Alleviation of dimethylnitrosamine-induced liver injury and fibrosis by betaine supplementation in rats

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\textbf{A B S T R A C T}

Previous studies suggested that betaine intake might antagonize the induction of oxidative stress-mediated acute liver injury through regulation of the sulfur–amino acid metabolism. In this study we examined the protective effects of betaine on chronic liver injury and fibrosis induced by dimethylnitrosamine (DMN). Male rats were supplemented with betaine (1%, w/v) in drinking water from 2 weeks prior to the initiation of DMN treatment (10 mg/(kg day), i.p., 3 days/week, for 1, 2, or 4 weeks) until sacrifice. Induction of liver injury was determined by quantifying serum alanine aminotransferase, aspartate aminotransferase activities, bilirubin levels, hepatic xenobiotic-metabolizing capacity, histopathological changes and 4-hydroxyproline levels. Development of oxidative injury was estimated by malondialdehyde (MDA) levels and total oxyradical scavenging capacity (TOSC) of liver and serum toward hydroxyl, peroxyl radicals, and peroxynitrite. Progressive changes in the parameters of liver injury and fibrosis were evident in the rats challenged with DMN. Elevation of MDA levels in liver was significant before the onset of a change in any parameters determined in this study. Betaine supplementation markedly attenuated the induction of hepatotoxicity and fibrosis by DMN. Elevation of MDA and the reduction of TOSC were also depressed significantly. Development of liver injury corresponded well with the induction of oxidative stress in rats treated with DMN, both of which are inhibited effectively by betaine supplementation. It is suggested that betaine may protect liver from fibrogenesis by maintaining the cellular antioxidant capacity.

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1. Introduction

Hepatic fibrosis is a wound-healing process in chronic liver injury, which is characterized by excess production and deposition of extracellular matrix components. It is now evident that hepatic stellate cells in the space of Disse undergo transformation under inflammatory stimuli into α-smooth muscle actin-positive myofibroblast-like cells, leading to nodule formation during fibrogenesis [1,2]. Although the precise mechanism for the activation of hepatic stellate cells still remains unclear, a consistent body of evidence suggests an important role of oxidative stress in the pathogenesis of fibrosis both in animal models and patients with chronic liver injury [3,4]. Conversely, stellate cell activation and/or proliferation are/is depressed by antioxidants or glutathione (GSH) precursors such as (−)-epigallocatechin-3-gallate and N-acetylcysteine [5–7].

It has long been realized that cirrhotic patients often have an impairment of sulfur–amino acid metabolism [8], which is attributed to a reduction of activity of critical enzymes involved in the transsulfuration reactions including methionine adenosyltransferase (MAT), methionine synthase, betaine-homocysteine methyltransferase (BHMT) and cystathionine β-synthase [9,10]. Recently it was suggested that a deficiency in MAT activity may contribute further to the predisposition to hepatocellular carcinoma [11]. Also an elevation of homocysteine in liver was shown to promote oxidative stress, which may cause mitochondrial damages in association with the activation of hepatic stellate cells [10,12,13]. However, it is still obscure how the impaired sulfur–amino acid metabolism is linked with cellular oxidative stress and fibrogenesis in liver.
Betaine, an oxidative metabolite of choline, is involved in the synthesis of methionine from homocysteine in liver. This reaction, catalyzed by BHMT, has an important role in the maintenance of hepatic methionine [14]. It was shown that betaine intake prevented a rise in plasma homocysteine concentrations after methionine intake [15,16] and increased the hepatic S-adenosylmethionine (SAM) generation [17]. Also betaine is effective against oxidative stress-mediated hepatic injury induced by a hepatotoxicant such as ethanol [18,19], chloroform [17,20], lipopolysaccharide [21] and alpha-naphthylisothiocyanate [22]. Sulfur-containing intermediates or products in the transsulfuration reactions, including SAM [23], S-methylthioadenosine [24] and taurine [25], were shown to prevent the induction of experimental hepatic fibrosis. These results suggest a possibility that the hepatoprotection provided by betaine may be associated with the inhibition of oxidative stress via its effect on the sulfur–amino acid metabolism.

