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Inhibition of Cytochrome P450 2E1 Expression by 2-(Allylthio)pyrazine, a Potential Chemoprotective Agent: Hepatoprotective Effects

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ABSTRACT. Cytochrome P450 2E1 (P450 2E1) is active in both the detoxification and activation of small organic molecules. The effects of 2-(allylthio)pyrazine (2-AP) on P450 2E1-catalytic activity and the expression of rat hepatic P450 2E1 were examined. 2-AP competitively inhibited 4-nitrophenol hydroxylase activity *in vitro* (K_i, 12 μM). 2-AP treatment of rats (200 mg/kg/day, po, 1–3 days old) resulted in 20–30% decreases in the rates of P450 2E1-specific metabolic activities. Immunoblot analysis also revealed that hepatic microsomes isolated from 2-AP-treated rats showed substantial decreases in P450 2E1 level. 2-AP-suppressed isoniazid (INH)-inducible hepatic P450 2E1 levels, as shown by both metabolic activities and immunoblot analyses. Thus, 2-AP was effective in suppressing both constitutive and inducible P450 2E1 expression. Northern blot analysis showed that 2-AP transiently suppressed the hepatic P450 2E1 mRNA level, suggesting that suppression in P450 2E1 expression by 2-AP may be mediated in part by transcriptional inactivation. Hepatoprotective effects of 2-AP against toxicants were monitored in mice. 2-AP pretreatment prior to the administration of lethal doses of acetaminophen (AAP) or INH substantially reduced toxicant-induced mortality. Whereas serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were markedly elevated after AAP administration (i.e. 9–20-fold), 2-AP pretreatment of animals before AAP administration resulted in >95% decreases in elevated serum aminotransferase activities. 2-AP was also effective against CCl_4-induced hepatotoxicity. Whereas CCl_4 treatment caused 35–70-fold increases in aminotransferase activities, treatment of mice with 2-AP (>10 mg/kg) resulted in the blocking of CCl_4-induced liver toxicity. The hepatoprotective effect of 2-AP was in part due to 2-AP-induced elevation of hepatic GSH levels. Whereas AAP or CCl_4 treatment resulted in 70–80% reduction in hepatic GSH levels, pretreatment of mice with 2-AP caused a 40–210% elevation in hepatic GSH levels, as compared with either AAP or CCl_4 alone. 2-AP pretreatment also reduced AAP- or CCl_4-induced increases in lipid peroxidation in a dose-dependent manner. The results of these metabolic activities and of immunoblot and RNA blot analyses demonstrate that 2-AP is efficacious in suppressing constitutive and inducible P450 2E1 expression and effective in protecting against toxicant-induced liver toxicity. *Copyright © 1997 Elsevier Science Inc.* BIOCHEM PHARMACOL 53;3:261–269, 1997.

KEY WORDS. cytochrome P450 2E1; 2-(allylthio)pyrazine; hepatoprotective agent; enzyme inhibition

P450 2E1,¶ an ethanol-inducible form of P450, is active in the metabolism of a variety of small organic molecules. The reactive intermediates formed during the metabolism of organic molecules such as nitrosamines, benzene and phenol are associated with covalent binding, tissue necrosis and/or tumorigenesis [1–4]. For example, the hepatotoxicity of AAP and carbon tetrachloride appeared to be potentiated by organic P450 2E1 inducers and fasting and diabetes states, as shown in animal and clinical studies [5–6]. P450 2E1 catalyzes a number of other small molecules including acetone, alcohols, carbon tetrachloride and NDMA [7–9]. Thus, the level of P450 2E1 expression is likely correlated with the production of reactive metabolic intermediates generated from a wide variety of small organic compounds, and the induction of hepatic and renal P450 2E1 provides a high propensity to generate reactive oxygen species and promote lipid peroxidation with destruction of microsomal membranes and the other P450s, as shown in preoperative fasting and ether anesthesia [10].

Dietary anticarcinogenic compounds that prevent cancer include DAS, a component of garlic oil, which exhibits potent inhibitory activity toward 1,2-dimethylhydrazine-induced colon and liver cancer [11, 12]. DAS also suppresses P450 2E1 activity [13, 14]. Moreover, the organosulfur compounds including DAS, allylmercaptan and al-

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¶ *Abbreviations:* 2-AP, 2-(allylthio)pyrazine; AAP, acetaminophen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; DAS, diallylsulfide; GSH, glutathione; INH, isoniazid; MDA, malonedialdehyde; NDMA, N-nitrosodimethylamine; NMR, nuclear magnetic resonance; 4-NP, 4-nitrophenol; P450 2E1, cytochrome P450 2E1; PZ, pyrazine; SDS-PAGE, sodium dodecylsulfate–polyacrylamide gel electrophoresis.

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lylmethylsulfide are effective in suppressing both constitutive and chemical-inducible P450 2E1 expression in our laboratories [15–17].

The present study was designed to develop chemoprotective and/or hepatoprotective agents that potentially function through selective modulation of P450 2E1 and other drug-metabolizing enzymes. A series of experimental hepatoprotective agents including 2-AP were synthesized. The effects of 2-AP on the constitutive and inducible expression of P450 2E1 and the hepatoprotective effects of the agent against toxicants are described in this study. 2-AP was most effective in inhibiting P450 2E1-catalytic activities *in vitro* and suppressing P450 2E1 expression *in vivo* among the series of synthetic compounds. Suppression in P4502E1 expression by 2-AP, as supported by metabolic activities, immunoblot and RNA blot analyses, was consistent with its hepatoprotective effects against the insult of AAP, INH or carbon tetrachloride.

