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Protective Effect of Tetramethylpyrazine on Absolute Ethanol-Induced Renal Toxicity in Mice

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Key Words

Antioxidant · Absolute ethanol · Tetramethylpyrazine · Lipid peroxidation · Malonic dialdehyde · Cytochrome C

Abstract

Acute administration of absolute ethanol (10 ml/kg) per os to fasted mice produced extensive renal failure as measured by a rise in blood urea nitrogen and creatinine. Pretreatment with oral administration of tetramethylpyrazine (TMP) prevented such failure. The maximal effect against absolute ethanol-induced renal failure could be observed 1 h after TMP administration. In order to further investigate the renal protective mechanism of TMP, experiments on lipid peroxidation and superoxide scavenging activity were conducted. Renal homogenates made from mice treated with ethanol showed that TMP pretreatment had an antioxidant effect. Mice in acute renal failure had higher malonic dialdehyde concentrations than those pretreated with TMP. The renal protective mechanism of TMP was attributed, in part, to its prominent superoxide scavenging effect, which protects the kidney from superoxide-induced renal damage.

Reactive oxygen species have been implicated in the pathogenesis of a variety of acute injury models, including ischemia-reperfusion injury [1, 17, 22] and ethanol-induced renal failure [19]. Acute ingestion of absolute ethanol (5 g/kg) has been reported to lead to an accelerated increase in lipid peroxidation, an index of oxidative stress [9]. Protection against renal injury can be achieved by a variety of agents, including scavengers of hydroxylation [2, 3, 8, 16] and superoxide dismutase, which converts superoxide to hydrogen peroxide [7]. Previous studies have shown that renal failure is often associated with ischemic injury [12] and nephrectomy [14]. Absolute ethanol-induced renal failure is a useful model in mice [9], but the detailed mechanism of its pathogenesis is still not fully clear.

Oxygen is essential for life, but it may also be dangerous. Reduction of oxygen in tissue produces a number of oxygen free radicals which may induce cellular damage and even cell death. Oxygen-handling cells have different systems, e.g. superoxide dismutase, peroxidases and catalases, which protect them against the toxic effects of oxygen free radicals [24].

Tetramethylpyrazine (TMP) is a constituent of *Ligusticum wallichii* French [18]. It not only blocks the entry of extracellular calcium through calcium channels but also inhibits the release of intracellular stored calcium in vas-

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cular smooth muscle cells [4, 13, 14, 18]. In 1997, Feng et al. [4] reported that pretreatment with TMP in hypoxic isolated rat heart enhances prostaglandin I_2 outflow and attenuates the release of thromboxane A_2 in rat heart during normoxia, hypoxia and reoxygenation, and hence could significantly protect the myocardium from hypoxic injury. Actually, TMP could be useful as a therapeutic agent in ischemic heart disease with coronary artery disease by suppressing coronary vasoconstriction and ischemic changes in the tissues produced by endothelin-1 [6, 19, 29].

Since TMP has been confirmed to be a true calcium antagonist, it may play very important roles in the area of tissue protection and preservation. In addition, in its use as a highly potent antihypertensive drug, it may exert favorable effects on renal hemodynamics related to the reversal of renal vasoconstrictors [13]. Although the mechanisms of action of TMP in the setting of chronic renal failure are not yet fully established, its beneficial effects may be related to protective actions such as the reduction of renal hypertrophy, modulation of mesangial cell uptake of macromolecules, changes in the permselectivity of the glomerulus and decreased free radical formation.

Acute administration of absolute ethanol often leads to tissue damage, especially in the renal system [26]. The aims of the present study were to investigate whether TMP administration per os in mice could protect the kidney from absolute ethanol-induced lesions, and if TMP did offer protection, what its mechanism of action might be.

Methods

Animals and Treatment

Male ICR mice (about 20–25 g) were purchased from the animal center, College of Medicine, National Yang-Ming University, Taiwan. They were kept for at least 1 week on commercial diets (Fu-So Co., Taipei, Taiwan) under controlled environmental conditions (25 ± 1 °C, 55 ± 5% humidity) with free access to food and water. ICR mice were randomly divided into eight groups of 10 animals each. Group 1 (control) received saline (0.9% sodium chloride solution, 10 ml/kg p.o.), group 2 received absolute ethanol (10 ml/kg p.o.) and groups 3–5 received ethanol and TMP at doses of 10, 25 and 50 mg/kg p.o., respectively. TMP was administrated orally 30 min before oral administration of 10 ml/kg absolute ethanol. The animals were killed 1 h after administration of absolute ethanol. The procedure was described in detail in a previous report of Zhang et al. [30].