The present study was aimed to examine the contribution of oxidative stress to the progression of hepatic injury and fibrosis in rats challenged with dimethylnitrosamine (DMN) for 4 consecutive weeks. We also determined the effect of betaine intake on the DMN-induced oxidative stress and ensuing liver fibrosis.

2. Materials and methods

2.1. Animals and treatments

Adult male SD rats (Dae-Han Laboratory, Seoul, Korea) were used. The use of animals was in compliance with the guidelines established by the Animal Care Committee of this institute. Rats were housed in temperature (22±2°C) and humidity (55±5%) controlled rooms with a 12-h light/dark cycle for at least 1 week before experimentation. Regular laboratory chow and purified tap water were allowed ad libitum. Betaine was dissolved in tap water (1%, w/v) that replaced the drinking water from 2 weeks prior to the initiation of DMN treatment until sacrifice. This dose of betaine was determined to be both safe and effective in previous studies [17,21,22]. Rats weighed 250–290 g at the beginning of betaine supplementation. Rats were treated with DMN (1% pyrogen-free saline, 10 mg/(kg day), i.p.) three consecutive days in 1 week for 1, 2 or 4 weeks and sacrificed 5 days after the final treatment. The dosage regimen of DMN was identical to the one suggested by George and Chandrakasan [26] except that the treatment period was extended from 3 to 4 weeks for the induction of more pronounced fibrotic changes in rats.

2.2. Determination of hepatotoxicity and lipid peroxidation

Blood was sampled from abdominal aorta in rats under light ether anesthesia. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were determined by the method of Reitman and Frankel [27]. Total bilirubin concentrations were measured using a commercially available kit (Youngdong Pharmaceutical, Seoul, Korea). Serum protein levels were determined by the method of Lowry et al. [28].

Total collagen contents in liver were estimated by quantifying 4-hydroxyproline (4-HP) [29]. Liver samples were hydrolyzed in 6N HCl. After the particles were removed by centrifugation, 3,4-dehydropoline was added to the supernatant as an internal standard, and then 4-HP and 3,4-dehydroproline were derivatized with 9-fluorenymethyl chloroformate. A HPLC system equipped with a fluorescence detector (FP-920; Jasco, Tokyo, Japan) and a 5 μm Symmetry C18 reversed phase column (4.6 mm × 250 mm; Eka Chemicals, Bohus, Sweden) was used. The emitted light was monitored at 310 nm with an excitation wavelength of 260 nm.

GSH levels in liver and plasma were determined using a HPLC separation/fluorometric detection method [30]. Livers were homogenized in a threefold volume of cold 1 M perchloric acid with 2 mM EDTA. Plasma was mixed with 10% sulfosalicylic acid. The HPLC system was equipped with a fluorescence detector (FP-920; Jasco, Tokyo, Japan) and a 3.5 μm Symmetry C18 column (4.6 mm × 75 mm; Waters, Milford, MA). Isocratic elution over a reversed phase column with 7.5% methanol and 92.5% 0.15 M sodium acetate buffer, pH 7.0, was used for the separation. Detection was performed at excitation wavelength of 360 nm and emission at 470 nm.

Malondialdehyde (MDA) levels were measured using a HPLC method [31]. Liver was homogenized in a threefold volume of cold 1.15% KCl. An aliquot of lysate was mixed with 0.2% thiobarbituric acid in 2 M sodium acetate buffer, pH 3.5, containing 5% butylated hydroxytoluene in ethanol. The mixtures were incubated at 95°C for 45 min. After centrifugation, the supernatant was injected into HPLC equipped with a fluorescence detector (FP-920; Jasco, Tokyo, Japan) and a 5 μm Symmetry C18 reversed phase column (4.6 mm × 150 mm; Eka Chemicals, Bohus, Sweden). The mobile phase was composed of 35% methanol and 65% 50 mM sodium phosphate buffer, pH 7.0. The MDA–thiobarbituric acid complex was monitored by fluorescence detection with excitation at 515 nm and emission at 553 nm.

