The protective effect of taurine against cyclosporine A-induced oxidative stress and hepatotoxicity in rats

Hanan H. Hagar

Department of Pharmacology, College of Medicine and KHUH, King Saud University, P.O. Box 2925, Riyadh 11461, Saudi Arabia

Received 11 December 2003; received in revised form 24 February 2004; accepted 4 March 2004

Available online 5 May 2004

Abstract

Cyclosporine A (CsA) is the immunosuppressant which is most frequently used in transplant surgery and in the treatment of autoimmune diseases. Oxidative stress has been implicated as one of the possible mechanisms of CsA-induced hepatotoxicity. The present investigation examined the ability of taurine as an antioxidant to protect against CsA-induced oxidative stress and hepatotoxicity. CsA hepatotoxicity was induced by subcutaneous injection of CsA at a dose of 20 mg/kg body weight daily for 21 days. Hepatotoxicity was assessed by reduced serum total protein level and increased serum levels of gamma glutamyl transferase (GGT), alanine aminotransferase (ALT), and aspartate aminotransaminase (AST). CsA treatment increased lipid peroxidation measured as thiobarbituric acid reactive substances (TBARS) concentration and decreased reduced glutathione (GSH) content and activities of catalase and glutathione peroxidase (GSH-Px) in the rat liver. Taurine administration (1% in the drinking water) for 3 days before and concurrently during CsA injections improved liver functions, as indicated by decline of serum transaminases and GGT levels and elevation of serum total protein. Moreover, taurine significantly reduced hepatic TBARS and increased GSH content and catalase and GSH-Px activities in the hepatic tissue. These results indicate that taurine has a protective action against CsA hepatotoxicity and suggest that taurine may find clinical application against a variety of toxins where cellular damage is a consequence of reactive oxygen species.

Keywords: Cyclosporine A; Thiobarbituric acid reactive substances; Glutathione peroxidase; Catalase; Glutathione; Taurine; Hepatotoxicity

1. Introduction

Cyclosporine A (CsA), a fungal cyclic polypeptide, is widely used clinically as an immunosuppressive (Margreiter et al., 1983). CsA significantly improves graft survival following renal, cardiac, pancreatic, bone marrow, and hepatic transplantation; however, recipients have to maintain therapy for the rest of their lives. In addition, this drug is used in the treatment of a variety of autoimmune diseases such as idiopathic nephritic syndrome, inflammatory bowel disease, psoriasis, and rheumatoid arthritis (Berg et al., 1986). Unfortunately, CsA causes a number of side effects including renal, hepatic, cardiac, alimentary, and neural toxicity (Farthing and Clark, 1981; Sibley et al., 1983; Acito et al., 1995). Kidney damage is the most frequent toxicity observed, although, hepatic injury.
also limits the clinical application of CsA, especially after liver transplantation (Muraca et al., 1993).

Cholestasis, hyperbilirubinemia, hypoproteinemia, increased alkaline phosphatase, elevated transaminases, and bile salts in the blood, inhibition of protein synthesis, and disturbed lipid secretion in both human and experimental animals (Whiting and Thomson, 1989; Hillebrand et al., 1999) characterize hepatotoxicity of CsA. Inhibition of ATP-dependent bile salt export carrier in the canalicular membrane and P-glycoprotein transporter is probably involved in cholestasis caused by CsA (Bohme et al., 1994). Although, mechanisms by which CsA causes hepatic injury are still not clear and still a matter of debate, several reports have strongly suggested that reactive oxygen species (ROS) production and oxidative stress are possible mechanisms of CsA toxicity (Wolf et al., 1997; Andrés et al., 2000; Durak et al., 2002).

Taurine (2-aminoethanesulfonic acid) is the major intracellular free $\beta$-amino acid, which is normally present in most mammalian tissues (Chesney, 1985). Although taurine is not a constituent of any structural mammalian protein, it plays various important physiological roles including osmoregulation, bile acid conjugation, modulation of the functioning of the central nervous system, cell proliferation, viability, and prevention of oxidant-induced injury in many tissues (Chesney, 1985; Huxtable, 1992; Redmond et al., 1996). The beneficial effects of taurine as an antioxidant in biological systems have been attributed to its ability to stabilize biomembranes (Wright et al., 1986), scavenge reactive oxygen species (Wright et al., 1985), and reduce the production of lipid peroxidation end products (Huxtable, 1992).

Taurine may have a protective effect on the deteriorated hepatic function that results from oxygen free radicals in the CsA-induced hepatotoxicity. To test this hypothesis, the present investigation examined the ability of taurine to protect against CsA-induced oxidative stress and hepatotoxicity.

