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Therapeutic effect of *S*-allylmercaptocysteine on acetaminophen-induced liver injury in mice

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Abstract

S-allylmercaptocysteine is one of the water-soluble organosulfur compounds in ethanol extracts of garlic (*Allium sativum* L.). We had demonstrated earlier that treatment with *S*-allylmercaptocysteine before acetaminophen administration protects mice against acetaminophen-induced hepatotoxicity. In this study, we examined the therapeutic effect of *S*-allylmercaptocysteine treatment after acetaminophen administration. A single dose of *S*-allylmercaptocysteine (200 mg/kg, p.o.) to mice 0.5 h after acetaminophen administration (500 mg/kg, p.o.) significantly suppressed both the increase in plasma alanine aminotransferase activity and the hepatic necrosis, and also reduced acetaminophen-induced mortality from 43% to 0%. These data indicate that *S*-allylmercaptocysteine is useful as an antidote for acetaminophen overdose. *S*-allylmercaptocysteine significantly suppressed hepatic cytochrome *P*450 2E1 (CYP2E1) activity and induction of inducible 70-kDa heat shock protein, a marker of acetaminophen arylation of protein. These results suggest that *S*-allylmercaptocysteine exerts its protective effect by inhibition of CYP2E1 activity, which leads to the suppression of acetaminophen arylation of hepatic protein.

Keywords: Acetaminophen; Hepatotoxicity; Cytochrome *P*450 2E1; Heat shock protein; *S*-allylmercaptocysteine; Garlic

1. Introduction

Since ancient times, garlic (*Allium sativum* L.) has been used world-wide as a food and a folk medicine. In recent years, many studies have shown that garlic possesses antimicrobial (Cavallito and Bailey, 1944), antithrombotic (Ariga et al., 1981), antitumor (Sumiyoshi and Wargovich, 1989), antihyperlipidemic (Kamanna and Chandrasekhara, 1982; Lau et al., 1987; Steiner et al., 1996), antioxidant (Horie et al., 1989; Ide et al., 1996; Imai et al., 1994) and hepatoprotective (Hikino et al., 1986; Wang et al., 1996) properties.

S-allylmercaptocysteine is one of the water-soluble organosulfur compounds in the aged garlic extract obtained by ethanol extraction of sliced garlic bulbs. *S*-allylmercaptocysteine has been shown to inhibit cell proliferation (Sigounas et al., 1997) and to have radical scavenging (Imai et al., 1994) and intraocular pressure-lowering effects (Chu et al., 1999). Moreover, several investigators, including our group, have demonstrated that *S*-allylmercaptocysteine pretreatment protects the liver against a number of hepatotoxicants, such as acetaminophen, carbon tetrachloride and D-galactosamine (Hikino et al., 1986; Nakagawa et al., 1989; Sumioka et al., 1998).

Acetaminophen is widely used as an analgesic and antipyretic drug. Although acetaminophen is harmless at therapeutic doses, it can produce hepatic injury in both human and experimental animals when given in high doses. After therapeutic doses, it is biotransformed and eliminated as non-toxic glucuronic acid and sulfate conjugates (Black, 1980; Pacifici et al., 1988). Only a small proportion of acetaminophen is converted to *N*-acetyl-*p*-benzoquinoneimine (NAPQI) (Dahlin et al., 1984), a cytochrome *P*450 (CYP)-mediated intermediate metabolite, which is normally detoxified by conjugation with GSH. However, after high doses of acetaminophen, the capacity for its removal by hepatic conjugation with glucuronide and sulfate is exceeded, and more of the reactive metabolite NAPQI is formed. Consequently, more NAPQI is conjugated with GSH, and when the hepatic GSH is depleted, more NAPQI will bind

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covalently to cellular macromolecules (Jollow et al., 1973; Potter and Hinson, 1986). This adduction of proteins by NAPQI is thought to lead to liver injury, and to play a key role in triggering heat shock protein (HSP) induction (Salminen et al., 1998). Mice and hamsters have been shown to be very sensitive to the hepatotoxic effects of acetaminophen, developing fulminant centrilobular necrosis similar to that observed in the human (Hinson, 1980).