2.3. Histopathology

The left lateral lobe of liver was sliced and fixed in 10% neutral-buffered formalin. Tissues were processed routinely and embedded in paraffin. Sections of 2–3 μm in thickness were subjected to hematoxylin and eosin staining. The hepatitis activity index (HAI), proposed by Knodell et al. [32], was used to grade the severity of the necroinflammatory process and fibrosis. HAI comprises four separate scores including perportal necrosis with or without bridging necrosis (0–10), intralobular degeneration and focal necrosis (0–4), portal inflammation (0–4) and fibrosis (0–4). An arbitrary scope was given to each microscopic field viewed at a magnification of 100–200×. A minimum of 10 fields were scored per liver slice to obtain the mean value.

2.4. Total oxyradical scavenging capacity (TOSC) assay

The method developed by Regoli and Winston [33] was employed. This assay is based on the ethylene-yielding reaction of α-keto-γ-methiolbutyric acid with hydroxyl, peroxyl radicals and peroxyxinitrite. Peroxyl radicals were generated by thermal homolysis of 2,2′-azobis-amidinopropane. Hydroxyl radicals were generated by the iron plus ascorbate-driven Fenton reaction. Peroxyxinitrite was produced by spontaneous decomposition of SIN-1. An aliquot taken from the headspace of a reaction vial was injected into GC equipped with a flame ionization detector and a Poropack N column (Supelco, Bellefonte, PA). Nitrogen was used as the carrier gas. TOSC values were quantified from the equation \( TOSC = 100 - (SA/CA \times 100) \), where SA and CA were the integrated ethylene peak areas obtained from the sample and control reactions, respectively. The specific TOSC was calculated by dividing the experimental TOSC with the weight of liver or volume of serum used.

2.5. Determination of xenobiotic-metabolizing enzyme activities

Hepatic microsomal xenobiotic-metabolizing enzyme activities were determined using the method described elsewhere [34].
Briefly, cytochrome P450 and cytochrome b\textsubscript{5} were quantified from the CO difference spectrum and the NADH difference spectrum, respectively. The activity of NADPH-dependent cytochrome P450 reductase was determined by using cytochrome c as substrate. p-Nitrophenol hydroxylase activity was determined by measuring the formation of p-nitrocatechol. Reaction mixtures (final volume of 1 ml) contained 0.1 mM p-nitrophenol, 1.0 mM ascorbic acid, 0.1 ml of microsomal suspension and 1 mM NADPH in 0.1 M potassium phosphate buffer (pH 6.8). Incubation was conducted at 37 \degree C for 3 min. Aminopyrine N-demethylase and p-nitroanisole O-demethylase activities were determined by measuring the production of formaldehyde and p-nitrophenol, respectively. Reaction mixtures consisted of 0.1 ml microsomal suspension, 1 mM NADPH, the substrate (5 mM for aminopyrine; 0.1 mM for p-nitroanisole) and 0.1 M potassium phosphate buffer (pH 7.4), in a total volume of 1.0 ml. Incubation was carried at 37 \degree C for 10 min.

2.6. Data analysis

All results expressed as mean ± S.E.M. were analyzed by Mann-Whitney test (non-parametric) or one-way analysis of variance (ANOVA) followed by Newman–Keuls multiple range test.

![Fig. 1. Changes in ALT and AST activities (A), total bilirubin and serum protein levels (B), and the relative liver weight and hepatic 4-HP levels (C) in rats treated with DMN and betaine. Rats were treated with DMN (10 mg/(kg day), i.p.) three consecutive days a week for 1, 2, or 4 weeks and sacrificed 5 days after the final treatment. Betaine-treated group was provided with 1% betaine in drinking water from 2 weeks prior to the initiation of DMN challenge until sacrifice. Each value represents the mean ± S.E.M. for at least five rats. Values with different letters are significantly different from one another at P < 0.05 (ANOVA followed by Newman–Keuls test).](image-url)
(parametric). The acceptable level of significance was established at $P < 0.05$ except when otherwise indicated.