MATERIALS AND METHODS

Materials

[γ-^{32}P]dATP (3000 mCi/mmol) was purchased from Amersham (Arlington Heights, IL, USA). 5′-End and random prime labeling kits were purchased from BRL (Gaithersburg, MD, USA). Biotinylated donkey anti-goat IgG, biotinylated goat anti-rabbit IgG and streptavidin-conjugated horseradish peroxidase were purchased from BRL (Gaithersburg, MD, USA). *p*-Nitrophenol, aniline, 4-nitrocatechol, PZ, ALT and AST kits, and other reagents in the molecular studies were obtained from Sigma Chemical Co. (St. Louis, MO, USA). 2-AP was synthesized at Yuhan Research Center (Yuhan Corporation, Kumpo, Korea). The purity of 2-AP (>98%, d = 1.186, pale color) was confirmed by the analyses of NMR, infrared, ultraviolet and mass spectrum and high-pressure liquid chromatography. The chemical structure of 2-AP is shown in Fig. 1.

Animals

Male Sprague-Dawley rats (160–180 g, 6 weeks of age) and female ICR mice (20–25 g) were supplied by the Seoul National University Animal Care Facility. Rats were treated with 2-AP for 1–3 days (200 mg/kg body weight/day, po) and fasted 18 hr before being killed. Olive oil was used as an administration vehicle (1 mL/kg body weight, 20% w/v). Control animals were treated with vehicle only.

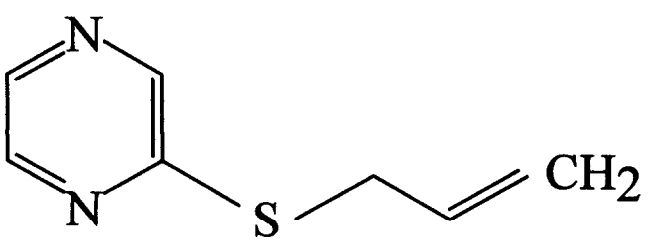

FIG. 1. Chemical structure of 2-AP.

Subcellular Fractionation

Hepatic microsomes were prepared by differential centrifugation, as described previously [17], washed in pyrophosphate buffer and stored at −70°C until used. Protein was assayed by the method of Lowry *et al.* [18].

4-NP Hydroxylase Assay

4-NP hydroxylase activity was assayed as described previously [19, 20]. Reaction mixtures contained 100 mM potassium phosphate buffer (pH 6.8), 1.0 mM ascorbic acid, 1 mM NADPH, 1 mg of hepatic microsomes and 100 μM 4-NP in a total volume of 1.0 mL. The 4-nitrocatechol formed was determined spectrophotometrically.

Aniline Hydroxylase Assay

Aniline hydroxylase was determined by measuring *p*-aminophenol formation as described by Brodie and Axelrod [21].

Gel Electrophoresis

SDS-PAGE analysis was performed according to Laemmli [22] using a BioRad Mini-Protean II apparatus. Microsomal proteins were analyzed using 7.5% separating gels.

Immunoblot Analysis

Immunoblot analysis was performed as described previously [17]. Microsomal proteins were separated by 7.5% SDS-PAGE and electrophoretically transferred to nitrocellulose paper that was immunoblotted with anti-P450 2E1 antibody, as shown previously [20]. Biotinylated donkey anti-goat IgG was used as the secondary antibody, and color was developed using streptavidin–horseradish peroxidase and 4-chloro-1-naphthol.

Isolation of Total RNA

Total RNA was isolated using the improved single-step method of thiocyanate-phenol-chloroform RNA extraction according to the method of Puissant and Houdebine [23].

Northern RNA Blot Hybridization

Northern blot analysis was performed as described previously [24]. Total RNA (20 μg) was separated on 1% agarose/2.2 M formaldehyde gel electrophoresis and transferred onto Hybond Nylon membrane. Blots were incubated in the hybridization buffer containing 6× SSC (1× SSC: 0.15 M NaCl, 15 mM sodium citrate, 100 μg/mL of sonicated salmon sperm DNA, 0.1% SDS and 5× Denhart's solution [0.1% Ficoll, 0.1% polyvinylpyrrolidine and 0.1% bovine serum albumin (Pentex Fraction V)] at 42°C for 1 hr without probe. Hybridization was performed in the same solution at 42°C for 18 hr with ^{32}P-labeled P450 2E1-specific

cDNA probe. A P450 2E1-specific cDNA probe was produced by reverse transcriptase-polymerase chain reaction, as described previously [25]. Blots were washed with 1× SSC/0.1% SDS for 15 min at room temperature, with 0.25× SSC/0.1% SDS for 15 min at 60°C, and with 0.1× SSC/0.1% SDS for 15 min at 60°C. Membranes were exposed to Kodak X-Omat AR film in a cassette containing intensifying screens at −70°C. The nylon membranes were stripped with 0.1× SSC/0.1% SDS at 90°C and probed with ^{32}P-labeled mouse β-actin cDNA probe (1.9-kb fragment). The band densities on films were quantified with a scanning laser densitometer (Molecular Dynamics). The P450 2E1 mRNA levels were normalized based on the β-actin mRNA expression.

Serum AST and ALT Assay

Serum AST and ALT activities were determined in female ICR mice (20–25 g) 18 hr following carbon tetrachloride treatment (80 mg/kg, po) with or without 3 day pretreatment with 2-AP.

Assays for Hepatic Lipid Peroxidation and GSH Contents

MDA and other degradation products of peroxidized lipids were determined as described previously [26]. GSH contents were measured as described previously [27].

