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Prevention of acetaminophen-induced liver toxicity by 2(*R,S*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid in mice☆

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Abstract

The cysteine (Cys) precursor 2(*R,S*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid (PTCA) was shown previously to maintain near normal levels of hepatic GSH and GSSG at 24 hr and to protect against hepatic necrosis and mortality at 48 hr after toxic doses of acetaminophen (APAP) in mice. Studies were performed in C57BL/6 mice to determine: (a) the time course of APAP-induced hepatic sulfhydryl depletion, and (b) the effectiveness of PTCA in preventing APAP-induced decreases in sulfhydryl concentrations at the time of maximal depletion. APAP (400–800 mg/kg in 50% propylene glycol; 2.65–5.29 mmol/kg) and PTCA (1–5 mmol/kg 30 min after APAP) were administered i.p. Hepatic GSH, GSSG, and Cys concentrations were determined by HPLC. Hepatocellular damage was assessed by elevations in serum glutamate-pyruvate transaminase (SGPT) activity and histopathologic examination. APAP and PTCA produced dose-dependent effects. At 4 hr after the highest dose of APAP, hepatic GSH and Cys concentrations were reduced to 5 and 14%, respectively, of values in vehicle-treated controls, and the GSSG concentration was below the sensitivity of the analytical method. At 24 hr, recovery of hepatic sulfhydryls was incomplete, and there was hepatic necrosis with an approximately 100-fold increase in SGPT activity. At the highest dose of PTCA, the concentrations of GSH, Cys, and GSSG at 4 hr after APAP (800 mg/kg) were 66, 116, and 111%, respectively, of vehicle controls. PTCA in doses of 1.75 to 5 mmol/kg attenuated the APAP-induced increases in SGPT activity. It was concluded that the protective effect of PTCA is most likely related to prevention of hepatic sulfhydryl depletion.

Keywords: Glutathione; Acetaminophen (APAP); 2(*RS*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid (PTCA); Cysteine; Hepatotoxicity

1. Introduction

APAP is a widely used analgesic and antipyretic drug that in toxic doses can cause potentially fatal hepatic necrosis [1, 2]. It is eliminated mainly by hepatic metabolism, and the liver damage occurring at toxic doses is thought to be due to a toxic metabolite. In therapeutic doses, APAP mainly undergoes glucuronidation and sulfation in the liver [3]. In addition, small amounts are oxidized by cytochrome P450-dependent enzymes [4] to the chemically reactive intermediate NAPQI [5], which undergoes conjugation (detoxification) with GSH. After large doses, increased amounts of NAPQI are formed, resulting in increased utilization of GSH and depletion of GSH stores [6]. The fraction of NAPQI that is not conjugated with GSH can bind covalently to vital macromolecules, resulting in hepatic necrosis. Hepatic GSH depletion of 70% or greater is required before significant covalent binding of APAP to proteins occurs [6]. The biotransformation and mechanism of hepatotoxicity of APAP have been reviewed recently [7, 8]. There is considerable species variation in susceptibility to APAP-induced hepatic toxicity; the mouse is among the more susceptible species [9] and is frequently used in studies of APAP toxicity.

Administration of l-Cys, the limiting substrate for GSH biosynthesis [10], within a certain time period after toxic doses of APAP, can reduce hepatic GSH depletion [11] and prevent the development of hepatic necrosis [11, 12]. How-

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Abbreviations: APAP, acetaminophen; Cys, cysteine; MPA, metaphosphoric acid; MTCA, 2(*R,S*)-methylthiazolidine-4(*R*)-carboxylic acid; NAPQI, *N*-acetyl-*p*-benzoquinone imine; PTCA, 2(*R, S*)-*n*-propylthiazolidine-4(*R*)-carboxylic acid; SGPT, serum glutamate-pyruvate transaminase; and SFU, Sigma Frankel Units.

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ever, because l-Cys in large doses causes neurotoxicity [13, 14], it is not a practical antidote for APAP overdose. *N*-Acetyl-l-cysteine, apparently serving as a Cys precursor, can also limit hepatic GSH depletion and prevent hepatic toxicity [12, 15] without producing neurotoxicity, and is clinically useful in the management of APAP poisoning [16, 17].

In addition to *N*-acetyl-l-cysteine, other Cys precursors, including certain 2-alkylthiazolidine carboxylic acids, have been shown to protect against APAP-induced hepatotoxicity and also to prevent cataract formation, which is associated with GSH depletion in the ocular lens [18, 19]. In contrast to *N*-acetyl-l-cysteine, which is converted to l-Cys enzymatically [20], there is evidence that Cys is released from these compounds nonenzymatically [21]. The alkylthiazolidine carboxylic acids MTCA and PTCA decrease APAP-induced hepatic necrosis and mortality in mice [18, 21]. Furthermore, MTCA has been shown to attenuate APAP-induced depletion of hepatic GSH and covalent binding of the drug in mice [22]. In mice receiving PTCA, hepatic GSH and GSSG concentrations were maintained at near normal levels at 24 hr after administration of toxic doses of APAP [19], but the effects at the time of maximum depletion have not been reported. Also, the effects of these compounds on possible APAP-induced changes in hepatic Cys levels have not been reported.

