Role of taurine in preventing acetaminophen-induced hepatic injury in the rat

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Waters, Eoin, Jiang Huai Wang, H. Paul Redmond, Qiong Di Wu, Elaine Kay, and David Bouchier-Hayes. Role of taurine in preventing acetaminophen-induced hepatic injury in the rat. Am J Physiol Gastrointest Liver Physiol 280: G1274–G1279, 2001.—Acetaminophen overdose causes acute liver injury in both humans and animals. This study was designed to investigate the potential role of the conditionally essential amino acid taurine in preventing acetaminophen-induced hepatotoxicity. Male Sprague-Dawley rats were administered acetaminophen (800 mg/kg) intraperitoneally. Taurine (200 mg/kg) was given 12 h before, at the time of, and 1 or 2 h after acetaminophen injection. Acetaminophen treatment increased the plasma levels of aspartate transaminase, alanine aminotransferase, and alkaline phosphatase and caused hepatic DNA fragmentation and hepatocyte necrosis. Taurine administered before, simultaneously with, or 1 h after acetaminophen resulted in significant improvement in hepatic injury as represented by decrease of hepatocellular enzyme release and attenuation of hepatocyte apoptosis and necrosis, and this correlated with taurine-mediated attenuation of hepatic lipid peroxidation. These results indicate that taurine possesses prophylactic and therapeutic effects in acetaminophen-induced hepatic injury.

cell apoptosis; cell necrosis; hepatocellular enzymes; lipid peroxidation

ACETAMINOPHEN (also known as paracetamol) is a commonly used and effective analgesic and antipyretic agent for relief of mild and moderate pain and is available as an over-the-counter medication. However, acetaminophen-induced hepatotoxicity, as the result of either deliberate overdose or accidental overdose with chronic alcohol abuse (24), is now the most frequent cause of fulminant liver failure in both the United Kingdom (7) and the United States (24) and can have a mortality rate of 90% (18).

Acetaminophen at therapeutic doses is rapidly metabolized in the liver principally through glucuronidation and sulfation, and only a small portion is oxidized by cytochrome P-450 2E1 to generate a highly reactive and cytotoxic intermediate, N-acetyl-p-benzoquinone-imine (NAPQI) (15, 25), which is quickly conjugated by hepatic glutathione to yield a harmless water-soluble product, mercapturic acid. However, after acetaminophen overdose the capacity for glucuronidation and sulfation is exceeded and a large amount of NAPQI is formed via cytochrome P-450 2E1. After glutathione is depleted, NAPQI binds covalently to hepatic parenchymal cell proteins and DNA with resultant liver injury (15, 25).

The precise mechanisms by which acetaminophen mediates hepatotoxicity in both humans and experimental animals still remain to be elucidated. Metabolic activation of acetaminophen and NAPQI binding to target proteins and DNA appear to be necessary but are not sufficient for toxicity (3). Recent evidence indicates that the generation of reactive oxygen species (1) and nitric oxide (9), lipid peroxidation (14), mitochondrial dysfunction (5), disruption of calcium homeostasis (23), and induction of apoptosis (21) are all mechanisms that may be involved in acetaminophen-induced hepatotoxicity.

Taurine, a conditionally essential amino acid, possesses a number of cytoprotective properties through its actions as an antioxidant, osmoregulator, and intracellular calcium flux regulator (12). We previously demonstrated that taurine at a pharmacological dose abrogated endothelial cell apoptosis through its antioxidant activity and regulation of intracellular calcium homeostasis (26) and attenuated apoptosis and necrosis in hepatocytes through inhibition of reactive oxygen intermediates, nitric oxide, and peroxynitrite formed by superoxide anion and nitric oxide reaction (22). These findings led us to investigate the hypothesis that taurine may exert a beneficial effect in preventing acetaminophen-induced hepatotoxicity.

MATERIALS AND METHODS

Animals. Adult male pathogen-free Sprague-Dawley rats obtained from Charles River were housed in barrier cages under controlled environmental conditions (12:12-h light/dark cycle, 55 ± 5% humidity, 23°C) and had free access to

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standard laboratory chow and water. Animals were fasted 12 h before experiments and allowed water ad libitum. All animal procedures were conducted under a license from the Department of Health, Republic of Ireland.