2. Materials and methods

2.1. Animals

Normal adult male rats of the Wistar strain weighing between 220 ± 20 g were used in this experiment. All the animals were fed on a standard rat chow and water ad libitum and kept in a temperature controlled environment (20–22 °C) with an alternating cycle of 12 h light and dark. Experimental protocol was approved by the Institutional Animal Care and Use Committee of our University.

2.2. Experimental protocol and groups

Experiments were performed on four groups each consisting of eight rats. The control group was treated with olive oil, s.c. for 21 days. The second group received CsA (sandimmun infusion concentrate 50 mg/ml, Sandoz Ltd., dissolved in olive oil to a final concentration of 25 mg/ml) at a daily dose of 20 mg/kg s.c. for 21 days. The rats in the third group were supplemented with taurine (Sigma Chemical Co., St. Louis, MO, USA) at a concentration of 1% in the drinking water. The fourth group received taurine 3 days before and concurrently during CsA injections for 21 days. Twenty-four hours after the last dose of CsA, animals were decapitated; blood samples were obtained, centrifuged, and sera were used for enzyme assays. Livers were quickly removed, washed immediately with ice-cold physiological saline, frozen in liquid nitrogen and kept until assayed at −20 °C.

2.3. Measurement of liver functions

In serum samples, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using the commercially available kits (Spinreact Inc., France). Serum total protein and gamma glutamyl transferase (GGT) levels were determined using the kits available from (Biomérieux Inc., France). The results of ALT, AST, and GGT were expressed in international units (IU) per liter.

2.4. Determination of enzyme activities

2.4.1. Preparation of hepatic homogenate

Hepatic tissue samples were homogenized in ice-cold saline to produce 10% (w/v) homogenates, which were centrifuged at 1000 x g for 10 min at 4 °C. The supernatants were used for the measurement of MDA, GSH, catalase, and GSH-Px activities.
2.4.2. Determination of TBARS concentration

The amount of hepatic TBARS was measured as previously described by Buege and Aust (1978). Briefly, 0.5 ml of homogenates were reacted with 2 ml of TBA reagent containing 0.375% TBA, 15% trichloroacetic acid, and 0.25N HCl. Samples were boiled for 15 min, cooled and centrifuged. Absorbances of the supernatants were spectrophotometrically measured at 532 nm. TBARS concentrations were calculated by the use of 1,1,3,3-tetraethoxypropane as a standard. The results were expressed as nmol/g wet tissue weight.

2.4.3. Determination of catalase activity

Catalase activity was measured according to the method described by Higgins et al. (1978). The decomposition of hydrogen peroxide (H$_2$O$_2$, Aldrich, USA) was followed spectrophotometrically at 240 nm in 50 mM K-phosphate buffer (pH 7.05) with 19 mM H$_2$O$_2$. Twenty microliters of supernatant were used for 1 ml of reaction mixture. The specific activity of catalase was expressed as the number of mol H$_2$O$_2$ decomposed/min/mg hepatic tissue.

2.4.4. Determination of GSH-Px activity

Hepatic GSH-Px was estimated according to the method described by Paglia and Valentine (1967) using Bioxytech® GPs-340 kit (Oxis Inc., Portland, USA). GPH-Px activity was defined as the number of mol NADPH oxidized/min/g wet tissue.

2.4.5. Determination of GSH content

Hepatic GSH was determined as previously described by Ellman (1959) as modified by Nagi et al. (1992) by its reaction with Ellman’s reagent (5,5-dithio-2-nitrobenzoic acid) in phosphate buffer (pH 8.0), and the absorbance was measured at 412 nm. GSH concentration was calculated using a standard solution of GSH. The results were expressed as µmol/g wet tissue weight.

2.5. Statistical analysis

Values were expressed as mean ± S.E.M. Statistical evaluation of significant difference between means was performed with one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. The data were analyzed with SYSTAT statistical software (SYSTAT Inc.). $P < 0.05$ was considered significant.

3. Results

3.1. Effect of taurine on CsA-induced hepatotoxicity

As shown in Table 1, CsA-induced hepatotoxicity as reflected by elevated serum ALT, AST, and GGT levels ($P < 0.001$) and reduced serum total protein level ($P < 0.001$). No significant changes were observed after taurine treatment. On the other hand, rats treated with taurine and CsA had significantly increased serum total protein level ($P < 0.01$) and lower serum ALT, AST, and GGT levels compared with CsA group ($P < 0.001$).