3. Results

3.1. Effects of betaine on hepatotoxicity and fibrosis induced by DMN

Serum AST, ALT activities, total bilirubin and protein levels were all changed significantly from 2 weeks after the initiation of DMN treatment (Fig. 1A and B). The relative liver weight decreased and hepatic 4-HP was elevated progressively starting from 1 week after DMN treatment (Fig. 1C). Betaine intake for 6 weeks did not alter the body weight gain (control, $177 \pm 4$ g; betaine-treated rats, $181 \pm 5$ g). Nor were the above parameters changed, suggesting that administration of betaine is not toxic at the dose used.

Betaine supplementation attenuated the elevation of AST and ALT activities in the rats treated with DMN (Fig. 1A). Changes in total bilirubin and serum protein levels were blocked completely (Fig. 1B). Elevation of 4-HP and the reduction of the relative liver weight were also depressed significantly (Fig. 1C). Consistent with these results, ascitic fluid that overflowed at mid-abdominal incision was present in 77% of the rats (10/13) treated with DMN only, whereas in the rats supplemented with betaine the incidence decreased to 11% (1/9). The body weight gain for the DMN treatment period was also recovered significantly (DMN control, $55 \pm 12$ g; betaine + DMN group, $102 \pm 14$ g).

3.2. Histopathological examinations

The progression of liver fibrosis was examined histopathologically (Fig. 2). Betaine supplementation did not induce any pathological changes in liver. After 1 week of DMN treatment, diffuse centrilobular congestion with mild dilation of central veins was shown. Focal degeneration of hepatocytes and hemorrhage were also noted at this stage. There were mild bile duct hyperplasia and centrilobular necrosis with the infiltration of mononuclear leukocytes. Two weeks of DMN treatment resulted in distinctive centrilobular degeneration surrounded by fibrotic figures. Fibrotic tissues bridged from a central vein to a portal tract or to another

![Histopathological changes in liver of rats treated with DMN and betaine.](image-url)
vein. In rats treated with DMN for 4 weeks centriflobular necrosis with marked fibrosis was apparent. Discrete lobular architecture was pronounced. Erythroblasts, indicative of extramedullary hematopoiesis, were also observed. Central veins were surrounded by collagen fibers. Both Knodell and fibrosis scores were increased gradually by DMN treatment (Table 1). Betaine supplementation inhibited the fibrotic changes induced by DMN. Fibrotic figures were limited to adjacencies of central veins. Elevation of Knodell and fibrotic scores was also decreased by betaine supplementation.

3.3. Effects of DMN and betaine on xenobiotic-metabolizing enzyme activities

Betaine administration did not affect the hepatic xenobiotic-metabolizing enzyme system (Table 2). In rats challenged with DMN, the microsomal enzyme activities as well as the components of cytochrome P450 enzyme system, except for the NADPH-dependent cytochrome P450 reductase, were decreased. The DMN-induced changes in the microsomal xenobiotic-metabolizing enzyme system were either blocked or inhibited significantly by betaine supplementation.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Fibrosis score</th>
<th>Knodell score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Betaine (6 weeks)</td>
<td>0 ± 0</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>DMN (1 week)</td>
<td>0.8 ± 0.2</td>
<td>2.8 ± 0.2</td>
</tr>
<tr>
<td>DMN (2 weeks)</td>
<td>1.4 ± 0.4</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td>DMN (4 weeks)</td>
<td>1.6 ± 0.0</td>
<td>11.4 ± 3.0</td>
</tr>
<tr>
<td>Betaine (6 weeks) + DMN (4 weeks)</td>
<td>1.4 ± 0.4</td>
<td>8.0 ± 0.6</td>
</tr>
</tbody>
</table>

Rats were treated with DMN (10 mg/kg, i.p.), i.e., three consecutive days a week for 1, 2 or 4 weeks and sacrificed 5 days after the final treatment. Betaine-treated group was provided with 1% betaine in drinking water from 2 weeks prior to the initiation of DMN challenge until sacrifice. Each value represents the mean ± S.E.M. for five rats. Significant difference from the animals treated with DMN only for 4 weeks at P < 0.05 (Mann–Whitney test).