The present studies in mice were undertaken to: (a) further evaluate the toxicity of APAP by determining whether it causes depletion of hepatic Cys in addition to GSH and GSSG, and (b) further evaluate the protective effects of PTCA by determining (i) whether it attenuates the depletion of GSH (and possibly related compounds) at the time of maximum APAP-induced depletion, and (ii) ascertaining the dose dependence of its effects on hepatic sulfhydryl depletion and hepatic damage, as assessed by SGPT activity and histopathologic examination. The effects of butyraldehyde, which is released from PTCA along with Cys, also were investigated.

2. Materials and methods

2.1. Chemicals

APAP, GSH, Cys, GSSG, monochloroacetic acid, and heptanesulfonic acid were obtained from the Sigma Chemical Co. MPA was obtained from the Aldrich Chemical Co. PTCA was synthesized [18] and provided by Dr. Herbert T. Nagasawa (VA Medical Center, Minneapolis, MN).

2.2. Animals

Male C57BL/6 mice (Charles River Laboratories), weighing 23–28 g, were used. They were maintained on a 12-hr light–dark cycle and received food and water *ad lib.* Animals were treated between 8:00 and 9:30 a.m. to minimize the effect of diurnal variation in hepatic GSH concentration [23, 24] on the experimental results. Body weights were recorded before and after treatments.

2.3. Treatments

Mice were killed by cervical dislocation and decapitation at designated times after i.p. injection of 800 mg/kg (5.29 mmol/kg) of APAP in 50% propylene glycol in water (pH 6–7; total volume injected, 8 mL/kg) to determine the time course of depletion of hepatic sulfhydryl and disulfide compounds. In separate experiments, mice were killed either 4 or 24 hr after i.p. injection of various doses of APAP in 50% propylene glycol (400–800 mg/kg; total volume injected, 8 mL/kg) to determine the optimal dose for further experiments. In experiments on the protective effect of PTCA, mice received different i.p. doses (1.0, 1.75, 2.5, or 5.0 mmol/kg) of the compound in 0.9% NaCl (pH 7; total volume injected, 10 mL/kg) 30 min after the administration of APAP (800 mg/kg). The dose range and time of administration of PTCA after APAP were based on the finding of Nagasawa *et al.* [18] that PTCA (2.45 mmol/kg), administered i.p. 30 min or 2 hr after APAP, provides complete protection against mortality in mice, but is less effective if administered at later times. The PTCA solution was injected immediately after preparation. In control experiments, mice received comparable volumes of the vehicle alone. In some experiments, PTCA (5 mmol/kg) was administered to mice that had not received APAP. In other studies, mice received an i.p. dose (5 mmol/kg) of butyraldehyde in 0.9% NaCl 30 min after the administration of APAP (800 mg/kg). Animals were killed at 4 hr after APAP administration to determine the extent of depletion of sulfhydryl and disulfide compounds, or at 24 hr to assess hepatic sulfhydryl and disulfide concentrations and hepatocellular damage.

2.4. Assessment of hepatocellular damage

Damage was assessed by elevations in SGPT activity and histopathologic examination. Blood samples (approximately 0.5 mL) were collected from the retro-orbital sinus. Serum was separated and kept at 4° until the time of analysis. SGPT activity was determined spectrophotometrically [25], using a commercial reagent kit (Sigma Chemical Co.). For histopathologic studies, portions of liver were placed in cassettes and fixed in neutral buffered formalin for 16–18 hr. Specimens were dehydrated and embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin for histopathological examination. Hepatic necrosis was graded on a scale of 0 to 4+, as described by Mitchell *et al.* [26], in which 0 represented no necrosis; 1+, necrosis of $<$ 6% of hepatocytes; 2+, necrosis of 6–25% of hepatocytes; 3+, necrosis of 26–50% of hepatocytes; and 4+, necrosis of $>$ 50% of hepatocytes. Microscopic evaluations were performed blinded by a pathologist (M.B.R.).

2.5. Tissue preparation

Mice were killed by cervical dislocation and decapitation after blood was drawn. The liver was excised, rinsed in ice-cold 0.9% NaCl, trimmed of adherent tissues, and weighed. Samples were kept on ice and processed as quickly as possible to prevent oxidation and degradation of sulfhydryl compounds. An 11% liver homogenate (w/v) was prepared in 4.4% MPA (final concentration, 4.0%), using an all-glass Tenbroeck homogenizer, and kept on ice. After standing for 20–40 min, the homogenate was centrifuged for 1 min (10,000 *g*), and the acid-soluble fraction was collected for measurement of sulfhydryl and disulfide compounds.