Protection against acetaminophen-induced liver injury can be achieved by various mechanisms. Cysteine prodrugs, including *N*-acetylcysteine, the most widely used antidote for acetaminophen overdose, have been reported to protect the liver against acetaminophen-induced injury. The mechanism responsible for this protection may be metabolism of these prodrugs to L-cysteine, which is incorporated into hepatic GSH (Corcoran and Wong, 1986; Hazelton et al., 1986a; Lauterburg et al., 1983; Miners et al., 1984; Roberts et al., 1987). Moreover, several CYP enzymes have been reported to play important roles in the bioactivation of acetaminophen to NAPQI (Harvison et al., 1988; Morgan et al., 1983; Patten et al., 1993; Raucy et al., 1989). Studies on CYP2E1 knockout mice have made it clear that CYP2E1 is the most important factor in acetaminophen bioactivation (Lee et al., 1996; Zaher et al., 1998). In addition, CYP2E1 inhibitors such as diallyl sulfide, phenethyl isothiocyanate and 2-(allylthio)pyrazine protect the liver against acetaminophen-induced injury (Kim et al., 1997; Li et al., 1997; Wang et al., 1996). This evidence supports the crucial role of the CYP2E1 enzyme in acetaminophen-induced liver damage. Furthermore, results of recent studies suggest that acetaminophen-induced liver injury is also caused by cellular oxidative stress, resulting in hepatic lipid peroxidation (Albano et al., 1983; Amimoto et al., 1995; Wendel et al., 1979). Several antioxidants and antioxidative enzymes, such as coenzyme Q_{10}, α-tocopherol, ascorbic acid and superoxide dismutase, have been shown to prevent acetaminophen-induced liver injury in vivo (Amimoto et al., 1995; Mitra et al., 1991; Nakae et al., 1990). Finally, enhancing the capacity for conjugation with glucuronic acid may protect against acetaminophen-induced liver injury, as shown in an experiment that used butylated hydroxyanisole (Hazelton et al., 1986b).

In a previous study, we demonstrated that *S*-allylmercaptocysteine pretreatment before acetaminophen administration protected mice against acetaminophen-induced liver injury (Nakagawa et al., 1989; Sumioka et al., 1998). Considering the clinical possibility for the use of *S*-allylmercaptocysteine for acetaminophen overdose, it is very important to examine whether *S*-allylmercaptocysteine treatment after acetaminophen overdose can relieve hepatotoxicity. In this study, we, therefore, examined the therapeutic effect of *S*-allylmercaptocysteine against acetaminophen-induced hepatotoxicity. Since the mechanisms responsible for this hepatoprotection may be inhibition of CYP2E1 activity (Sumioka et al., 1998), we assumed that formation of the CYP-mediated reactive metabolite, NAPQI and subsequent acetaminophen arylation of protein would be decreased by *S*-allylmercaptocysteine treatment. However, we had no data to support our assumption. To clarify this, we examined whether *S*-allylmercaptocysteine treatment affected acetaminophen-induced hepatic-inducible 70-kDa heat shock protein (HSP70i) induction. This induction is strongly associated with the hepatic intralobular sites of acetaminophen arylation of protein (Salminen et al., 1997a).

2. Materials and methods

2.1. Chemicals

S-allylmercaptocysteine was synthesized as described previously (Hikino et al., 1986). Acetaminophen, 2-vinylpyridine and phenylmethylsulfonyl fluoride were purchased from Wako (Osaka, Japan). Glutathione reductase, isocitric dehydrogenase and *N*-acetylcysteine were purchased from Sigma (St. Louis, MO, USA). NADPH and NADP were purchased from Boehringer Mannheim Biochemicals (Mannheim, Germany). *N*-nitrosodimethylamine was purchased from Tokyo Kasei Industry (Tokyo, Japan). 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) was purchased from Katayama Chemical Industries (Osaka, Japan). Mouse anti-HSP70 monoclonal antibody was purchased from Stressgen (Victoria, BC, Canada). All the other chemicals used were of analytical grade.