3.4. Changes in oxidative stress and antioxidant capacity

Hepatic MDA levels were elevated after 1 week of DMN treatment and remained at a level significantly higher than normal throughout the treatment period (Table 3). Hepatic GSH was not altered for the first week, but decreased thereafter. Plasma GSH was decreased concurrently. Betaine supplementation alone did not affect the GSH, GSSG or MDA levels, which is consistent with our previous observations [17,21,22]. The DMN-induced changes in MDA, GSH and GSSG were prevented completely by betaine intake.

The antioxidant capacity of liver and serum was measured using the TOSC assay. DMN treatment decreased the TOSC of liver cytosol toward hydroxyl, peroxyl radicals, and peroxynitrites progressively (Fig. 3A). The specific TOSC of microsomes was generally less than 20% of the cytosolic TOSC (Fig. 3B), indicating that the cytosol has a significantly greater antioxidant capacity than does the microsomal fraction. The TOSC of microsomes and serum was increasingly diminished by DMN treatment (Fig. 3B and C). The TOSC of liver or serum was not altered in the rats treated with betaine only. But the decrease in the TOSC by DMN was inhibited significantly in the rats supplemented with betaine. Betaine provided almost complete protection of antioxidant capacity in the cytosolic

### Table 2

<table>
<thead>
<tr>
<th>Control</th>
<th>Betaine</th>
<th>DMN</th>
<th>Betaine + DMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomal protein (mg/g liver)</td>
<td>201 ± 0.7a</td>
<td>19.6 ± 10a</td>
<td>12.5 ± 0.4b</td>
</tr>
<tr>
<td>Cytochrome P450 (nmol/mg protein)</td>
<td>0.91 ± 0.04a</td>
<td>0.96 ± 0.03a</td>
<td>0.69 ± 0.03b</td>
</tr>
<tr>
<td>Cytochrome b5 (nmol/mg protein)</td>
<td>0.35 ± 0.01a</td>
<td>0.34 ± 0.01a</td>
<td>0.30 ± 0.01b</td>
</tr>
<tr>
<td>NADPH-dependent cytochrome P450 reductase (nmol/(min mg) protein)</td>
<td>171.9 ± 9.7a</td>
<td>161.5 ± 5.7a</td>
<td>169.1 ± 8.2a</td>
</tr>
<tr>
<td>Aminopyrine N-demethylation (nmol/(min mg) protein)</td>
<td>6.92 ± 0.31a</td>
<td>6.97 ± 0.08a</td>
<td>3.49 ± 0.20c</td>
</tr>
<tr>
<td>p-Nitroanisole O-demethylation (nmol/(min mg) protein)</td>
<td>1.48 ± 0.03a</td>
<td>1.51 ± 0.06a</td>
<td>1.19 ± 0.05b</td>
</tr>
<tr>
<td>p-Nitrophenol hydroxylation (nmol/(min mg) protein)</td>
<td>1.14 ± 0.08a</td>
<td>1.31 ± 0.06a</td>
<td>0.65 ± 0.08b</td>
</tr>
</tbody>
</table>

Rats were treated with DMN (10 mg/kg, i.p.), i.e., three consecutive days a week for 4 weeks and sacrificed 5 days after the final treatment. Betaine-treated group was provided with 1% betaine in drinking water from 2 weeks prior to the initiation of DMN challenge until sacrifice. Each value represents the mean ± S.E.M. for five or more rats. Values with different letters (a, b, c) in the same row are significantly different from one another at P < 0.05 (ANOVA followed by Newman–Keuls test).