2.6. Chemical analyses

GSH, GSSG, and Cys were quantified by HPLC with electrochemical detection according to the method of Richie and Lang [27], with slight modifications. In brief, 20-μL samples were injected onto a reversed-phase C18 column (Val-U-Pak HP, fully endcapped ODS, 5 μm, 250 x 4.6 mm; Chrom Tech Inc.). The mobile phase, which consisted of a solution of 0.1 M monochloroacetic acid and 2 mM heptanesulfonic acid at pH 2.8 (98%) and acetonitrile (2%), was delivered at a flow rate of 1 mL/min. The compounds were detected in the eluant with a Bioanalytical Systems dual LC4B or LC4C amperometric detector, using two Au/Hg electrodes in series with potentials of -1.2 V and $+0.15$ V for the upstream and downstream electrode, respectively. Standard curves for the analytes were plotted as peak height versus concentration of the analyte. PTCA eluted from the column later than any of the standards and did not interfere with the analyses.

In preliminary studies, MPA, which was used in the preparation of tissue homogenates, did not cause significant release of Cys from PTCA. The measured amount of Cys in an ice-cold solution (10 mL), which initially contained 4% MPA and 20 μmol PTCA, was 0.07 μmol at time zero, 0.09 μmol after 1 hr, and 0.09 to 0.1 μmol after 4 hr.

2.7. Statistical analyses

Analyses were performed using the SPSS for Windows computer program (SPSS Inc.). Differences between the means of two groups were evaluated by a two-tailed *t*-test for independent samples. The equality of variances between groups was evaluated by Levene's test; if the test indicated significant differences in variance, then separate variance *t*-tests were used. Differences between the means of three or more groups were evaluated by ANOVA. If the ANOVA indicated a significant difference among the groups, then the *post hoc* test was determined by a test of homogeneity of variances. If the test indicated equal variances, then the Tukey test was employed. If the test indicated nonequal variances, then the Dunnett T3 test was employed. A value of $P < 0.05$ was considered statistically significant.

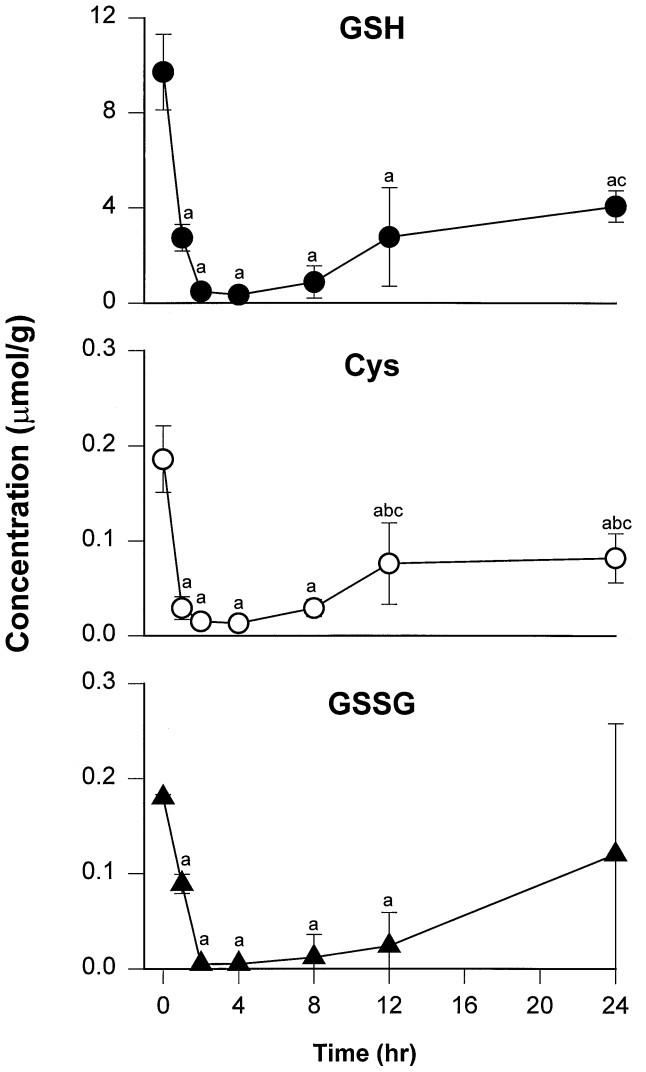

Fig. 1. Time course of depletion of hepatic sulfhydryl and disulfide compounds by APAP. APAP (800 mg/kg; 5.29 mmol/kg) in 50% propylene glycol was injected i.p. at time zero. Symbols represent means ± SD (N = 3–17). GSSG concentrations at 2 and 4 hr were below the sensitivity of the analytical method and are plotted as 5 nmol/g (the lower limit of the method). Key: (a) significantly different from the value in the untreated group (time zero value); (b) significantly different from the value at 2 hr; and (c) significantly different from the value at 4 hr. Level of significance: $P < 0.05$.