RESULTS

2-AP Inhibition of P450 2E1-Metabolic Activities in vitro

The rates of 4-NP hydroxylase activity were monitored in PZ-induced rat hepatic microsomes in the presence of 1 μM to 2 mM 2-AP. Dixon plot analysis revealed that 2-AP competitively inhibited 4-NP hydroxylase activity in the PZ-induced rat hepatic microsomes, with K_i = 12 μM (Fig. 2).

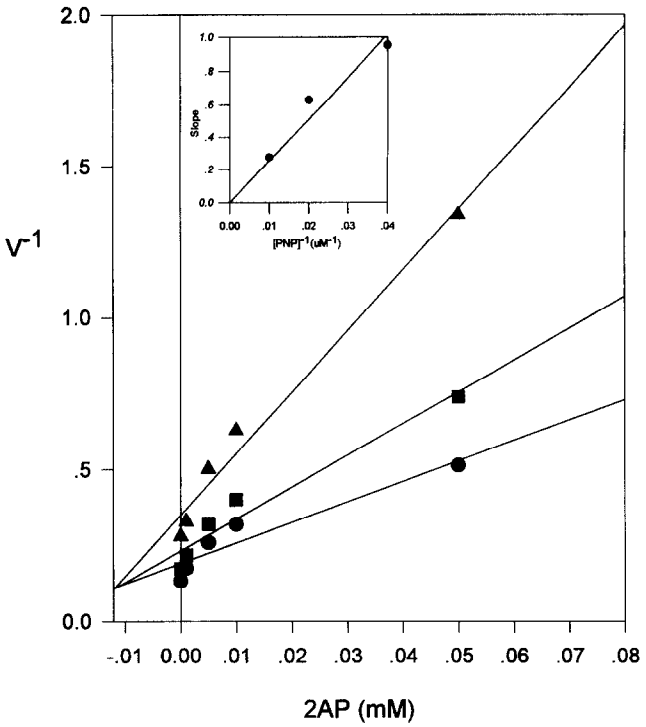

FIG. 2. Dixon plot of velocity (nmol of 4-nitrocatechol/min/mg of protein) *vs.* 2-AP concentration for the inhibition of 4-nitrocatechol formation by 2-AP at 4-NP concentrations of 25 (triangle), 50 (square) and 100 (circle) μM. Dixon plot analysis yielded a K_i value of 12 μM. Inset: Replot of the slopes of the lines from the Dixon plot confirmed competitive inhibition.

Effect of 2-AP Treatment on Constitutive Hepatic P450 2E1 Expression

Rats were treated with 2-AP to assess the effect of 2-AP on the expression of hepatic P450 2E1. 2-AP treatment at the dose of 200 mg/kg for 1–3 days resulted in 20–30% decreases in the rates of 4-NP, aniline hydroxylase or NDMA demethylase activities relative to control (Fig. 3A).

Western immunoblot analyses revealed that P450 2E1 levels in the hepatic microsomes produced from rats treated with 2-AP for 1–3 consecutive days were decreased below the limit of detectability (Fig. 3B). In contrast, PZ-induced rat hepatic microsomes, used for comparative purposes, exhibited marked time-dependent increases in P450 2E1 levels (Fig. 3B). To determine any difference in P450 2E1 expression after different routes of 2-AP administration, hepatic microsomes produced from the rats treated with the daily dose of 10–200 mg/kg of 2-AP for 3 days through either intraperitoneal or oral administration were subject to immunoblot analyses (Fig. 3C). 2-AP was effective in suppressing P450 2E1 expression at even 10 mg/kg of daily dose via either route of administration (Fig. 3C).

Effect of 2-AP Treatment on INH-Inducible P450 2E1 Expression

INH was employed as an inducer of P450 2E1 to examine whether 2-AP was effective in suppressing the inducible P450 2E1 expression. Whereas INH treatment at the daily dose of 200 mg/kg for 3 days resulted in 140%, 140% and 80% increases in the oxidation of 4-NP, aniline and NDMA, respectively, concomitant 2-AP treatment of animals at the dose of 200 mg/kg for 3 days caused complete blocking of INH-inducible microsomal oxidations below those in uninduced hepatic microsomes (Fig. 4A). Because the decreased rates of metabolic activity could reflect 2-AP inactivated P450 2E1 and possibly decreased levels in P450 2E1 protein (i.e. suppression of P450 2E1 expression), studies were carried out to examine the basis of altered activity.

Immunoblot analyses were performed to determine immunochemically detectable P450 2E1 levels in the hepatic microsomes. Rat hepatic microsomes produced after INH treatment showed notable time-dependent increases in the level of P450 2E1 with the maximal ~4-fold increase being noted at 3 days posttreatment (Fig. 4B). In contrast, hepatic microsomes prepared at day 1 after treatment of rats with