2.2. Animals and treatments

Male ddY mice (5 weeks old) were purchased from Japan SLC (Shizuoka, Japan) and housed five or six per cage in plastic cages. The animals were maintained on a 12-h light/dark cycle under controlled temperature (23 ± 3 °C) and humidity ($55 \pm 5\%$) for 1 week before experimental use. They were allowed free access to standard laboratory food and water, but were fasted the night before the experiment. During food deprivation and experimentation, they were housed in cages with wire-mesh floors to prevent them ingesting their bedding material and excreta.

Acetaminophen at 500 mg/kg (p.o.) was administered to the mice, and then *S*-allylmercaptocysteine at 200 mg/kg (p.o.) was given 0.5 or 1 h after acetaminophen administration. Acetaminophen was administered between 9 and 11 a.m. to avoid any circadian variation. *S*-allylmercaptocysteine and acetaminophen were suspended in 1% gum arabic and 1% carboxymethyl cellulose sodium salt solutions, respectively. The dose volume of each compound and vehicle combined was 10 ml/kg. Mice in the 'vehicle' group were fasted and given acetaminophen but not *S*-allylmercaptocysteine; instead of *S*-allylmercaptocysteine they received only the 1% gum arabic vehicle.

The mice were anesthetized with diethyl ether and blood samples were taken from the right ventricle with a heparinized syringe and then their livers were removed 0, 1, 3, 6 or

9 h after acetaminophen administration. The livers were frozen immediately and stored in liquid nitrogen until assayed.

These studies were approved by the Institutional Animal Care and Use Committee at Tottori University, and were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3. Histopathological examination

Mice were allowed free access to food 9 h after acetaminophen administration, and survival was monitored until 48 h after the acetaminophen had been given. Mice surviving 48 h after acetaminophen administration were anesthetized with diethyl ether and their livers were removed for histopathological examination. The livers were fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin. The extent of liver necrosis was quantitated by the point-counting method (Mori et al., 1980). To estimate the extent of necrosis, the specimen was observed under a light microscope with an eyepiece grid (441 points/cm^2). The extent of necrosis was expressed as the percentage of test points lying on the profile of the necrotic parenchyma within the fields observed.

2.4. Measurement of plasma alanine aminotransferase activity

Plasma alanine aminotransferase activity was determined spectrophotometrically with a commercially available kit (Wako), based on the method of Wróblewski and LaDue (1956) which involves calculating the enzyme activity from the decrease in NADH absorbance at 340 nm. Alanine aminotransferase activity was expressed as international units (IU)/liter.

2.5. GSH and GSSG assays

Frozen liver tissue was homogenized in 5% trichloroacetic acid/5 mM EDTA under a stream of nitrogen gas, then centrifuged at 20,000 × *g* for 10 min at 4 °C. Trichloroacetic acid in the supernatant was removed by extracting three times with diethyl ether. The total hepatic glutathione level, the sum of the GSH and GSSG was determined with a glutathione reductase DTNB recycling assay by the method of Griffith (1980). The GSSG level was determined after GSH had been allowed to react with 2-vinylpyridine, and the GSH level was calculated by subtracting the GSSG level from the total glutathione level.

2.6. Western blot analysis

Frozen liver tissue (about 100 mg) was homogenized in 1.5 ml of sample buffer [50 mM Tris–HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 10 mM dithiothreitol, 10% glycerol and 1 mM phenylmethylsulfonyl fluoride], boiled for 5 min, passed through a 22-gauge needle three times to shear the DNA and stored at − 80 °C until use. The protein content of each sample was determined and adjusted to a concentration of 2.5 mg/ml, and bromophenol blue was added to each sample to a final concentration of 0.01%. The samples were boiled again for 5 min, then 50 μg of protein from each sample was separated by SDS polyacrylamide gel electrophoresis with 12.5% polyacrylamide gel and electroblotted on to a polyvinylidene difluoride (PVDF) membrane (Bio-Rad Laboratories, Hercules, CA, USA). After non-specific binding sites had been blocked in blocking buffer (20 mM Tris–HCl, pH 7.5, 500 mM NaCl and 5% skim milk) for 18 h, the PVDF membrane was incubated for 2 h with mouse anti-HSP70 monoclonal antibody at room temperature with occasional shaking. The antibody was used at a 1:1000 dilution in washing buffer (20 mM Tris–HCl, pH 7.5, 500 mM NaCl and 0.05% Tween 20) containing 5% skim milk. It was then washed four times with washing buffer, incubated for a further hour at room temperature (with occasional shaking) with horseradish peroxidase-conjugated sheep anti-mouse antibody (Amersham Pharmacia Biotech, Buckinghamshire, England) at a 1:3000 dilution in washing buffer containing 5% skim milk, and then washed four times with washing buffer. The immunoblot was revealed with an ECL™ Western blotting analysis system (Amersham Pharmacia Biotech). The PVDF membrane was exposed to Hyperfilm-ECL (Amersham Pharmacia Biotech, Buckinghamshire, England) to localize antibody binding. Western blots were quantitated using Image-Pro® Plus version 3.0 software (Media Cybernetics, Silver Spring, MD, USA).