3. Results

3.1. Depletion of hepatic sulfhydryl and disulfide compounds by APAP

The time course of changes in hepatic GSH, Cys, and GSSG concentrations after the administration of APAP (800 mg/kg) is shown in Fig. 1. The concentration of each com-

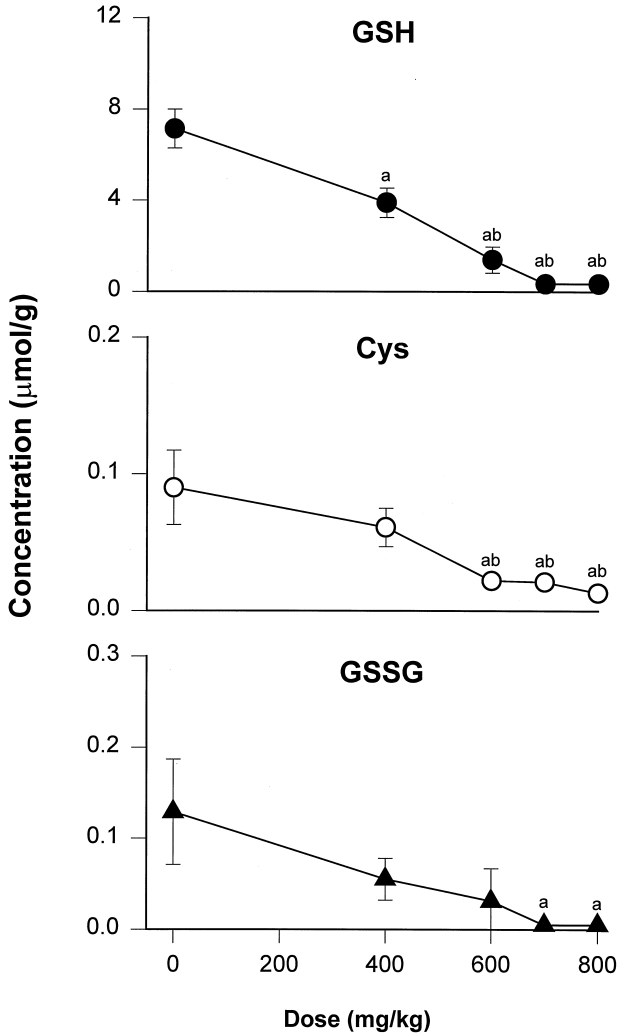

Fig. 2. Dose-dependent depletion of hepatic sulfhydryl and disulfide compounds by APAP. Concentrations were determined at 4 hr after the administration of APAP (800 mg/kg) in 50% propylene glycol. Symbols represent means ± SD (N = 3–17). GSSG concentrations at APAP doses of 700 and 800 mg/kg were below the sensitivity of the analytical method and are plotted as 5 nmol/g. Key: (a) significantly different from the value in the vehicle-treated group (dose zero value); and (b) significantly different from the value in the APAP (400 mg/kg) group. Level of significance: $P < 0.05$.

pound decreased to < 10% of the untreated control (time zero) value at 2–4 hr, and then increased gradually over the next 20–22 hr. By 24 hr, GSH, Cys, and GSSG concentrations were 43, 47, and 67%, respectively, of the control values. In subsequent experiments, sulfhydryl and disulfide depletion was determined at 4 hr after APAP administration.

The relationship between APAP dose and depletion of hepatic sulfhydryl and disulfide compounds at 4 hr after APAP administration is illustrated in Fig. 2. The concentration of GSH at an APAP dose of zero, which represents the concentration at 4 hr after the administration of 50% propylene glycol, the APAP vehicle, was about 74% of the value in untreated animals (value at time zero in Fig. 1). The corresponding concentrations of Cys and GSSG in vehicle-

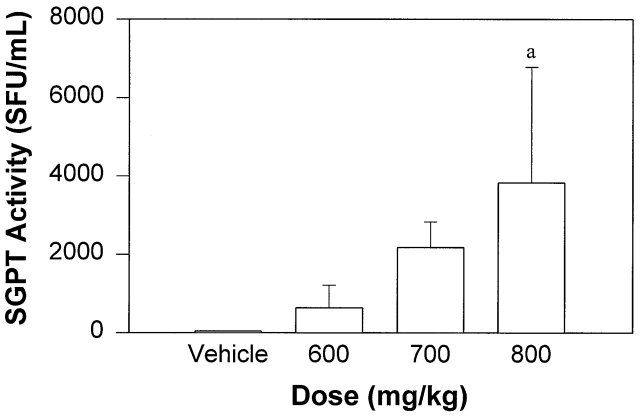

Fig. 3. Dose-dependent increases in SGPT activity by APAP. SGPT activity was determined at 24 hr after APAP administration. Data bars represent means ± SD (N = 3–17). SGPT activity in the vehicle-treated group was 38 ± 7 SFU/mL. Key: (a) significantly different ($P < 0.05$) from activity in the vehicle-treated group and the APAP (600 mg/kg) group.

treated animals were 48 and 72% of the values in untreated animals. Over the dosage range of 400–700 mg/kg, APAP produced dose-related decreases in the concentrations of the measured compounds. At an APAP dose of 800 mg/kg, there was a further small decrease in the concentration of Cys but not the other compounds. At this dose, Cys concentration was 14% of the concentration in vehicle-treated animals; the concentrations of the other compounds were ≤ 5% of the concentrations in vehicle-treated animals.