3.2. Effect of taurine on CsA-induced oxidative stress

3.2.1. Effect of taurine on lipid peroxidation

As presented in Fig. 1, TBARS concentration was increased in CsA-treated rats ($179.66 ± 13.85$ µmol/g wet tissue, $P < 0.001$) as compared to normal controls.

### Table 1

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CsA (20 mg/kg)</th>
<th>Taurine (1% in the drinking water)</th>
<th>CsA + Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (U/l)</td>
<td>18.04 ± 2.74</td>
<td>49.87 ± 6.29$^{*}$</td>
<td>18.69 ± 0.93</td>
<td>30.5 ± 2.66$^{**}$</td>
</tr>
<tr>
<td>AST (U/l)</td>
<td>40.04 ± 1.50</td>
<td>134.5 ± 5.54$^{***}$</td>
<td>38.21 ± 1.53</td>
<td>63.08 ± 3.09$^{***}$</td>
</tr>
<tr>
<td>GGT (U/l)</td>
<td>2.46 ± 0.17</td>
<td>4.06 ± 0.14$^{**}$</td>
<td>2.32 ± 0.09</td>
<td>6.05 ± 0.17$^{***}$</td>
</tr>
<tr>
<td>Total protein (g/dl)</td>
<td>6.97 ± 0.11</td>
<td>3.88 ± 0.11$^{***}$</td>
<td>6.77 ± 0.15</td>
<td>6.65 ± 0.13$^{***}$</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E.M., n = 8.

$^{*}$ $P < 0.01$ compared with control group.

$^{**}$ $P < 0.001$ compared with control group.

$^{***}$ $P < 0.001$ compared with CsA group.
Fig. 1. Effect of taurine supplementation (1% in the drinking water) for 3 weeks on hepatic TBARS concentration in cyclosporine A-induced hepatotoxicity in adult male rats. Values are expressed as mean ± S.E.M., n = 8. *P < 0.05, **P < 0.001 compared with control group. ***P < 0.001 compared with CsA group.

3.2.2. Effect of taurine on catalase activity

As depicted in Fig. 2, the activity of catalase was significantly decreased in CsA group (27.52 ± 1.62 μmol/min/mg wet tissue, P < 0.001) as compared to normal control group (82.68 ± 3.97 μmol/min/mg wet tissue).

Fig. 2. Effect of taurine supplementation (1% in the drinking water) for 3 weeks on hepatic catalase activity in cyclosporine A-induced hepatotoxicity in adult male rats. Values are expressed as mean ± S.E.M., n = 8. *P < 0.05, **P < 0.001 compared with control group. ***P < 0.001 compared with CsA group.
Fig. 3. Effect of taurine supplementation (1% in the drinking water) for 3 weeks on hepatic GSH-Px activity in cyclosporine A-induced hepatotoxicity in adult male rats. Values are expressed as mean ± S.E.M., n = 8. **P < 0.01, ***P < 0.001 compared with control group. 

[min/mg wet tissue]. Taurine alone increased catalase activity (100.2 ± 6.6 μmol/min/mg wet tissue, P < 0.05). Taurine supplementation reduced CsA-induced catalase inhibition and increased catalase activity up to (50.31 ± 2.95 μmol/min/mg wet tissue, P < 0.01) as compared to CsA group alone.

3.2.3. Effect of taurine on GSH-Px activity

As demonstrated in Fig. 3, GSH-Px activity was reduced in CsA-treated rats (3.48 ± 0.077 μmol/min/g wet tissue, P < 0.001) as compared to normal control rats (5.94 ± 0.115 μmol/min/g wet tissue). Taurine alone enhanced GSH-Px activity (6.82 ± 0.313 μmol/
min/g wet tissue, \( P < 0.01 \). Treatment with taurine resulted in a significant increase in GSH-Px activity (5.07 ± 0.072 μmol/min/g wet tissue, \( P < 0.001 \)) as compared to CsA group alone.

### 3.2.4. Effect of taurine on GSH concentration

As given in Fig. 4, CsA produced a depletion of hepatic GSH content (0.27 ± 0.037 μmol/g wet tissue, \( P < 0.01 \)) as compared to normal control group (1.13 ± 0.282 μmol/g wet tissue). Taurine alone induced a remarkable increase in its level (3.151 ± 0.159 μmol/g wet tissue, \( P < 0.001 \)) compared with the control group. Treatment with taurine abrogated CsA-induced GSH depletion and increased GSH concentration (1.068 ± 0.071 μmol/g wet tissue, \( P < 0.05 \)) as compared to CsA group alone.