2.7. Subcellular fractionation and CYP2E1 assay

A single dose of *S*-allylmercaptocysteine (200 mg/kg, p.o.) was given to mice 2.5 or 5.5 h before removal of the livers, and the livers were perfused with chilled 154 mM KCl, then removed and homogenized in a homogenizing buffer (50 mM Tris–HCl, 154 mM KCl, pH 7.4). The homogenates were centrifuged at 9000 × *g* for 20 min at 4 °C, and the microsomal fraction was separated from the supernatant by centrifugation at 105,000 × *g* for 90 min at 4 °C. The microsomal pellet was washed with homogenizing buffer, centrifuged again at 105,000 × *g* for 90 min at 4 °C and then suspended in 250 mM sucrose. The microsomal fraction was stored at − 80 °C until assayed.

N-nitrosodimethylamine demethylase activity was determined as an index of CYP2E1 activity by the method of Peng et al. (1982). *N*-nitrosodimethylamine demethylase activity was expressed as nanomoles of HCHO formed per minute per milligram of protein.

2.8. Protein assay

Protein contents were determined by the method of Lowry et al. (1951), with bovine serum albumin as a standard.

Table 1
Therapeutic effect of *S*-allylmercaptocysteine on acetaminophen-induced liver injury in mice

Treatment group	Mortality (%)	
	Treatment given 0.5 h after acetaminophen	Treatment given 1 h after acetaminophen
Vehicle	13/30 (43%)	22/32 (69%)
S-allylmercaptocysteine	0/20 (0%) [a]	14/25 (56%)

S-allylmercaptocysteine (200 mg/kg, p.o.) or vehicle (1% gum arabic solution) was given 0.5 or 1 h after acetaminophen administration (500 mg/kg, p.o.). Mortality was determined 48 h after acetaminophen administration.
[a] Significantly different from the vehicle group ($P<0.01$).

2.9. Statistical analysis

The results are expressed as means ± S.E.M. Differences between means were evaluated by one-way analysis of variance or the Kruskal–Wallis method for multiple comparisons, and Student's *t*-test or the Aspin–Welch method for single comparisons. Mortality was analyzed with the χ^2 test. Statistical analyses were performed with SAS/STAT™ Software Release 6.12 (SAS Institute, Cary, NC, USA). Differences at $P<0.05$ were considered to be significant.

3. Results

3.1. Effect of S-allylmercaptocysteine on acetaminophen-induced hepatotoxicity

The effect of *S*-allylmercaptocysteine on acetaminophen-induced mortality is shown in Table 1. Treatment with only the vehicle 0.5 h after acetaminophen administration gave a mortality of 43% by 48 h after acetaminophen administration. *S*-allylmercaptocysteine treatment 0.5 h after acetaminophen administration completely blocked acetaminophen-induced mortality. However, *S*-allylmercaptocysteine treatment 1 h after acetaminophen administration did not improve acetaminophen-induced mortality. Therefore, we further investigated the effects of *S*-allylmercaptocysteine treatment 0.5 h after acetaminophen admi- nistration.

The livers from vehicle-treated mice 48 h after acetaminophen showed severe centrilobular necrosis, which in some cases was also accompanied by congestion. In contrast, hepatic necrosis and congestion appeared less marked in *S*-allylmercaptocysteine-treated mice. The extent of hepatic necrosis in the *S*-allylmercaptocysteine group was significantly lower than that in the vehicle group (Fig. 1).