3.2. APAP-induced hepatocellular damage

APAP produced dose-related increases in SGPT activity at 24 hr (Fig. 3). At the highest dose (800 mg/kg), SGPT activity was significantly higher (3838 ± 2946 SFU/mL; mean ± SD, N = 17) than the control value (38 ± 7 SFU/mL, N = 8). Histopathologic changes produced by APAP are illustrated in Fig. 4A. Liver sections stained with hematoxylin and eosin showed extensive paracentral necrosis and degeneration of liver cells. Hepatocyte nuclei were absent or pyknotic, and the cytoplasm was homogeneous and glassy in appearance. Mean necrotic scores at 24 hr in mice receiving 50% propylene glycol (vehicle) and APAP were 0.8+ (range 0 to 1+, N = 5) and 2.0+ (range 1+ to 3+, N = 5), respectively. Based on these results, an APAP dose of 800 mg/kg was selected for use in subsequent experiments on the effects of PTCA.

3.3. Effects of PTCA on APAP-induced depletion of hepatic sulfhydryl and disulfide compounds

The effects of PTCA on APAP-induced depletion of GSH, Cys, and GSSG at 4 hr are illustrated in Fig. 5. The data on the sulfhydryl and disulfide compound concentrations in vehicle- and APAP-treated animals are the same as

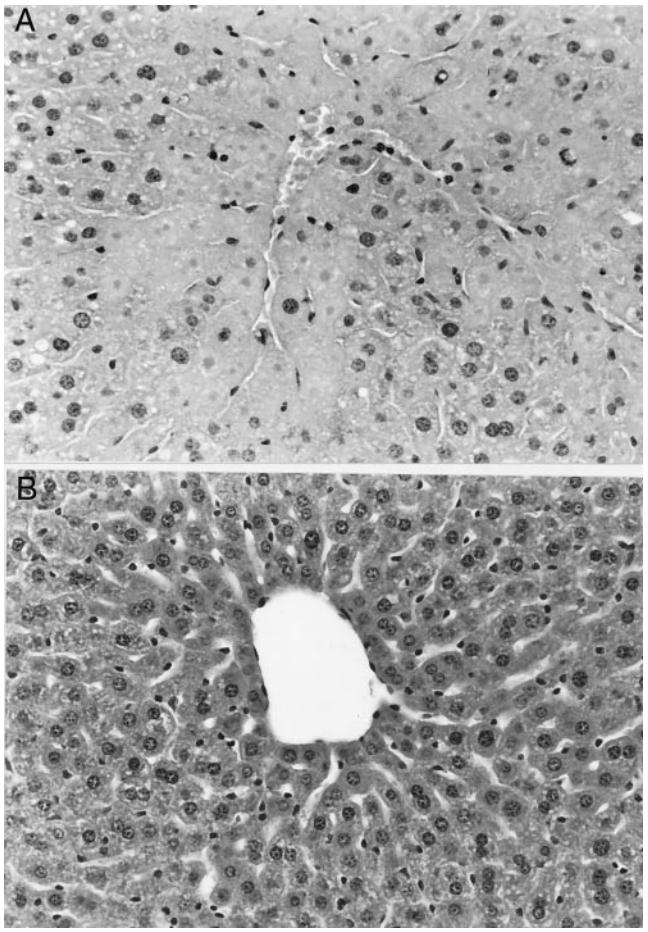

Fig. 4. Effects of PTCA on APAP-induced liver histopathology. Animals were killed at 24 hr after APAP administration. Representative photomicrographs of liver sections stained with hematoxylin and eosin (x400). (A) APAP (800 mg/kg); (B) APAP (800 mg/kg) *plus* PTCA (5 mmol/kg).

