0.001 compared with control group. **Statistically significant difference at p < 0.01 compared with control group. †Statistically significant difference at p < 0.001 compared with AFB1 group. ††Statistically significant difference at p < 0.05 compared with AFB1 group.
Increased MDA concentrations and decreased hepatic GSH levels were evident after infusion of 1 ppm concentration of AFB1. In the group pretreated with PTX before administration of AFB1, the decrease of MDA concentration was significant in comparison with the group given AFB1 only, but the increase of GSH levels was not significant. Protein aggregates were produced in cell membranes, which underlie lipid peroxidation induced by AFB1. These proteins impair the physiological function of tissues and are metabolized in the liver. It seems that PTX protects against AFB1-induced oxygen free radicals but could not compensate for reduced GSH. PTX is a nonselective inhibitor of phosphodiesterase capable of increasing cAMP and cGMP levels (Radfar et al., 2005). These nucleotides prevent oxidative stress by reducing lipid peroxidation and thus PTX reduces hepatic cellular damage due to free radicals (Radfar et al., 2005). PTX also has anti-inflammatory effects and prevents transcription of TNF-α. This cytokine plays a role in the augmentation of AFB1-induced liver damage, and its inhibition by PTX and anti-TNF-α antibodies prevents further damage (Abdin et al., 2010; Abdel-Salam et al., 2003).

Conclusion

One of the underlying mechanisms of AFB1-induced hepatic cell injury is oxidative damage that can be prevented by various natural antioxidants. In the present study, exposure to AFB1 caused an increase in MDA concentration as an indicator of cellular lipid peroxidation, and a decrease in the GSH content of lipid peroxidation. PTX also has anti-inflammatory effects and prevents transcription of TNF-α. This cytokine plays a role in the augmentation of AFB1-induced liver damage, and its inhibition by PTX and anti-TNF-α antibodies prevents further damage (Abdin et al., 2010; Abdel-Salam et al., 2003).

References

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