Assessment of Renal Failure Index

All blood samples were collected by cutting the carotid artery and were allowed to coagulate at room temperature for 1 h. A serum sample was used in the determination of blood urea nitrogen (BUN) and creatinine levels [26].

Determination of Lipid Peroxidation by Measurement of Thiobarbituric Acid Reactive Substance in vivo

The effect of TMP on mice renal homogenate with lipid peroxidation was determined using malonic dialdehyde (MDA)-thiobarbituric acid according to the modified method described by Yuda et al. [28]. Both the kidneys of the animals were removed and placed in an incubator for 1 h at 37 °C for homeostasis. After incubation, 9 ml of distilled water and 2 ml of 0.6% thiobarbituric acid were added to the incubated mixture, which was then subjected to vigorous shaking. The mixture was heated for 30 min in a boiling water bath. After cooling, 5 ml of n-butanol was added and the mixture was again shaken vigorously. The n-butanol layer was separated by centrifugation at 1,000 *g* for 10 min, and MDA production was measured at 532 nm [27].

Cytochrome C Test in vitro

Superoxide anions were assayed spectrophotometrically according to the reduction method described by McCord and Fridovich [11]. Xanthine oxidase converts uric acid to yield superoxide anions, followed by direct reduction of ferricytochrome C to ferrocytochrome C, which has a specific UV absorbance at a wavelength of 550 nm. When a compound shows superoxide scavenging activity, there is a decrease of the UV absorbance spectra in the reduction of ferricytochrome C.

Drugs and Chemicals

Absolute ethanol, BUN kit, creatinine kit, thiobarbituric acid, sodium dodecyl sulfate, ferric chloride, n-butanol, xanthine oxidase and cytochrome C were all purchased from Sigma Chemical Company (St. Louis, Mo., USA). Acetic acid was obtained from a local company in Taipei, Taiwan. TMP was a gift of the Institute of Chinese Materia Medica, China Academy of Traditional Chinese Medicine, Beijing, PROC.

Statistical Analysis

All data are shown as mean ± SE (n = 10). Statistical analysis was assessed by one-way analysis of variance coupled with Dunnett's test or the Newman-Keuls test. The level of significance was chosen as $p < 0.05$.

Results

Renal Protective Effect of TMP on Absolute Ethanol-Induced Acute Renal Toxicity

The results show a rise in BUN and creatinine values produced by ethanol (table 1); however, when various doses (10, 25 and 50 mg/kg) of TMP were administered as pretreatment, these values were significantly reduced.

Positive Control by TMP

TMP at 10, 25 and 50 mg/kg was able to significantly improve renal function. It is interesting to find that TMP could inhibit lipid peroxidation-induced MDA formation, even in normal control kidney (table 2).

Table 1. Effect of TMP on absolute ethanol-induced serum BUN and creatinine increases in mice

Groups	BUN, g/dl	Creatinine, g/dl
Normal saline p.o.	17.9 ± 1.4	0.07 ± 0.04
AE p.o.	27.9 ± 1.7***	0.21 ± 0.01***
AE + TMP (10 mg/kg) p.o.	18.1 ± 1.3##	0.20 ± 0.01##
AE + TMP (25 mg/kg) p.o.	13.6 ± 1.2###	0.06 ± 0.03##
AE + TMP (50 mg/kg) p.o.	13.4 ± 1.3###	0.06 ± 0.03##

Each value represents mean ± SE (n = 10). AE = Absolute ethanol. *** $p < 0.001$ compared to the normal control group; ## $p < 0.05$, ### $p < 0.001$ compared to the absolute ethanol group (Newman-Keuls test).

Table 2. Effect of various doses of TMP (10, 25, 50 mg/kg) on lipid peroxidation in vivo

Groups	MDA, nmol/mg protein
Normal control	0.194 ± 0.030
TMP (10 mg/kg)	0.197 ± 0.007###
TMP (25 mg/kg)	0.179 ± 0.005###
TMP (50 mg/kg)	0.133 ± 0.004###

Each value represents mean ± SE (n = 10). ### $p < 0.001$ compared to the normal control group (one-way analysis of variance coupled with Dunnett's test).