Plasma alanine aminotransferase activity was measured 0, 1, 3, 6 and 9 h after acetaminophen administration as an index of the early stages of liver damage. Plasma alanine aminotransferase activity started to increase 3 h after acetaminophen administration and rose steeply from 3 h onward. *S*-allylmercaptocysteine treatment significantly

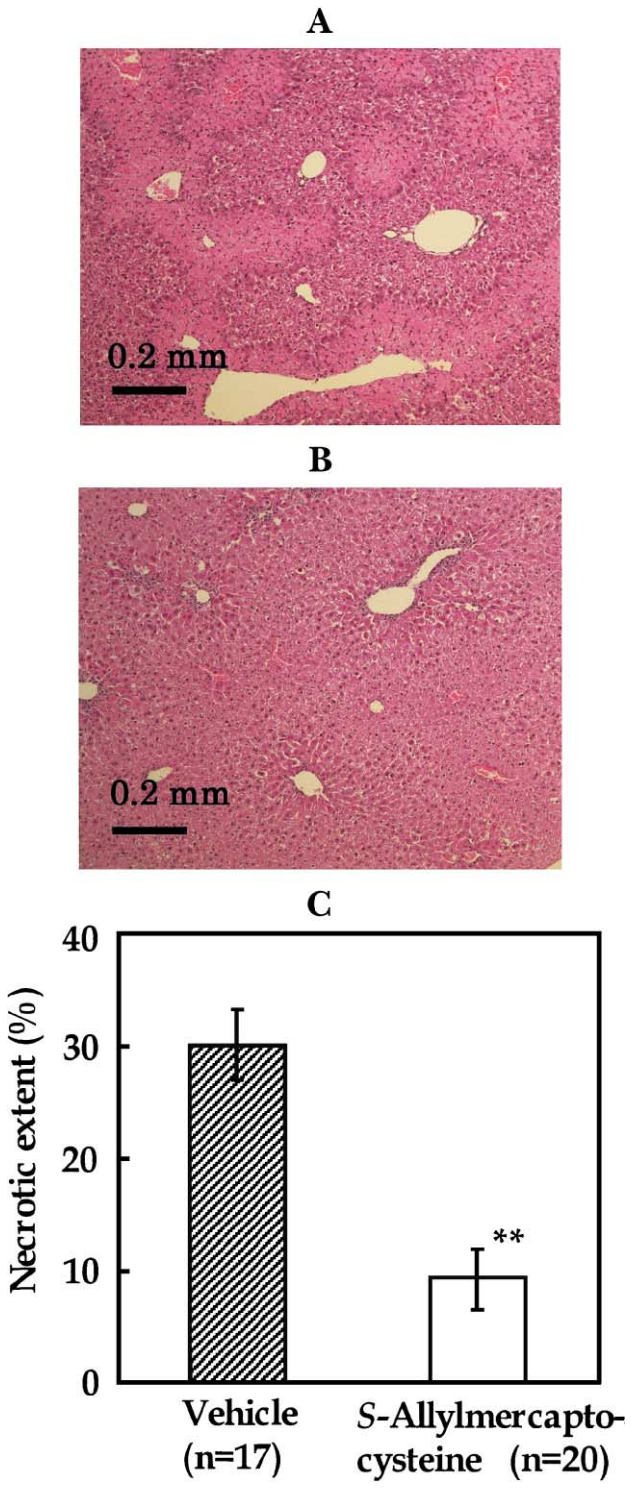

Fig. 1. Histopathological examination 48 h after acetaminophen administration (500 mg/kg, p.o.). *S*-allylmercaptocysteine (200 mg/kg, p.o.) or vehicle (1% gum arabic solution) was given to mice 0.5 h after acetaminophen. Liver sections were stained with hematoxylin and eosin. (A) Liver treated with vehicle has areas of severe and extensive necrosis. (B) Liver treated with *S*-allylmercaptocysteine shows less necrosis. (C) Quantitative examination of hepatic necrosis 48 h after acetaminophen administration. The data are expressed as means ± S.E.M. ** Significantly different from the vehicle group ($P<0.01$).