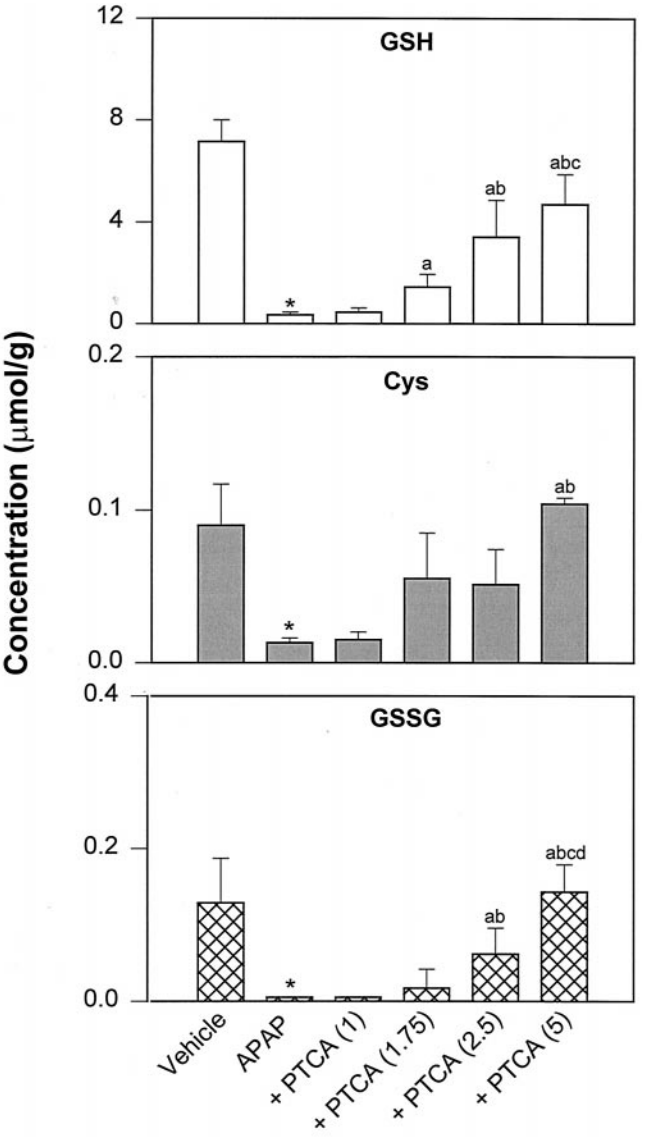

Fig. 5. Effects of PTCA on hepatic concentrations of sulfhydryl and disulfide compounds at 4 hr after administration of APAP. PTCA was injected i.p. 30 min after APAP (800 mg/kg, 5.29 mmol/kg). + PTCA denotes groups receiving APAP *plus* PTCA; the PTCA dose (mmol/kg) is shown in parentheses. Data bars represent means ± SD (N = 3–17). GSSG concentrations in the *APAP alone* and + *PTCA (1)* groups were below the sensitivity of the analytical method and are plotted as 5 nmol/g. Key: (*) significantly different from the vehicle-treated group; (a) significantly different from *APAP alone*; (b) significantly different from + *PTCA (1)*; (c) significantly different from + *PTCA (1.75)*; and (d) significantly different from + P*TCA (2.5)*. Level of significance: $P < 0.05$.

in Fig. 2. At the lowest dose tested (1.0 mmol/kg), PTCA had no demonstrable effect. At a dose of 1.75 mmol/kg, the concentration of GSH, but not the other measured compounds, was significantly higher (20% of value in vehicle-treated group) than in animals receiving APAP alone (5% of value in vehicle-treated group). At a PTCA dose of 2.5 mmol/kg, the concentrations of all measured compounds were higher than in animals receiving APAP alone, although only the GSH and GSSG concentrations were significantly different. At the highest dose (5 mmol/kg), there were significant increases in the concentrations of GSH (66% of value in vehicle-treated group), Cys (116%), and GSSG (111%). The hepatic concentrations of GSH, Cys, and GSSG at 4 hr after the administration of PTCA (5 mmol/kg) alone were 7.2 ± 1.6, 0.09 ± 0.04, and 0.14 ± 0.04 μmol/g (N = 4–5), respectively, which were not significantly different from the corresponding values in the vehicle-treated controls.

The effects of PTCA on the recovery of hepatic sulfhydryl and disulfide compounds at 24 hr after APAP administration are shown in Fig. 6. In animals receiving APAP alone, the concentration of GSSG at 24 hr had returned to levels observed in vehicle-treated animals. However, the concentrations of GSH and Cys remained significantly decreased (48 and 44%, respectively, of values in vehicle-treated group). PTCA in doses of 1.75 to 5 mmol/kg produced significantly higher GSH concentrations (89–100% of value in the vehicle-treated group) than observed in animals receiving APAP alone. PTCA had no demonstrable effect on the concentration of Cys or GSSG at 24 hr after APAP administration. The hepatic concentrations of GSH,