**Experimental protocol.** Acetaminophen was dissolved in pathogen-free normal saline to make a concentration of 30 mg/ml. Animals were anesthetized by inhalation of halothane (May & Baker, Dagenham, UK), and acetaminophen was administered intraperitoneally in an hepatotoxic dose of 800 mg/kg as determined in our preliminary study. Taurine (Sigma, St. Louis, MO) dissolved in normal saline (20 mg/ml) was injected intraperitoneally into animals at a dose of 200 mg/kg.

Sixty rats were randomly divided into six groups (n = 10 for each group): a control group that received an equivalent volume of saline, an acetaminophen-only group, a group that received taurine 12 h before acetaminophen injection, a group that received taurine and acetaminophen simultaneously, a group that received taurine 1 h after acetaminophen injection, and a group that received taurine 2 h after acetaminophen injection. Blood samples and liver specimens were collected 24 h after acetaminophen treatment.

**Measurement of hepatocellular enzyme levels in plasma.** Heparinized blood samples were taken 24 h after experiments by cardiac puncture using a 22-gauge needle under anesthesia with inhalation of halothane. Plasma aspartate transaminase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALK) were determined using a standard clinical automatic analyzer (Beckman, Brea, CA). The results of ALT, AST, and ALK activity were expressed in international units (IU) per liter.

**Assessment of DNA fragmentation in liver homogenates.** A cell death detection ELISA kit (Boehringer Mannheim, Mannheim, Germany), which quantitatively detects cytosolic histone-associated DNA fragments, was used to assess DNA fragmentation in liver homogenates (16). Briefly, liver samples collected 24 h after experiments were immediately homogenized. The cytosolic fraction was separated from liver homogenates by centrifugation at 13,000 g at 4°C for 20 min and used as antigen source in a sandwich ELISA with a primary anti-histone monoclonal antibody coated to the microtitration plate and a second anti-DNA monoclonal antibody coupled to peroxidase. The percentage of DNA fragmentation was calculated according to the following formula

\[
\text{% DNA fragments} = \left( \frac{\text{OD Value}_{\text{stimulated cell}} - \text{OD Value}_{\text{blank}}}{\text{OD Value}_{\text{control cell}} - \text{OD Value}_{\text{blank}}} \right) \times 100
\]

**Determination of hepatic lipid peroxidation.** An LPO-586 colorimetric assay kit (Oxis International, Portland, OR), which quantitatively measures the end products malonaldehyde and (E)-4-hydroxy-2-nonenal of the lipid peroxidative reaction (6), was used to assess lipid peroxidation in tissue homogenates according to the manufacturer’s instructions. Briefly, liver samples were homogenized in ice-cold 20 mM Tris-HCl buffer (pH 7.4). After centrifugation at 3,000 g at 4°C for 20 min, an aliquot of 200 μl of supernatant was used to detect a stable chromophore produced by the reaction of a provided chromogenic reagent with malonaldehyde in the supernatant at 45°C for 60 min. The absorbances of this chromophore were measured at 590 nm on a microtitrator plate reader (Dynex Technologies, Chantilly, VA). Lipid peroxidation was determined according to a malonaldehyde standard curve and expressed in micromoles per milligram of protein.

**Assessment of plasma taurine levels.** Thirty Sprague-Dawley rats were randomly divided into six groups (n = 5 per group) as described in Experimental protocol. Heparinized blood samples were collected 6 h after acetaminophen treatment by cardiac puncture using a 22-gauge needle under anesthesia by inhalation of halothane. Blood samples were centrifuged and treated with 10% 5-sulfosalicylic acid (Sigma). Plasma taurine levels were then measured by amino acid analysis on an AminoTac Amino Acid Analyser (JEOL).

**Histological examination and quantification of necrotic cells.** One lobe of the liver was removed after completion of experiments and was cut into longitudinal sections 2–4 mm in thickness. Liver slices were then fixed in 10% buffered formalin and embedded in paraffin. Hematoxylin-eosin staining was performed according to standard histological procedures on 4-μm sections. Quantification of necrotic cells was performed by image analysis (Interactive Image Processing, Alcatel, Paris, France) under light microscopy by two histopathologists who were blinded as to the study groups. Ten representative areas from each section consisting of five perportal and five perivenous zones were examined. The area of each high-power field was maintained at 1.27 mm². Areas of hepatic necrosis were delineated using the image analysis software, and the percent area of each high-power field affected was determined.

**Statistical analysis.** All data are presented as means ± SD. Statistical analysis was performed using ANOVA. Differences between groups were judged to be statistically significant when the P value was <0.05.