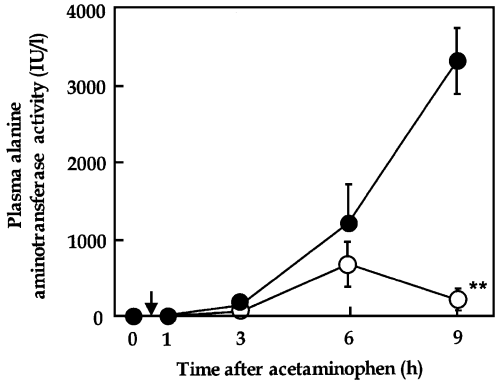

Fig. 2. Time courses of changes in plasma alanine aminotransferase activity in mice 0–9 h after acetaminophen administration (500 mg/kg, p.o). *S*-allylmercaptocysteine (200 mg/kg, p.o.; ○) or vehicle (1% gum arabic solution; ●) was given to mice 0.5 h after acetaminophen. The data are expressed as means ± S.E.M. for at least five animals. ↓: Treatment with *S*-allylmercaptocysteine or vehicle. * * Significantly different from the vehicle group ($P < 0.01$).

suppressed the increase in plasma alanine aminotransferase activity (Fig. 2).

3.2. Effect of S-allylmercaptocysteine treatment on hepatic GSH and GSSG levels

As GSH plays an important role in the detoxification of acetaminophen, the hepatic GSH and GSSG levels were examined 0, 1, 3, 6 and 9 h after acetaminophen administration. *N*-acetylcysteine, the most widely used antidote for acetaminophen overdose, was also given (molar equivalent dose of *S*-allylmercaptocysteine 200 mg/kg) 0.5 h after acetaminophen administration, and the effect of *S*-allylmercaptocysteine or *N*-acetylcysteine treatment on hepatic GSH and GSSG levels were examined.

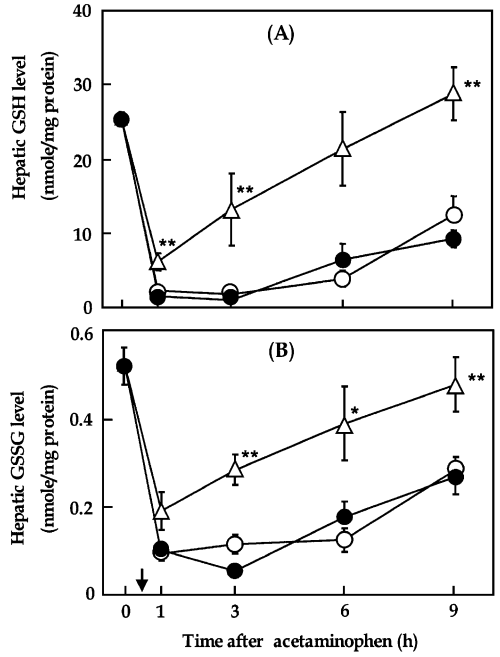

Fig. 3. Time courses of changes in hepatic GSH (A) and GSSG (B) levels in mice 0–9 h after acetaminophen administration (500 mg/kg, p.o). *S*-allylmercaptocysteine (200 mg/kg, p.o.; ○), *N*-acetylcysteine (molar equivalent dose of *S*-allylmercaptocysteine 200 mg/kg, p.o.; △) or vehicle (1% gum arabic solution; ●) was given to mice 0.5 h after acetaminophen. The data are expressed as means ± S.E.M. for at least five animals. ↓: Treatment with *S*-allylmercaptocysteine, *N*-acetylcysteine or vehicle. *, * * Significantly different from the vehicle group ($P < 0.05$ and 0.01, respectively).

The time courses of the changes in hepatic GSH and GSSG levels are shown in Fig. 3. The hepatic GSH level of the vehicle-treated group decreased rapidly to 6% of the initial (0 h) level 1 h after acetaminophen administration, with no reciprocal increase in the GSSG level. These results suggest that GSH loss may have resulted from the detoxification of NAPQI by GSH conjugation, rather than from the consumption of GSH by glutathione peroxidase. The hepatic GSH level increased slightly from 6 h onward, but never reached the initial level. *S*-allylmercaptocysteine treatment produced GSH and GSSG results that were not significantly different from those in the vehicle group. On the other hand, *N*-acetylcysteine treatment significantly suppressed the reduction of hepatic GSH and GSSG levels.