### 3.3. Correlation analysis

Using the combined results from all animals, TBARS was directly correlated with the biochemical parameters of liver injury, indicating the role of lipid peroxidation in CsA-induced hepatic injury. On the other hand, TBARS was inversely correlated with internal antioxidants. Moreover, the parameters of hepatotoxicity were inversely correlated with internal antioxidant enzymes, suggesting the importance of internal antioxidants in protecting against ROS (Table 2).

### 4. Discussion

CsA is the drug most frequently used in transplant surgery because of its potent immunosuppressive action (Sigal and Dumont, 1993). However, its clinical use is accompanied by adverse side effects such as hyperpertension, nephrotoxicity, and hepatotoxicity (Galan et al., 1995; Remuzzi and Perico, 1995). Previous studies established that ROS production and oxidative stress situation are involved in CsA hepatotoxicity (Wolf et al., 1997; Andrés et al., 2000). The present work investigated the effect of taurine supplementation on the severity of CsA-induced oxidative stress and hepatotoxicity.

In the present study, CsA-induced hepatotoxicity was characterized by significant increases in serum ALT, AST, and GGT levels and a decrease in serum total protein (Table 1). Moreover, CsA treatment produced a significant elevation in hepatic TBARS and a decline in GSH, GSH-Px, and catalase concentrations suggesting a role of oxidative stress in CsA hepatotoxicity. The relationship between oxidative stress and hepatotoxicity is patent in the present investigation, where an increase in indices of oxidative stress is positively correlated with biochemical parameters characteristic of hepatotoxicity (Table 2).

Lipid peroxidation is a chemical mechanism capable of disrupting the structure and the function of the biological membranes that occurs as a result of free radical attack on lipids. The ability of CsA to produce ROS was indicated in our study by the increased amount of hepatic lipid peroxides measured as TBARS. Other studies have demonstrated that intracellular generation of peroxides, mainly hydrogen peroxide (\( H_2 O_2 \)), could be involved in the initiation of CsA hepatotoxicity in vitro (Andrés et al., 2000; Andrés and Cascales, 2002) and in vivo (Kwak and Mun, 2000; Durak et al., 2002). Moreover, lipid peroxidation has been shown to contribute to CsA nephrotoxicity (Padi and Chopra, 2002).

The excess production of ROS by CsA may be explained by its ability to produce alterations in mitochondrial by blocking the permeability transition pore (Nicoli et al., 1995), originating an increase in mitochondrial Ca\(^{2+}\) concentration (Fournier et al., 1999), and alterations in mitochondrial electron transport chain. These events cause the oxidative phosphorylation uncoupling (Salducci et al., 1992) and the subsequent increase in ROS production. Nevertheless, the ROS generation is not exclusively at the mitochondrial level, since CsA is metabolized by cytochrome P-450 3A that can also generate ROS (Serino et al., 1994).

When ROS begin to accumulate, hepatic cells exhibit a defensive mechanism by using various antioxi-

### Table 2

Correlation coefficient (\( r \)) among oxidative stress and hepatotoxicity biochemical parameters

<table>
<thead>
<tr>
<th>Catalase</th>
<th>GSH-Px</th>
<th>GSH</th>
<th>TBARS</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>−0.836</td>
<td>−0.796</td>
<td>−0.737</td>
</tr>
<tr>
<td>ALT</td>
<td>−0.751</td>
<td>−0.841</td>
<td>−0.684</td>
</tr>
<tr>
<td>GGT</td>
<td>−0.877</td>
<td>−0.819</td>
<td>−0.823</td>
</tr>
<tr>
<td>Total protein</td>
<td>0.679</td>
<td>0.702</td>
<td>0.542</td>
</tr>
<tr>
<td>MDA</td>
<td>−0.708</td>
<td>−0.619</td>
<td>−0.695</td>
</tr>
</tbody>
</table>

\* \( P < 0.0001 \)
hepatotoxicity, e.g. melatonin (Kwak and Mun, 2000), antioxidants have been shown to reduce CsA-induced acid (Wolf et al., 1997). Vitamin E (Andres and Cascales, 2002), and ascorbic acid (Milei et al., 1992). In addition, other free radicals, thus inhibiting lipid peroxidation, oxidative stress, and hepatotoxicity. In the current study, CsA depleted GSH stores and reduced catalase and GSH-Px activities. These results are in harmony with other investigations (Duruibe et al., 1989; Wolf et al., 1994; Al Khader et al., 1996). For example, Duruibe et al. (1989) found that the total amount of liver GSH content decreased in CsA-treated rats. Moreover, CsA-induced hepatotoxicity was exacerbated by GSH depletion (Inselman et al., 1994). In the current study, depletion of GSH stores can account for the inhibition of GSH-Px activity. In addition, high levels of peroxides may explain catalase activity inhibition (Ghadermarzi and Moosavi-Movahedi, 1996).