**RESULTS**

After acetaminophen treatment of rats for 24 h, plasma levels of hepatocellular enzymes AST, ALT, and ALK were found to be significantly elevated compared with the control group (P < 0.05; Table 1). Hepatic DNA fragmentation in the acetaminophen-treated group was also significantly increased (P < 0.05 vs control group; Table 1). Histological examination showed that acetaminophen induced the typical changes of confluent centrilobular necrosis (Fig. 1). There was also some scattered lobular chronic inflammation with individual necrotic hepatocytes and occasional confluent hepatocyte necrosis. Figure 2 illustrates the percentage of necrotic hepatocytes as determined by image analysis. Acetaminophen induced significant centrilobular hepatocyte necrosis compared with controls (P < 0.05). This necrosis was significantly attenuated with the administration of taurine 12 h before or simultaneously with acetaminophen (P < 0.05). Furthermore, a significant reduction in hepatocyte necrosis was observed when taurine was administered 1 h after acetaminophen (P < 0.05; Table 1).

**Table 1. Hepatotoxicity induced by acetaminophen treatment in the rat**

| Control | 73 ± 16 | 47 ± 7 | 698 ± 78 | 100 ± 19 |
| Acetaminophen | 175 ± 75* | 67 ± 25* | 984 ± 103* | 498 ± 84* |

Values are means ± SD; n = 10 rats. Plasma aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALK), and DNA fragments in liver homogenates were measured 24 h after animals were treated with saline as control or acetaminophen (800 mg/kg) as described in MATERIALS AND METHODS. Statistical significance compared with control group: *P < 0.05.
Fig. 1), whereas taurine administration 2 h after acetaminophen had no effect.

When taurine was given 12 h before or simultaneous with acetaminophen, there was greatest significant improvement of plasma AST, ALT, and ALK compared with the acetaminophen-only group ($P < 0.05$), with no significant differences from control levels in these enzymes (Fig. 3). More importantly, when taurine was administered 1 h after acetaminophen, hepatocellular enzyme levels in plasma were again significantly amended compared with the acetaminophen-only group ($P < 0.05$; Fig. 3). However, taurine administered 2 h after acetaminophen failed to attenuate acetaminophen-induced elevation in plasma AST and ALT (Fig. 3).

In the present study, we found that administration of acetaminophen to rats resulted in a significant increase in hepatic DNA fragmentation ($P < 0.05$ vs. control group), indicating that acetaminophen is capable of inducing hepatocyte apoptosis, which is a form of cell death that differs from necrosis and has been demonstrated to be responsible for acetaminophen-, tumor necrosis factor-$\alpha$, and Fas-induced hepatic failure (8, 17, 21). As shown in Fig. 4, taurine given 12 h before, at the time of, or 1 h...
after acetaminophen significantly reduced acetaminophen-induced accumulation of DNA fragments ($P < 0.05$ vs. acetaminophen-only group), although DNA fragmentation in these three taurine-treated groups was still significantly increased relative to the control group ($P < 0.05$). Administration of taurine 2 h after acetaminophen had no effect on attenuating acetaminophen-induced DNA fragmentation.

The formation of lipid peroxidation indicates cellular injury mediated by reactive oxygen intermediates with resultant destruction of membrane lipids and production of lipid peroxides (13). This study showed that acetaminophen significantly enhanced hepatic lipid peroxidation in the rat ($P < 0.05$ vs. control group; Fig. 5), whereas taurine administered 12 h before, at the time of, and 1 h after acetaminophen significantly inhibited hepatic lipid peroxidation ($P < 0.05$ vs. acetaminophen-only group).

As shown in Fig. 6, there were no significant differences in plasma taurine levels between the groups treated with taurine administered 12 h before, simultaneously with, or 1 h and 2 h after acetaminophen and the groups treated without taurine in which animals were given normal saline as control or acetaminophen only. Furthermore, the plasma taurine level 6 h after exogenous taurine administration in normal animals was $143 \pm 24$ mol/l, very similar to the values of plasma taurine found in the above groups. These results indicate that a rapid intracellular flux of taurine or quick intake of taurine by hepatocytes occurs after exogenous taurine administration.
sustains the potential of taurine as a treatment option for preventing acetaminophen poisoning by incorporation of taurine with acetaminophen.