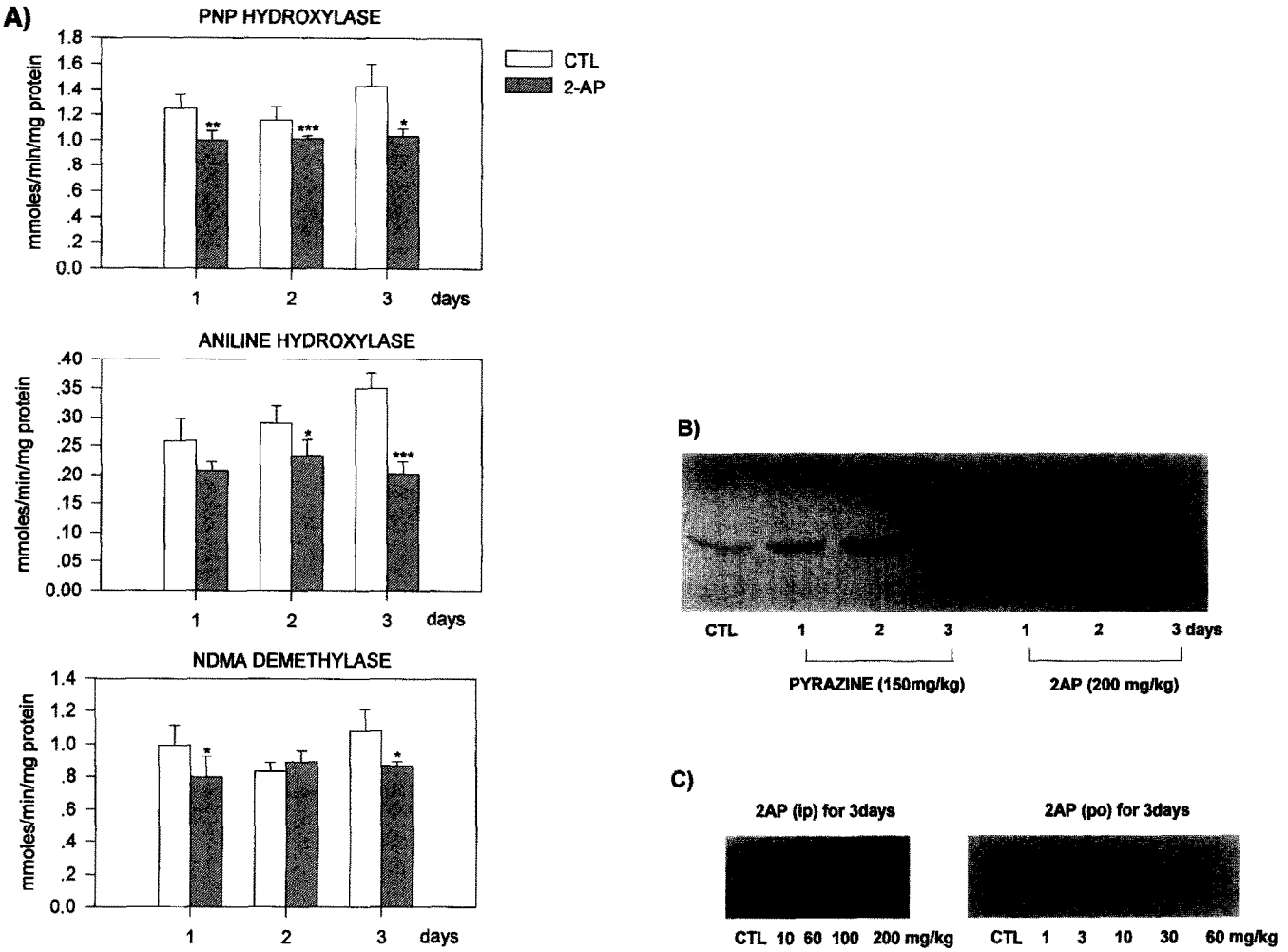

FIG. 3. The effects of 2-AP on constitutive hepatic P4502E1 levels. (A) The effects of 2-AP on 4-NP (paranitrophenol, PNP), aniline hydroxylase and NDMA demethylase activities as a function of time. The rate of metabolic activities was assayed in the hepatic microsomes produced from vehicle-treated rats or rats treated with 2-AP at the dose of 200 mg/kg (ip). Values are mean ± SD (N = 4) and are significantly different from the vehicle-treated group at *$P < 0.05$, **$P < 0.01$; ***$P < 0.001$. (B) Immunoblot analyses of rat hepatic microsomal proteins with anti-P450 2E1 antibody. Hepatic P450 2E1 in rats after consecutive daily treatment with either PZ or 2-AP. Each lane was loaded with 20 μg of rat liver microsomes produced after treatment with olive oil (CTL), PZ (150 mg/kg), or 2-AP (200 mg/kg) for 1–3 days. (C) Immunochemically detectable hepatic P450 2E1 levels after treatment with different doses of 2-AP either intraperitoneally or by oral gavage. Animals were treated with 2-AP at the daily doses of 1, 3, 10, 30, 60, 100 or 200 mg/kg for 3 days.

both 2-AP and INH exhibited significant decreases in P450 2E1 band intensity, which were even less than those in vehicle-treated animals (Fig. 4B). These results indicate that 2-AP was capable of efficiently blocking INH-inducible P450 2E1 expression. Hepatic P450 2E1 levels at day 2 or 3 after consecutive daily 2-AP treatment were not immunochemically detectable (Fig. 4B), demonstrating that 2-AP completely suppressed the expression of P450 2E1 throughout the treatment regimen. The results of metabolic activities and immunoblot analyses showed that 2-AP was effective in suppressing both constitutive and inducible P450 2E1 expression.

RNA Blot Hybridization Analyses of P450 2E1 mRNA

RNA blot hybridization analysis was performed with the RNA isolated from rat hepatic tissue after 2-AP treatment (Fig. 5A,B). 2-AP treatment transiently suppressed P450 2E1 mRNA levels at day 2 posttreatment, whereas the P450 2E1 mRNA level returned to the pretreatment level in untreated animals at day 3 posttreatment. Animals treated with different doses of 2-AP showed minimal changes in the P450 2E1 mRNA level after multiple treatment for 3 days (Fig. 5B). These results suggest that 2-AP transiently suppressed P450 2E1 mRNA levels, possibly by transcriptional inactivation, although the exact mechanism for this change in mRNA level should be clarified.