Table 3. Inhibitory effect of TMP on absolute ethanol (10 ml/kg)-induced lipid peroxidation in mice renal homogenate in vivo

Groups	Concentration of PEE, %	MDA nmol/mg protein	Inhibition rate, %
Normal control	–	0.194 ± 0.030	–
AE	–	0.248 ± 0.010*	–
AE + TMP	10	0.156 ± 0.020##	37
AE + TMP	25	0.149 ± 0.004##	40
AE + TMP	50	0.119 ± 0.006##	52

Each value represents mean ± SE (n = 10). PEE = Propolis ethanol extract; AE = absolute ethanol. * $p < 0.05$ compared to the normal control group; ## $p < 0.05$ compared to the absolute ethanol group (Newman-Keuls test).

Table 4. IC_{50} of three different concentrations of TMP in the in vitro assay of the inhibition of lipid peroxidation

Groups	IC_{50}, μM
TMP (0.01 mg/ml)	0.114 ± 0.001
TMP (0.1 mg/ml)	0.112 ± 0.002
TMP (1.0 mg/ml)	0.102 ± 0.001

The data shown are those derived from a concentration response tested with three different concentrations of TMP. Each value represents the mean ± SE of three independent assays in concentration determination studies; each assay was done in triplicate.

Inhibitory Effect of TMP on Tissue Lipid Peroxidation in Absolute Ethanol-Induced Acute Renal Toxicity in vivo

It has been reported that absolute ethanol stimulates lipid peroxidation in the kidney [9]. Ethanol treatment in the present study caused a rise in the MDA value (table 3). Various doses of TMP (10, 25, 50 mg/kg) inhibited absolute ethanol-stimulated lipid peroxidation in mice kidney (table 3).

Cytochrome C Test in vitro

In the cytochrome C test, the IC_{50} of three different concentrations of TMP in the in vitro assay of the inhibition of lipid peroxidation ranged from 0.114 ± 0.001 to 0.102 ± 0.002 μM. TMP at 1.0 mg/ml exhibited the strongest superoxide scavenging activity (table 4).

Discussion

In the present study, the renal protective effect of TMP on absolute ethanol-induced renal injury was investigated in ICR mice. Serum levels of BUN and creatinine and superoxide scavenging activity were used as indicators of renal protection. As one might suspect, an increase in the serum BUN level may also be induced by muscle protein breakdown [10, 21], but muscle protein breakdown could not lead to an increase in the creatinine level [15]. As many authors have reported that an increase in both serum BUN and creatinine levels could be used clinically to imply renal insufficiency [5, 20], we decided to choose the tests of serum BUN and creatinine levels as the indication of renal damage.

The acute administration of absolute ethanol (10 ml/kg) to mice leads to a marked elevation of serum BUN and creatinine levels. This elevation of BUN and creatinine levels reflects the degree of renal injury. In the

present study, pretreatment of absolute ethanol-intoxicated mice with TMP significantly decreased renal toxicity and the serum BUN and creatinine levels (table 1). It can be concluded from these results that TMP possesses a remarkable protective effect on absolute ethanol-induced renal injuries.

With respect to the lipid peroxidative product MDA, many researchers have reported that overproduction of MDA may also lead to renal damage [23, 25]. Compared to the positive control, TMP, administered orally at various concentrations (10, 25 and 50 mg/kg), exhibited significant inhibition of lipid peroxidation and hence significantly decreased MDA formation in vivo (table 2). From table 3, it can be seen that TMP also exhibited significant inhibition of absolute ethanol-induced lipid peroxidation in vivo. These findings indicate that the decrease in MDA formation is likely to play an important role in the prevention of renal injuries induced by absolute ethanol.

Taken together, the results of the present study indicate that the production of free radicals may be involved in the pathogenesis of renal injuries induced by absolute ethanol. They also show that TMP significantly inhibits the formation of renal injuries induced by absolute ethanol, probably through its inhibitory effect on membrane lipid peroxidation and free radical formation or due to its free radical scavenging ability, and that it can thereby simultaneously decrease BUN and creatinine increases induced by absolute ethanol.

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