3.3. Effect of S-allylmercaptocysteine treatment on hepatic CYP2E1 activity

As the hepatotoxicity of acetaminophen results from the formation of NAPQI, we examined the activity of CYP2E1, which is a major enzyme responsible for acetaminophen bioactivation. *S*-allylmercaptocysteine treatment 2.5 or 5.5 h before removal of the liver significantly reduced the activity of *N*-nitrosodimethylamine demethylase as an indicator of CYP2E1 activity (Fig. 4). These times were equivalent to 3

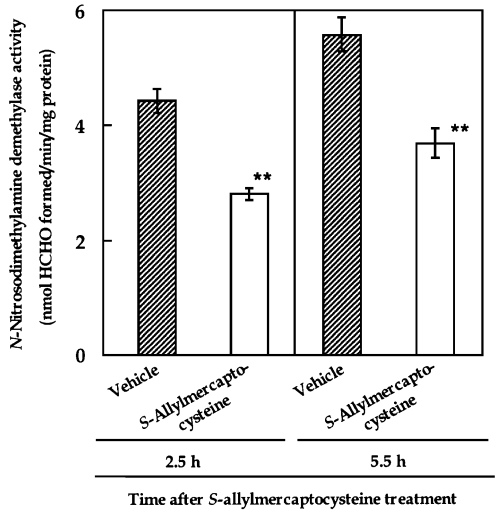

Fig. 4. Effect of *S*-allylmercaptocysteine treatment on hepatic CYP2E1 activity in mice. *S*-allylmercaptocysteine (200 mg/kg, p.o.) was given 2.5 or 5.5 h before the liver was removed. The vehicle group received 1% gum arabic solution (10 ml/kg). *N*-nitrosodimethylamine demethylase activity was used as an indicator of CYP2E1 activity. The data are expressed as means ± S.E.M. for at least five animals. * * Significantly different from the vehicle group ($P < 0.01$).

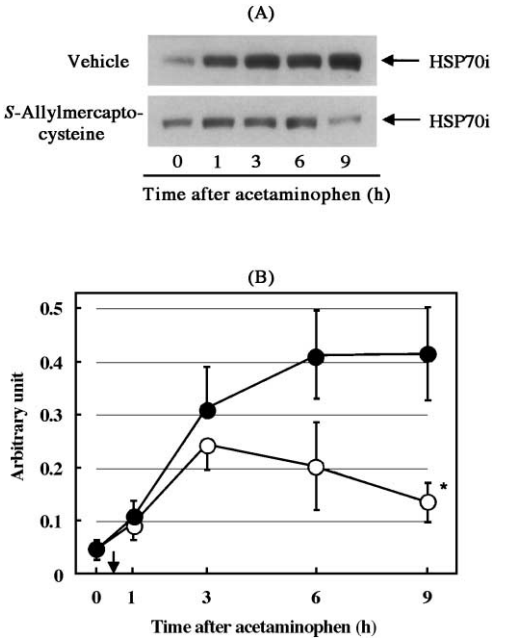

Fig. 5. Effect of *S*-allylmercaptocysteine treatment on induction of HSP70i in mouse liver 0–9 h after acetaminophen administration (500 mg/kg, p.o.). (A) Representative Western blots. (B) Densitometric analysis. *S*-allylmercaptocysteine (200 mg/kg, p.o.; ○) or vehicle (1% gum arabic solution; ●) was given to mice 0.5 h after acetaminophen. The data are expressed as means ± S.E.M. for five animals. ↓: Treatment with *S*-allylmercaptocysteine or vehicle. * Significantly different from the vehicle group ($P < 0.05$).

and 6 h after acetaminophen administration, respectively, because the *S*-allylmercaptocysteine was given 0.5 h after acetaminophen administration.

3.4. Effect of S-allylmercaptocysteine treatment on induction of HSP70i by acetaminophen administration

Western blot analysis was used to analyze the induction of HSP70i expression in the mouse liver by acetaminophen administration. Representative Western blots and densitometric analyses are shown in Fig. 5. Hepatic HSP70i induction started 1 h after acetaminophen administration, and HSP70i expression increased in a time-dependent manner. *S*-allylmercaptocysteine treatment significantly suppressed this induction.