Effects of 2-AP on Toxicant-induced Mortality

To verify the hypothesis that the inhibition of P450 2E1 catalytic activity and suppression of P450 2E1 expression by 2-AP is associated with hepatoprotective effects, the protective effects of 2-AP on AAP- or INH-induced toxicity

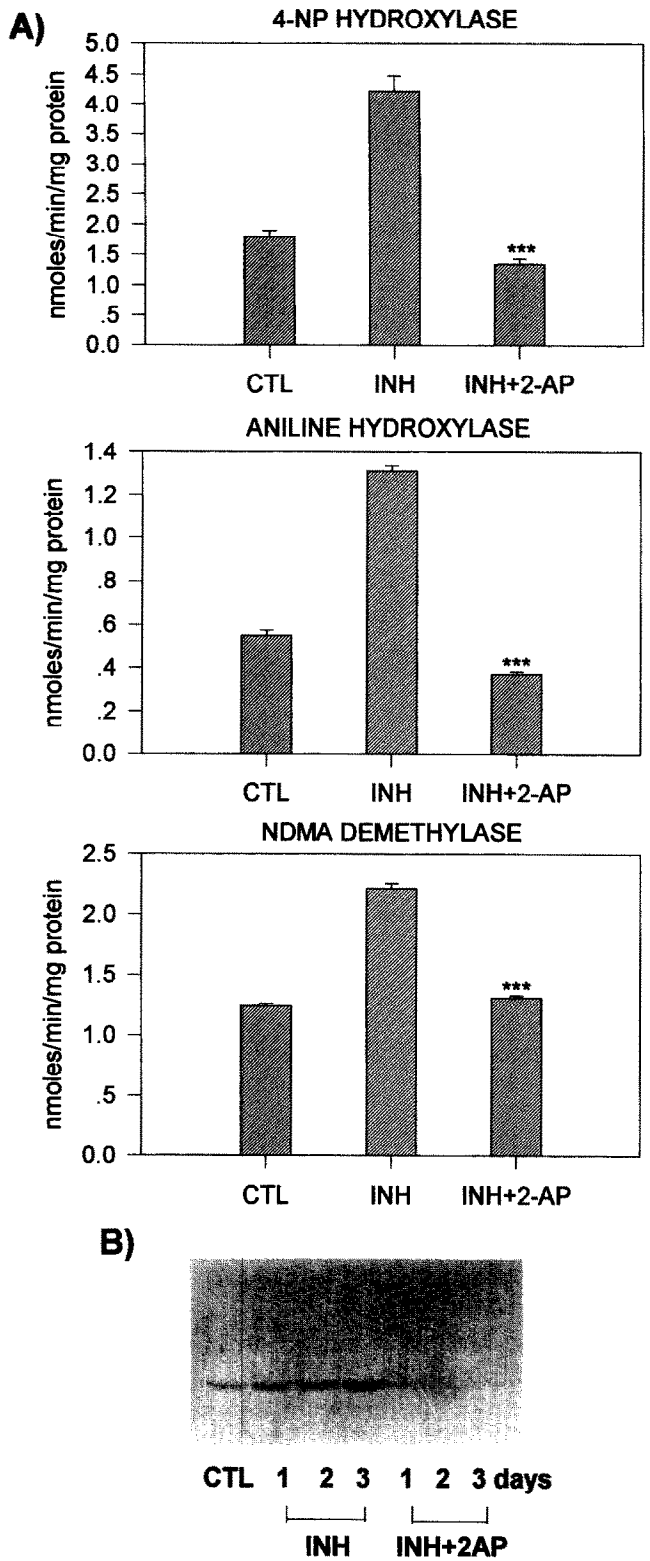

FIG. 4. The effects of 2-AP on INH-inducible P450 2E1 levels. (A) Effects of 2-AP on INH-inducible monooxygenase activities. Values are mean ± SD (N = 4) and are significantly different from the INH-treated animal group at ***$P < 0.001$. (B) Immunoblot analysis of rat hepatic microsomes with anti-P450 2E1 antibody. Each lane was loaded with 20 μg of rat liver microsomes isolated from rats treated with olive oil (CTL), INH (150 mg/kg, ip) or INH + 2-AP (200 mg/kg) for 1–3 consecutive days.

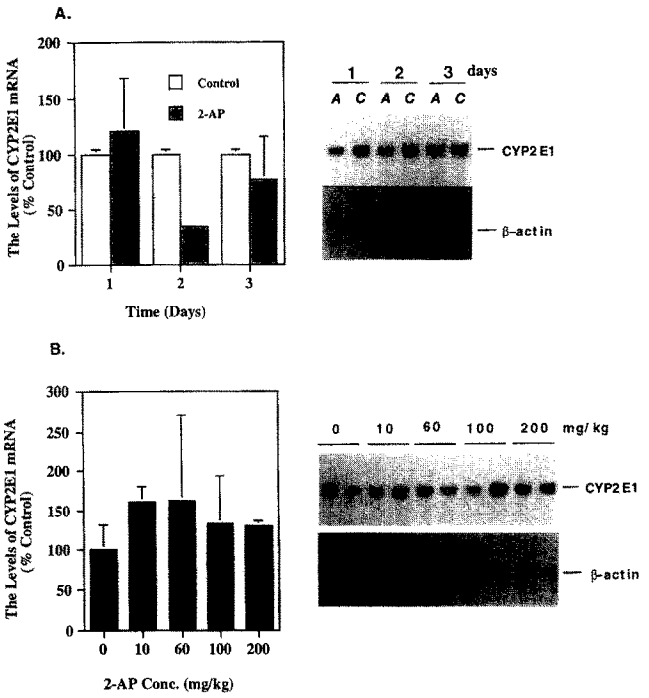

FIG. 5. Northern RNA blot analyses of P450 2E1 mRNA in rat liver after treatment with 2-AP. (A) The levels of P450 2E1 mRNA were assessed in the total RNA fractions isolated from rats treated with olive oil (C) or 2-AP (A) for 1, 2 or 3 consecutive days. The mRNA level for β-actin was used to determine mRNA loading. (B) Northern blot analyses were carried out with the samples derived from rats treated with different doses of 2-AP for 3 days. The Northern blot shows duplicate determinations. Bar graphs represent mean ± SD (N = 3).