Taurine supplementation in our study significantly mitigated CsA-induced oxidative stress and hepatotoxicity. This was clearly manifested by the improvement in all the biochemical variables determining CsA hepatotoxicity (Table 1). In addition, taurine inhibited lipid peroxidation, diminished the decrease in catalase and GSH-Px activities, and abrogated GSH depletion induced by CsA.

Consistent with our finding, taurine has been demonstrated to protect against hepatotoxicity induced by several free radicals generating insults including lipopolysaccharide (Kim and Kim, 2002), acetaminophens (Waters et al., 2001), thiocetamide (Dogru-Abbasoglu et al., 2001), and ischemia/reperfusion (Chen, 1993). Moreover, the antioxidant effect of taurine was shown in other organs including the lung (Banka et al., 1992), kidney (Saad and Al-Rikabi, 2002), and heart (Milei et al., 1992). In addition, other antioxidants have been shown to reduce CsA-induced hepatotoxicity, e.g. melatonin (Kwak and Mun, 2000), Vitamin E (Andres and Cascales, 2002), and ascorbic acid (Wolf et al., 1997).

Taurine has been demonstrated to function as a direct antioxidant that scavenges or quenches oxygen free radicals, thus inhibiting lipid peroxidation, and as an indirect antioxidant that prevents the increase in membrane permeability resulting from oxidant injury in many tissues including liver (Chen, 1993). Taurine might stimulate s-nitrosylation of GSH producing s-nitrosglutathione, which is approximately 100 times more potent than the classical GSH. In addition, s-nitrosoylation of cysteine residues by nitrosoglutathione can inactivate caspase-3, thus preventing hepatic cell apoptosis (Chiueh and Rauhala, 1999). Moreover, taurine might lessen CsA-induced oxidative injury either by forming chloramines, known to be more stable and less reactive molecules, with hypochlorous (HOCl) and HOCl-metalloproteins, or by binding free metal ions such as Fe^{2+} by its sulfonic acid group (Trachman et al., 1992; Schuller-Levis et al., 1994).

As an indirect antioxidant, taurine has been proposed as a membrane stabilizer that can maintain membrane organization, prevent ion leakage and water influx, and subsequently, avoid cell swelling (Chen, 1993; Milei et al., 1992). The stabilizing effect of taurine on cellular membrane has been suggested to be associated with the interaction between taurine and polyunsaturated fatty acids in the membrane, which results in an increase in the affinity of taurine for its carrier transport and the interaction between taurine and the sites related to anion transport and water influx. This property of taurine may also partly account for its protection against CsA-induced hepatocyte necrosis.

On the other hand, taurine can also function as a regulator of intracellular calcium homeostasis (Huxtable, 1992) that can be disturbed due to CsA toxicity (Fournier et al., 1999). Taurine has been shown to protect against endothelial cell death by modulating intracellular calcium fluxes (Wang et al., 1996). Finally, taurine may ameliorate CsA-induced hepatic injury by enhancing the activities of endogenous antioxidants. Support for this concept comes from our results, which show that taurine produced a remarkable significant increase in hepatic GSH level and GSH-Px and catalase activities. This could be attributed to the role of taurine in maintaining a normal IGF-I level (Dawson et al., 1999) and its antioxidant action against lipid peroxidation, thus conserving the internal antioxidants system. The stimulatory effect of taurine on endogenous antioxidants was reported by others (Erdem et al., 2000; Saad and Al-Rikabi, 2002). Together, the results of the present study demonstrate that administration of taurine has a therapeutic role...
in preventing cyclosporine-induced hepatotoxicity, possibly through its unique cytoprotective properties such as antioxidant activity.

In conclusion, we suggest that (a) the imbalance between production of oxygen free radicals and the endogenous antioxidant defense system, as a result of the effect of CsA, is the main mechanism responsible for peroxide accumulation and hepatotoxicity; and (b) taurine reduces the oxidative stress through the inhibition of lipid peroxidation (a widely known mechanism), but also through the increase of the activity of catalase and GSH-Px which replenish GSH stores and allow for the correct cell defense against ROS.

References


graft rejection refractory to high-dose methylprednisolone.

Transplantation 36, 203–204.