### Table 3

<table>
<thead>
<tr>
<th>Control</th>
<th>Betaine (6 weeks)</th>
<th>DMN (1 week)</th>
<th>DMN (2 weeks)</th>
<th>DMN (4 weeks)</th>
<th>Betaine (6 weeks) + DMN (4 weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g liver)</td>
<td>5.2 ± 0.1a</td>
<td>4.5 ± 0.3a</td>
<td>9.4 ± 0.4b</td>
<td>8.4 ± 0.4b</td>
<td>9.8 ± 1.0b</td>
</tr>
<tr>
<td>Total GSH (μM)</td>
<td>6.81 ± 0.13a</td>
<td>6.80 ± 0.18a</td>
<td>6.45 ± 0.21ab</td>
<td>6.29 ± 0.40ab</td>
<td>6.29 ± 0.40ab</td>
</tr>
<tr>
<td>Hepatic GSSG/GSH (%)</td>
<td>2.01 ± 0.06a</td>
<td>1.99 ± 0.08a</td>
<td>2.43 ± 0.15a</td>
<td>3.44 ± 0.41b</td>
<td>3.56 ± 0.36b</td>
</tr>
<tr>
<td>Plasma GSH (μM)</td>
<td>8.8 ± 0.5a</td>
<td>8.6 ± 0.2a</td>
<td>7.8 ± 0.5a</td>
<td>4.0 ± 0.3b</td>
<td>3.0 ± 0.4b</td>
</tr>
</tbody>
</table>

Rats were treated with DMN (10 mg/kg, i.p.), i.e., three consecutive days a week for 1, 2 or 4 weeks and sacrificed 5 days after the final treatment. Betaine-treated group was provided with 1% betaine in drinking water from 2 weeks prior to the initiation of DMN challenge until sacrifice. Each value represents the mean ± S.E.M. for five or more rats. Values with different letters (a, b) in the same row are significantly different from one another at P < 0.05 (ANOVA followed by Newman–Keuls test).
fraction and serum, which appeared to be somewhat less in microsomes.

3.5. Effects of betaine on early changes in liver toxicity induced by DMN

Effects of betaine administration on the early change in the parameters of DMN-induced liver injury were determined (Table 4). Serum ALT, AST activity or hepatic 4-HP was not altered by a single dose of DMN. The relative liver weight was increased slightly. The most significant change induced by a single DMN dose was the elevation of hepatic MDA levels. The elevation of hepatic MDA was markedly inhibited by betaine supplementation initiated 2 weeks prior to the DMN challenge.

4. Discussion

Hepatic stellate cells have been identified as the primary source of excess extracellular matrix production in liver fibrosis. During the development of fibrosis, these cells proliferate and undergo a process of activation. Oxidative stress that results from the increased production of reactive oxygen species and lipid peroxides is suggested to be associated with the proliferation and activation of stellate cells either directly or through paracrine stimulation of neighboring cells including injured hepatocytes, platelets, Kupffer cells and neutrophils [1–3,35,36]. Therefore, a number of studies have focused on the pathogenetic significance of oxidative stress in liver injury as well as on the therapeutic intervention of this process with antioxidant and metabolic scavengers [37,38].
In this study, DMN administration provoked fibrotic changes in liver as early as 1 week after initiation of the treatment as determined by an increase in hepatic 4-HP contents and histopathological examination. Serum parameters for liver injury were changed after 2 weeks of DMN treatment. On the other hand even a single dose of DMN increased hepatic lipid peroxidation significantly before any changes in 4-HP or other parameters were noted. Also the TOSC of liver and serum was generally decreased from 1 week after the initiation of DMN treatment. These results indicate that, in the development of liver injury by DMN, the elevation of oxidative stress precedes a change in liver collagen contents or other biochemical parameters for hepatotoxicity. It is suggested that oxidative stress may have a causal role in the induction of hepatic fibrosis by DMN.

The present results show that betaine supplementation may prevent or significantly attenuate the fibrotic changes associated with DMN treatment. Betaine administration depressed the elevation of serum hepatotoxic parameters and hepatic 4-HP, the incidence of ascites, and the histopathological changes. Deterioration of hepatic function estimated by xenobiotic-metabolizing enzyme system was also inhibited in rats supplemented with betaine. These results indicate that betaine intake may effectively antagonize the DMN-induced hepatic injury.