were assessed. ICR mice were used to assess hepatoprotective effects of 2-AP because mice were more sensitive than rats to the toxicants. Pretreatment of mice with 2-AP at the dose of 25, 50 or 100 mg/kg for 2 days prior to a single lethal dose of AAP injection (800 mg/kg, ip) reduced the AAP-inducible mortality rate by 78%, 80% or 100%, respectively. Treatment of animals with 100 mg/kg of 2-AP completely blocked the AAP-induced mortality rate (Table 1).

Whereas treatment of animals with INH at the dose of 150 mg/kg resulted in an 88% death rate within 72 hr

TABLE 1. The protective effects of 2-AP against AAP- or INH-induced mortality in ICR mice

2-AP	AAP	INH
0 mg/kg	50% (4/8)	88% (7/8)
25 mg/kg	22% (2/9)	0% (0/8)
50 mg/kg	20% (2/10)	0% (0/8)
100 mg/kg	0% (0/8)	0% (0/8)

Female ICR mice were pretreated with 2-AP at the doses of 25, 50 or 100 mg/kg for 2 days (po) prior to the administration of AAP (800 mg/kg, ip) or INH (150 mg/kg, ip). Mortality was normally assessed at 3 days after the insult of AAP or INH. The reduction in mortality was confirmed subsequent to the insult of AAP, indicating that the lethality was not simply delayed. Numbers in parentheses represent the number of dead animals per total number of animals used.

posttreatment, INH-induced mortality was completely reduced by pretreatment of animals with 2-AP at the dose of 25 mg/kg or more (Table 1). Thus, the AAP- or INH-inducible mortality rate was substantially reduced in mice by prior treatment with 2-AP.

2-AP Protection Against Toxicant-induced Hepatotoxicity

The effect of 2-AP on AAP- or CCl_4-induced hepatotoxicity was monitored in mice by assaying serum AST and ALT activities (Fig. 6). Serum AST and ALT levels were elevated ~9- and 20-fold after the administration of AAP at the dose of 400 mg/kg (ip). Pretreatment of animals with 2-AP before AAP administration resulted in 95% and 96% decreases in AAP-induced elevation of serum AST and ALT activities, respectively.

2-AP was also effective against CCl_4-induced hepatotoxicity. Treatment of mice with CCl_4 at the dose of 50 μL/kg caused 35- and 70-fold increases in AST and ALT activities, respectively. Serum AST activity was ~90% reduced after treatment of animals with 80 mg/kg or greater doses of 2-AP. The decrease in CCl_4-inducible elevation of ALT activity by 2-AP pretreatment was dose related. Treatment of mice with 10, 50 or 100 mg/kg of 2-AP resulted in 75%, 81% or 91% reduction in ALT activity. These results demonstrate that 2-AP was effective in protecting the liver from toxicant-induced liver injury.

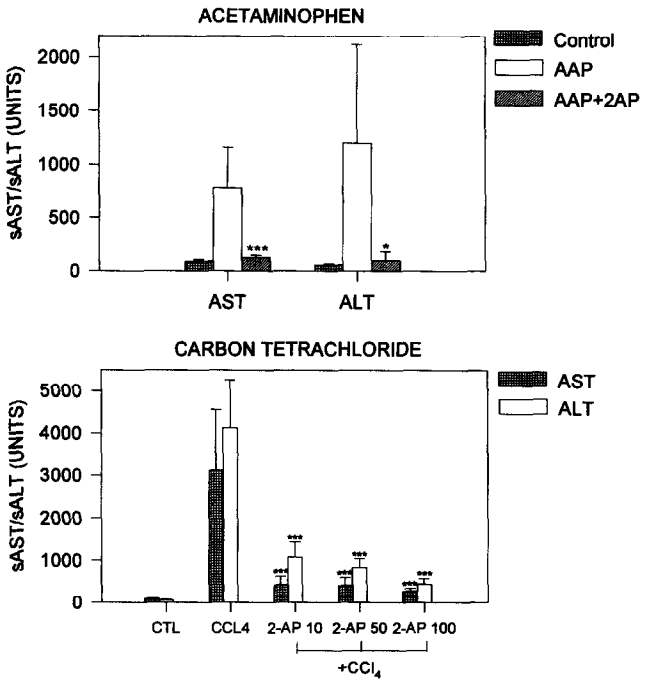

FIG. 6. Protective effects of 2-AP on AAP- or CCl_4-induced hepatotoxicity. Mice were pretreated with 2-AP at the dose of 200 mg/kg before an intraperitoneal injection of AAP (400 mg/kg) or with 2-AP at the doses of 10, 50 or 100 mg/kg before an oral gavage of CCl_4 (50 μL/kg). Serum AST or ALT activities were determined 24 hr after the injection of toxicant. Values represent mean ± SD (N = 8–10) and are significantly different from AAP- or carbon tetrachloride-treated group at *P < 0.05, ***P < 0.001.

Hepatic GSH Levels after 2-AP Treatment

GSH concentrations in the liver were determined to establish whether 2-AP-induced reduction in the mortality rate is associated with hepatic GSH contents (Fig. 7A). Hepatic GSH levels in mice were elevated by 31% or 48% at 20 hr after a single dose of 50 or 200 mg/kg 2-AP, respectively. A single intraperitoneal injection of AAP caused a 73% reduction in hepatic GSH levels. Pretreatment of mice with 50 and 200 mg/kg of 2-AP caused a 40% and 113% elevation, respectively, in hepatic GSH levels, as compared with the animals treated with AAP alone. 2-AP was also effective in elevating CCl_4-induced decreases in hepatic GSH contents in mice (Fig. 7A).