Acetaminophen overdose results in the accumulation of a highly reactive and cytotoxic intermediate, NAPQI, which may cause centrilobular necrosis of hepatocytes (2). Hepatocyte apoptosis has also been shown to be a significant contributor to acetaminophen-induced liver injury and to precede necrosis (21). Reactive oxygen species (1), nitric oxide (9), lipid peroxidation (14), and disordered calcium homeostasis (23) are mechanisms that may have a contributory role in the development of liver injury after acetaminophen excess.

Results from plasma taurine measurements showed that exogenous administration of taurine in experimental animals did not result in significantly high levels of taurine in the plasma. Furthermore, normal animals that received exogenous taurine also had no significant changes in plasma taurine levels. These results indicate that the rapid disappearance of exogenous taurine in the plasma is caused by an intracellular flux of plasma taurine rather than a direct binding of plasma taurine to acetaminophen. Elevated intracellular taurine possesses the ability to protect against acetaminophen-induced hepatotoxicity through its cytoprotective mechanisms.

Recently, evidence has accumulated that supports a role for taurine in several critical functions in cell metabolism including osmoregulation, membrane protection, antioxidant defense, and regulation of cellular calcium homeostasis. Taurine has been demonstrated to function as a direct antioxidant that scavenges or quenches oxygen free radicals and inhibits lipid peroxidation and as an indirect antioxidant that prevents the increase in membrane permeability resulting from oxidant injury in many tissues including liver (4, 10). Therefore, the possible mechanism by which taurine prevents acetaminophen-induced hepatotoxicity may be associated with its antioxidant property. As clearly demonstrated in the present study, taurine significantly attenuated acetaminophen-mediated hepatic lipid peroxidation, which is mediated by the reaction of reactive oxygen intermediates with cell membrane phospholipids, indicating that taurine blocks acetaminophen-associated oxidative damage in the liver. Consistent with our finding, another study has also shown the beneficial effect of the reactive oxygen intermediate-scavenging capacity of taurine, specifically in relation to attenuation of lipid peroxidation in ischemia-reperfusion of the liver (4). Furthermore, we found previously (22) that taurine protects against lipopolysaccharide-mediated hepatocyte necrosis through inhibition of reactive oxygen intermediate and subsequent peroxynitrite formation, which may partly explain the beneficial role of taurine in preventing acetaminophen-induced hepatotoxicity as observed in the present study. On the other hand, taurine, as an indirect antioxidant, has been proposed as a membrane stabilizer, which has been shown to maintain membrane organization, prevent ion leakage and water influx, and subsequently avoid cell swelling (4, 19). 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 acetaminophen-induced hepatocyte necrosis.

Nitrergic oxide has recently been recognized as an important mediator in acetaminophen-induced hepatotoxicity (9). We demonstrated previously (22) that taurine abrogated hepatocyte apoptosis through its capacity for inhibiting nitric oxide production in an in vitro hepatocyte culture model. Hepatocyte apoptosis is significantly increased, which accounts for up to 40% of cell death within 24 h after acetaminophen treatment, and precedes hepatocyte necrosis (21). Increased nitric oxide production induced by acetaminophen is responsible not only for cell apoptosis but also for cell necrosis when nitric oxide interacts with superoxide anion to form peroxynitrite. On the other hand, acetaminophen-induced hepatotoxicity is characterized by disruption of calcium homeostasis that is strongly correlated with hepatic DNA damage accompanied by cell apoptosis and necrosis (23). Taurine can also function as a regulator of intracellular calcium homeostasis (12). Taurine appears to affect cell metabolism through a calcium biphasic effect that depends on calcium concentration. Taurine is positively inotropic at low calcium concentration and is negatively inotropic at high calcium concentration. We showed previously (26) that taurine protects against endothelial cell death by modulating intracellular calcium fluxes. Therefore, the findings in the present study that taurine prevented hepatocyte apoptosis and necrosis after acetaminophen treatment may relate to the capacity of taurine to inhibit nitric oxide production and regulate calcium homeostasis.

Together, the results of the present study demonstrate that administration of taurine has a prophylactic as well as a therapeutic role in preventing acetaminophen-induced hepatotoxicity, possibly through its unique cytoprotective properties such as antioxidant activity, inhibition of nitric oxide, and modulation of calcium homeostasis. These findings indicate that taurine deserves further consideration as a potential option for preventing acetaminophen overdose-related fulminant liver injury.

REFERENCES