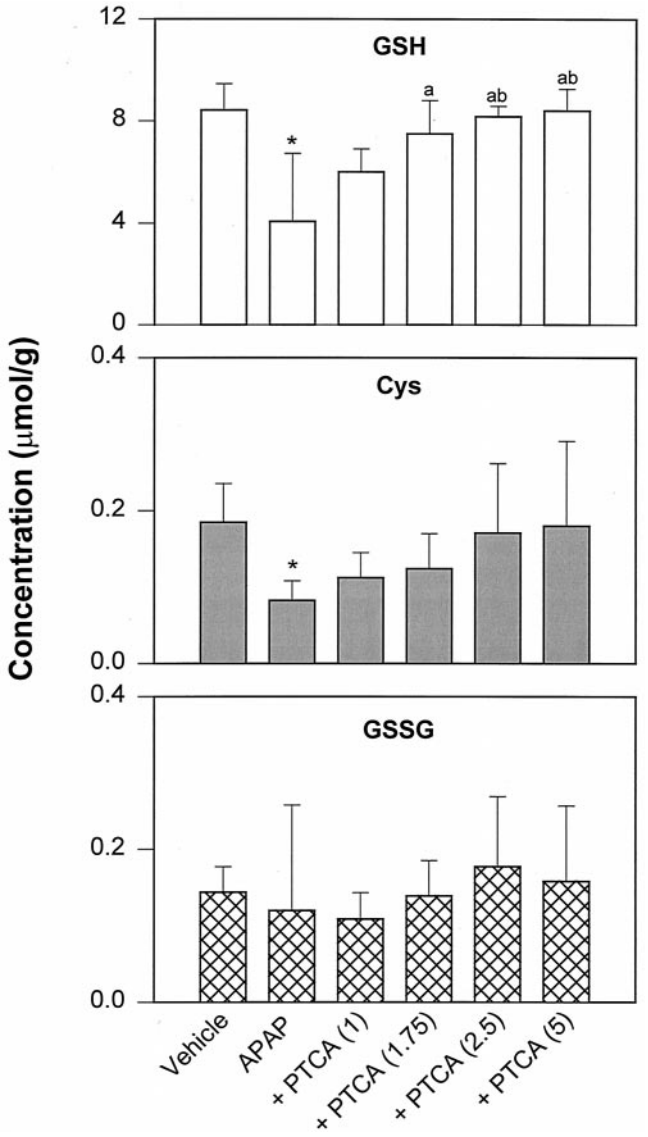

Fig. 6. Effects of PTCA on hepatic concentrations of sulfhydryl and disulfide compounds at 24 hr after administration of APAP. Data are presented as described in the legend for Fig. 5. Key: (*) significantly different from the vehicle-treated group; (a) significantly different from *APAP alone;* and (b) significantly different from + *PTCA (1).* Level of significance: $P < 0.05$.

Cys, and GSSG at 24 hr after the administration of PTCA (5 mmol/kg) alone were not significantly different from the corresponding values in vehicle-treated animals (data not shown).

3.4. Effects of PTCA on APAP-induced hepatocellular damage

PTCA (1.75 to 5 mmol/kg) protected against APAP-induced increases in SGPT activity. Activities at 24 hr after APAP administration in mice receiving PTCA in doses of 1.75, 2.5, and 5 mmol/kg were 56 ± 32 (N = 5), 50 ± 12 (N = 7), and 52 ± 17 SFU/mL (N = 4), respectively, which were significantly lower than the value with APAP alone (3838 ± 2946 SFU/mL). At a lower dose (1 mmol/kg), PTCA had no significant effect on SGPT activity (1439 ± 821 SFU/mL, N = 4). PTCA (5 mmol/kg) also protected against APAP-induced hepatic necrosis (Fig. 4B). Liver sections stained with hematoxylin and eosin showed fairly normal-appearing hepatocytes around the central vein. Binuleate liver cells and mild nuclear pleomorphism were present, suggesting a regenerative process. The mean necrotic score at 24 hr in mice receiving APAP *plus* PTCA was 0.2+ (N = 6) in comparison to the value of 2.0+ in mice that did not receive PTCA after APAP administration.

3.5. Effects of butyraldehyde on APAP-induced hepatocellular damage and hepatic sulfhydryl compound depletion

The mean SGPT activity at 24 hr in mice receiving APAP *plus* 5 mmol/kg butyraldehyde (1372 ± 319 SFU/mL; mean ± SD, N = 4) was lower than the value in mice receiving APAP alone (3838 ± 2946 SFU/mL, N = 17; $P < 0.05$) but remained much higher than the values in vehicle controls (36-fold higher; $P < 0.05$) and in mice receiving APAP *plus* PTCA in the lowest effective dose of 1.75 mmol/kg (25-fold higher, $P < 0.05$).

In APAP-treated mice, butyraldehyde had no significant effect on hepatic GSH concentration at 4 hr (0.33 ± 0.02 μmol/g, N = 7; 5% of vehicle control) or 24 hr (4.39 ± 0.84 μmol/g, N = 5; 52% of vehicle control). The apparent Cys concentration was increased at 4 hr (0.12 ± 0.04 μmol/g, N = 7; 137% of vehicle control; $P < 0.05$ vs APAP alone) but not at 24 hr (0.11 ± 0.02 μmol/g, N = 5; 58% of vehicle control). Similar effects of butyraldehyde were observed at 4 hr in mice that had not received APAP. In these mice, butyraldehyde had no effect on hepatic GSH concentration at 4 hr (6.50 ± 0.49 μmol/g; N = 3; 91% of vehicle control), but caused an apparent decrease in GSH concentration at 24 hr (6.19 ± 0.85 μmol/g, N = 5; 74% of vehicle control, $P < 0.05$). The apparent Cys concentration was increased at 4 hr (0.18 ± 0.04 μmol/g, N = 3; 198% of vehicle control; $P < 0.05$) but not at 24 hr (0.15 ± 0.07 μmol/g, N = 5; 83% of vehicle control).

4. Discussion

4.1. Sulfhydryl and disulfide concentrations in control animals

The observation that hepatic concentrations of sulfhydryl and disulfide compounds 4 hr after the administration of the APAP vehicle (50% propylene glycol) were lower than the concentrations at time zero in untreated animals (Fig. 1) is consistent with the diurnal variation in hepatic non-protein sulfhydryl levels in mice and rats [23, 24]. Some other possible explanations include a response to the stress of i.p.

injection or an effect of propylene glycol. However, the latter possibility is unlikely since McRae *et al.* [28] found that orally administered 50% propylene glycol (0.01 mL/g) does not affect hepatic GSH concentration at 4 hr in fasted mice.