The production of MDA was assayed to examine AAP- or CCl_4-induced lipid peroxidation. 2-AP pretreatment at the dose of 50 or 200 mg/kg reduced AAP-induced increases in MDA production by 38% and 72%, respectively (Fig. 7B). Comparable dose-related decreases were observed in CCl_4-induced elevation in lipid peroxidation after 2-AP treatment (Fig. 7B). Whereas CCl_4 treatment stimulated MDA production by 1.8-fold relative to control, 2-AP pretreatment of mice at the doses of 25 and 200 mg/kg for 2 days reduced CCl_4-induced MDA production by 32% and 64%, respectively.

DISCUSSION

Hepatic cytochromes P450 contribute to overall production of reactive oxygen species more significantly than do other enzymes such as cyclooxygenases and xanthine oxidase [28]. Among the P450s, ethanol-inducible P450 2E1 is especially capable of affecting oxidative events because it has a high rate of oxidase activity even in the absence of substrate [28, 29]. P450 2E1, a constitutive enzyme in uninduced immature and adult liver, consists of 8–11% of total cytochromes P450. Moreover, this enzyme is inducible by a number of agents and by certain pathophysiological conditions [30]. The pathology of alcoholic liver disease is also worsened by increasing polyunsaturated fatty acids in the diet under the conditions of increases in P450 2E1 by high blood-alcohol levels [31–33]. P450 2E1 induction plays a central role in the pathogenesis of alcoholic liver disease due to the potential for free radical generation and results in a pronounced increase in the rate of NADPH-dependent microsomal lipid peroxidation. Lipid peroxides are also elevated in plasma and red blood cells of rats and humans during high ethanol intake. Thus, an increase in the hepatic P450 2E1 level is likely to be associated with lipid peroxidation and play a significant role in oxygen-mediated tissue toxicity. Protein adducts of lipid peroxidation products appeared to be increased, which is further supported by the enhancement of this process by INH treatment [33].