It has been shown that the DMN-induced toxicity is mediated by its reactive metabolites produced mainly by cytochrome P450 2E1 [39]. Therefore, an intact microsomal enzyme system is needed for the induction of DMN toxicity. In this study, betaine administration did not affect the xenobiotic-metabolizing enzyme system in liver. Cytochrome P450-mediated microsomal metabolizing activities including hydroxylation of p-nitrophenol, a substrate considered to be selective for cytochrome P450 2E1, were not altered by betaine supplementation. Thus, the antifibrotic effect of betaine may not be ascribed to the inhibition of DMN activation by cytochrome P450.

Treatment of rats with DMN resulted in a progressive increase in oxidative stress as measured by changes in GSH, MDA and TOSC values in liver and blood. Betaine supplementation blocked the changes in GSH and MDA completely, and also inhibited the reduction of TOSC significantly in rats treated with DMN. These results suggest that the preservation of antioxidant defense capacity may account for the hepatoprotective activity of betaine. Betaine prevented the reduction of TOSC more efficiently in the cytosolic fraction. Therefore, the inhibition of oxidative stress provided by betaine appeared to be mediated via its effects on the cytosolic substances and/or enzymes involved in the antioxidant defense.

Betaine, up to 20 mM, does not exhibit any oxi-radical scavenging capacity (unpublished observation in this laboratory), indicating that betaine per se does not have a direct antioxidant activity. Inhibition of oxidative stress by betaine is most probably expressed via its effect on the metabolism of sulfur-containing substances in the transsulfuration pathway. It has been shown that betaine serves as a regulator of hepatic metabolism of sulfur–amino acids in the previous studies conducted in our laboratory [17,40]. In those studies betaine was shown to enhance the recycling of homocysteine for synthesis of methionine, resulting in increased SAM synthesis while the generation of cystathionine is reduced. It has been reported that a change in hepatic levels of sulfur-containing metabolites is closely associated with the development of hepatic fibrosis [9,10]. Recent studies suggest that the elevation of hepatic homocysteine levels may trigger activation of stellate cells through oxidative stress [10,12,13]. On the other hand SAM administration was shown to inhibit the development of liver fibrosis induced by carbon tetrachloride [23].

Another possibility is that betaine would inhibit the production of reactive oxygen species and nitrogen species through the regulation of Kupffer cells. Betaine has been identified as an important organic osmolyte in Kupffer cells [41]. In both acute and chronic liver disease, Kupffer cells become activated to produce cytokines and reactive oxygen radicals [3,42]. It has been shown that betaine decreases lipopolysaccharide–stimulated cyclooxygenase–2 induction and prostaglandin E2 formation [41], and protects liver against ischemia-reoxygenation injury [43]. These results are compatible with our previous study showing that betaine prevented the lipopolysaccharide-induced liver injury, which was accompanied with a decrease in the elevation of tumor necrosis factor-alpha and nitric oxide [21].

Although oxidative stress is suggested to be an important fibrogenic stimulus and the levels of antioxidants such as carotenoids and tocopherol are frequently decreased in chronic liver disease [44], conflicting results have been obtained in clinical trials with vitamin E and C [45,46]. The reason for this discrepancy may be due, in part, to a compensatory decrease in antioxidant enzyme expression in patients treated with antioxidants. Cellular antioxidant enzymes and low molecular weight antioxidants work cooperatively to protect cells against various endogenous and exogenous oxidants. Therefore, administration of antioxidants for some duration could adversely affect the activities of antioxidant enzymes and vice versa. In fact an aggravation of cellular redox state associated with oxidative stress has been reported to result in transcriptional activation of antioxidant enzymes [47]. Also a low molecular antioxidant such as GSH was shown to inhibit the expression of antioxidant enzymes in response to oxidative stress [48]. The present results show that betaine supplementation may inhibit the development of oxidative liver injury and fibrosis induced by DMN. Betaine seems to have a distinct mechanism of action in that this substance inhibits oxidative stress without a direct interaction with oxidants. The inhibitory effect of betaine on the DMN-induced oxidative stress may be associated with its regulatory role in the metabolism of sulfur-containing substances in liver. Further studies to determine the effects of betaine on the impairment of the sulfur–amino acid metabolism in fibrotic livers are being conducted in this laboratory.

Conflict of interest

None declared.

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