4.2. Effects of APAP

The absence of mortality within 24 hr of administration of APAP in a dose of 800 mg/kg in this study contrasts with substantial 24-hr mortality in some other studies employing lower doses [e.g. Refs. 21, 22, and 26]. A probable factor in the lack of mortality is inhibition of the metabolic activation of APAP by the vehicle propylene glycol. There is evidence that cytochrome P450IIE1 is involved in the metabolic activation of APAP in humans [4] and that propylene glycol inhibits hepatic cytochrome P450IIE1-dependent metabolic activity in mice both *in vitro* [29] and *in vivo* [30]. Further, propylene glycol has been shown to reduce APAP-induced GSH depletion [30], hepatotoxicity [30, 31], and mortality [31] in mice. Another factor that may have been involved in the absence of mortality in the present study is the nutritional status of the animals. For example, Ginsberg *et al.* [32] found that APAP in 50% propylene glycol in water (800 mg/kg, p.o.; 10 mL/kg) causes high mortality within 18 hr in mice that have been fasted for 16–20 hr. However, this dose administered i.p. produced no mortality within 24 hr in the present study in which mice received food and water *ad lib.* Fasting has been shown to cause depletion of hepatic GSH and to increase APAP-induced hepatotoxicity [33, 34] and mortality [34, 35] in mice. Additional factors that could contribute to the differences in mortality in various studies with APAP include the time of day the drug was administered (due to diurnal variation in hepatic GSH concentration) and differences in susceptibility of different strains of mice.

The time course of APAP-induced hepatic GSH depletion (Fig. 1) was in general agreement with previous reports, although the minimum concentration occurred somewhat later (at 2–4 hr) than in some studies employing lower doses (1–2 hr) [6, 9, 12, 22, 36]. APAP-induced decreases in GSSG concentration as observed in the present study have also been reported previously [19, 37]. However, Arnaiz *et al.* [37] found that the decrease in GSSG concentration at 30 and 60 min after APAP administration in female Swiss mice was preceded by an increase at 15 min. GSSG concentrations prior to 1 hr after APAP administration were not determined in the present study. The present studies demonstrated that APAP causes depletion of Cys as well as GSH and GSSG, suggesting possible utilization of this compound for GSH synthesis following GSH depletion.

4.3. Protective effects of PTCA

Nagasawa and coworkers demonstrated that PTCA reduces hepatic necrosis [18] and prevents death [18, 19] in mice receiving large doses of APAP. They also determined hepatic GSH concentrations in mice that had been pretreated with β-napthoflavone to induce cytochrome P450 isozymes and thus enhance the metabolic activation of APAP. In those studies [19], animals receiving PTCA maintained nearly normal hepatic GSH levels at 24 hr after the administration of APAP. The present study extends these observations by demonstrating that, in addition to protecting against hepatic necrosis (Fig. 4), PTCA, in a dose-dependent manner, also attenuated both the APAP-induced SGPT elevation, which has been associated with hepatic necrosis [38], and the decrease in hepatic GSH concentration at the time of maximal depletion. Further, PTCA attenuated the decreases in Cys and GSSG at the time of maximal APAP-induced depletion.

The results support the hypothesis that the protective effects of PTCA are due to the release of l-Cys, which can be utilized for GSH synthesis. The dose of PTCA required to attenuate GSH depletion significantly (1.75 mmol/kg) was lower than the dose required to attenuate Cys (5.0 mmol/kg) and GSSG (2.5 mmol/kg) depletion, suggesting that Cys released from PTCA was utilized for GSH synthesis. Another possible mechanism for the protective effect would be direct reaction of the Cys released from PTCA with the active metabolite of APAP. However, studies with stereoisomers of the Cys precursors MTCA [18, 22] and *N*-acetylcysteine [12] showed that isomers that release l-Cys but not those that release d-Cys protect against APAP-induced hepatic GSH depletion, liver necrosis, plasma transaminase elevation, and mortality in mice. Also, l-Cys but not d-Cys protects against APAP-induced plasma alanine aminotransferase (GPT) elevation [12]. Since d-Cys and l-Cys would be expected to be equally capable of direct reaction with NAPQI, these results do not favor such a direct mechanism.

Butyraldehyde, which is released along with l-Cys from PTCA [18], could, in theory, contribute to the observed protective effect of the drug against APAP-induced hepatocellular damage. In mice receiving APAP *plus* butyraldehyde (5 mmol/kg), SGPT activity was lower than in mice receiving APAP alone, but remained much higher ($\geq$25-fold) than in controls or mice receiving APAP *plus* the lowest effective dose of PTCA (1.75 mmol/kg). The results of these limited studies with butyraldehyde thus suggest that any contribution of the aldehyde to the protective effects of PTCA was small and that prevention of hepatocellular damage was due mainly to the increase in GSH concentrations, presumably from the Cys released from PTCA. Butyraldehyde decreased GSH concentration in mice that had not been treated with APAP, although it may have increased Cys concentrations in both of these experimental groups.

In summary, the results of the present study demonstrated that (a) APAP causes dose-dependent decreases in the hepatic concentrations of Cys as well as GSH and GSSG, and (b) PTCA attenuates the APAP-induced changes in GSH, Cys, and GSSG in a dose-dependent man-

ner and prevents APAP-induced liver damage. It was concluded that the protective effect of PTCA is most likely related to prevention of hepatic sulfhydryl depletion.

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