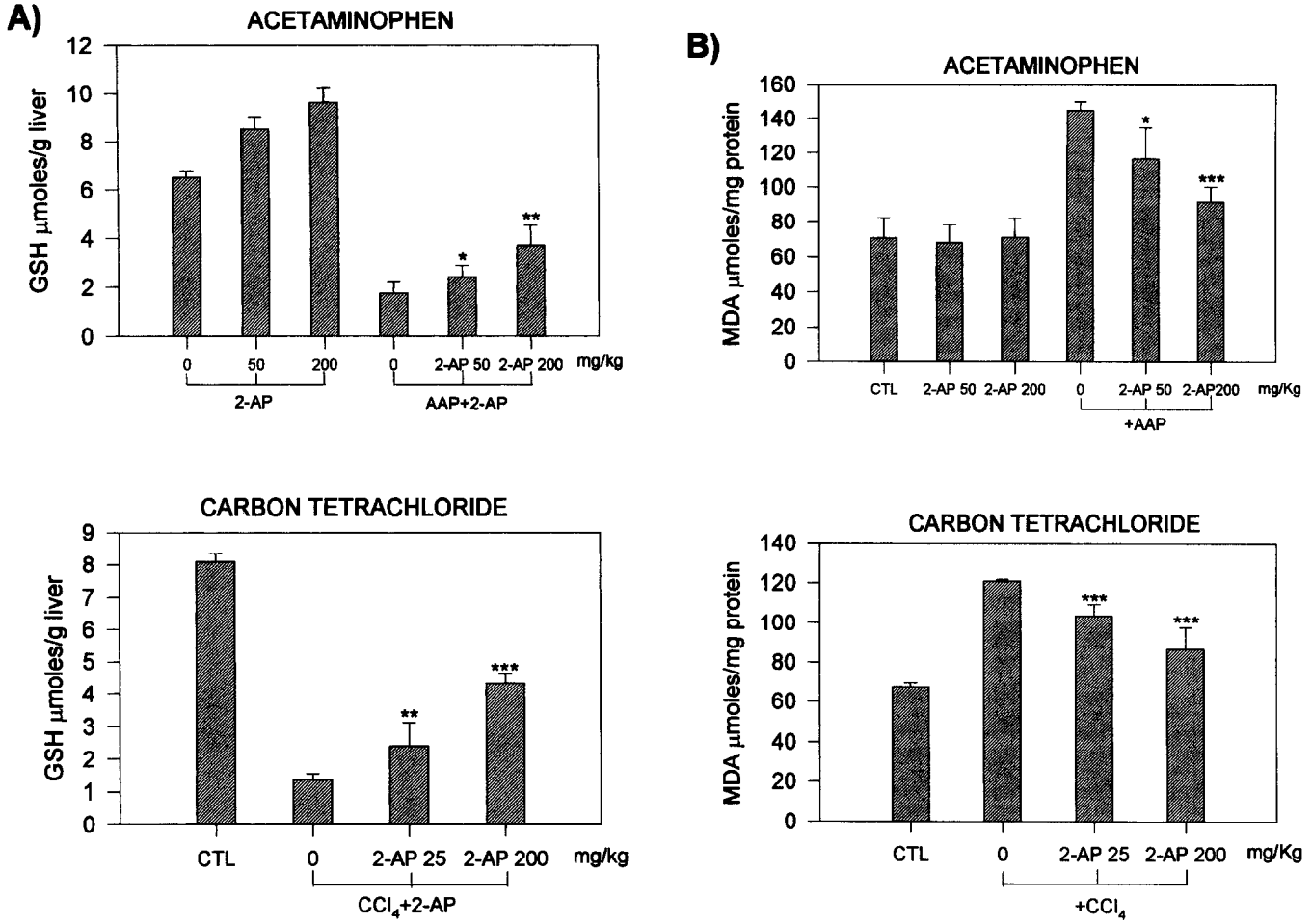

FIG. 7. The effects of 2-AP on hepatic GSH levels and on microsomal lipid peroxidation in mice. (A) Hepatic GSH levels. ICR mice were pretreated with 2-AP for 2 days at the doses of 25 through 200 mg/kg prior to the administration of AAP (400 mg/kg, ip) or CCl_4 (50 µL/kg). Values represent mean ± SD (N = 8–10) and are significantly different from AAP- or carbon tetrachloride-treated group at *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. (B) Hepatic microsomal lipid peroxidation. ICR mice were pretreated with 2-AP for 2 days at the daily doses of 25 to 200 mg/kg prior to the administration of AAP (400 mg/kg, ip) or CCl_4 (50 µL/kg). Values represent mean ± SD (N = 8–10) and are significantly different from the AAP- or carbon tetrachloride-treated group at *$P < 0.05$, ***$P < 0.001$.

These effects seem to be associated with Ito cells and Kupffer cells in conjunction with cytokine and collagen production [29]. Morimoto *et al.* showed that treatment with DAS, a P450 2E1 inhibitor, modifies toxicant-induced pathologic processes [31].

Certain nitrogen-containing heterocycles such as pyridine, thiazole and PZ inhibit P450 2E1-catalyzed metabolic activities *in vitro* and induce P450 2E1 *in vivo* after multiple treatment [20]. Interestingly, certain P450 2E1 inducers and inhibitors of P450 2E1 expression serve as inhibitors of P450 2E1 metabolic activities *in vitro*, as was observed with nitrogen heterocycles or certain organosulfur compounds. Based on these previous observations, a series of PZ derivatives covalently linked with allylmercaptan or structurally related organosulfur compounds was synthesized in this study. 2-AP was effective in suppressing P450 2E1 expression in the liver and in inhibiting P450 2E1 catalytic activity *in vitro*. 2-AP was also efficacious in protecting organs against AAP-, INH- or carbon tetrachloride-induced intoxication, as shown by a decrease in the mortality rate and blunting of toxicant-induced elevations in liver function tests. The mechanism for 2-AP protection against chemical-induced liver toxicity is probably associated with modulation of P450 2E1 levels and/or possibly with that of certain phase II detoxification enzyme expressions such as glutathione S-transferases.

Substituted 1,2-dithiol-3-thiones including oltipraz, the investigative chemopreventive compounds, reduce hepatic toxicity caused by AAP or carbon tetrachloride in animals and exhibit protective effects against the development of pulmonary and forestomach cancers induced by chemical carcinogens [34–36]. The chemopreventive effects of oltipraz appear to be associated with the elevation of electrophile detoxication systems such as glutathione S-transferases, epoxide hydrolase and NADP(H):quinone reductase activities. Preliminary studies have shown that 2-AP effectively induces phase II detoxification enzymes, including microsomal epoxide hydrolase and glutathione

S-transferases. The dual effects of 2-AP on P450 2E1 and phase II detoxifying enzyme expression may provide a pharmacological basis for hepatoprotective effects.

Administration of 2-AP to mice insulted with AAP or carbon tetrachloride caused elevations in hepatic GSH contents, as compared with those treated with toxicants alone. The effect of 2-AP on hepatic GSH levels was dose related, indicating that 2-AP protection against AAP-or CCl_4-induced elevations in serum aminotransferase activities may be associated with elevation of the hepatic GSH level. Increases in GSH concentrations were also observed in animals treated with oltipraz [37].

Previous studies have shown that treatment of animals with nitrogen heterocycles including PZ, pyridine or thiazole, which are strong P450 2E1 inducers, cause substantial decreases in P450 2E1 mRNA levels at 24 hr after treatment, returning to levels which approximated 60% of the RNA levels present in untreated animals at 48 and 72 hr, although the exact molecular mechanism was not established [20]. We have shown that the organosulfur compounds, including DAS, allylmercaptan and allylmethylsulfide, suppress P450 2E1 expression without transcriptional inactivation, as shown by the lack of significant changes in the levels of P450 2E1 mRNA [15]. The results were consistent with the observations reported by Yang and colleagues [14]. Suppression in P450 2E1 protein expression by 2-AP in this study accompanied a transient decrease in its mRNA levels at 48 hr posttreatment. P450 2E1 mRNA levels, however, returned to those of untreated animals after multiple treatment for 3 days. Thus, the molecular mechanism for P450 2E1 suppression by 2-AP may be related in part to changes in P450 2E1 mRNA at an early time point (i.e. transcriptional inactivation). Nonetheless, the exact molecular mechanism for P4502E1 suppression should be further established.

In this study, 2-AP was efficacious in blocking the toxicities induced by AAP, INH and carbon tetrachloride, which are substrates primarily metabolized by P450 2E1. Further metabolic, toxicological and mechanistic studies on 2-AP would facilitate development of this agent as a clinically useful drug. Patients with increased P450 2E1 activity caused by obesity or by INH therapy would need this additional pharmacological manipulation to decrease the risk of xenobiotic-induced toxicity. In addition, modified pharmacokinetic parameters should also be provided with this type of medication because of the altered metabolism of xenobiotics, along with the modulation of P450 2E1 expression and activity.

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