4. Discussion

In a previous study, we demonstrated that *S*-allylmercaptocysteine treatment (once a day for 2 days) before acetaminophen administration dramatically protects mice against acetaminophen-induced liver injury (Nakagawa et al., 1989; Sumioka et al., 1998). In the current study, we examined the therapeutic effect of *S*-allylmercaptocysteine given after acetaminophen and its potential application to clinical therapy.

A single dose of *S*-allylmercaptocysteine 0.5 h after acetaminophen administration significantly suppressed the increase in plasma alanine aminotransferase activity and dramatically improved acetaminophen-induced liver necrosis, as well as the mortality rate. These findings suggest that *S*-allylmercaptocysteine treatment, not only before but also after acetaminophen administration, can protect mice against acetaminophen-induced liver injury, and that a single dose of *S*-allylmercaptocysteine is enough to block acetaminophen-induced liver injury. A single dose of *S*-allylmercaptocysteine 1 h after acetaminophen administration significantly suppressed the increase in plasma alanine aminotransferase activity 6 h after acetaminophen administration (data not shown), but did not improve the mortality rate. Therefore, we consider that a single dose of *S*-allylmercaptocysteine 0.5 h after acetaminophen administration was the regimen needed to block acetaminophen-induced hepatotoxicity completely.

CYP2E1, 3A4 and 1A2 have been shown to be the major enzymes responsible for acetaminophen bioactivation in the human and rat liver (Harvison et al., 1988; Patten et al., 1993; Raucy et al., 1989). Recently, it became evident from experiments using CYP2E1 knockout mice (Lee et al., 1996; Zaher et al., 1998) and a variety of CYP2E1 inhibitors (Kim et al., 1997; Li et al., 1997; Wang et al., 1996) that CYP2E1 is the most important player in the bioactivation of acetaminophen to NAPQI. We have already shown that *S*-allylmercaptocysteine treatment (once a day for 2 days) suppresses CYP2E1 activity in a dose-dependent manner. In the present study, we demonstrated that a single dose of *S*-allylmercaptocysteine also suppressed CYP2E1 activity (Fig. 4). Therefore, we suggest strongly that the mechanism underlying the therapeutic effect of *S*-allylmercaptocysteine is associated with its inhibition of CYP2E1 activity. Another research group has demonstrated that *S*-allylcysteine ($CH_2=CH\text{-}CH_2\text{-}S\text{-}CH_2\text{-}CHNH_2\text{-}COOH$), whose chemical structure resembles that of *S*-allylmercaptocysteine ($CH_2=CH\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CHNH_2\text{-}COOH$), does not inhibit CYP2E1-dependent *N*-nitrosodimethylamine demethylase activity (Wang et al., 1996). *S*-allylmercaptocysteine contains a disulfide bond (−S−S−) in its chemical structure. In contrast, *S*-allylcysteine contains a sulfide bond (−S−). Therefore, the disulfide bond in the *S*-allylmercaptocysteine molecule may be important for the inhibition of CYP2E1 activity. CYP2E1 is also a key bioactivator of various carcinogens, such as azoxymethane and *N*-nitrosodimethylamine (Koop, 1992). In this context, *S*-allylmercaptocysteine could be a potential inhibitor of carcinogenesis by environmental carcinogens, and its clinical application could spread from only acetaminophen-induced liver injury to all CYP2E1-related disorders.

Many studies on the mechanisms of acetaminophen-induced liver injury have demonstrated that GSH plays an important role in the detoxification of NAPQI, a reactive and toxic metabolite of acetaminophen, and that liver necrosis begins when GSH stores are almost exhausted

(Mitchell et al., 1973). Cysteine prodrugs, such as *N*-acetylcysteine, have been reported to protect the liver against acetaminophen-induced injury. These prodrugs are thought to be metabolized to L-cysteine, thereby supplying a component for hepatic GSH synthesis and thus further liver protection (Corcoran and Wong, 1986; Hazelton et al., 1986a; Lauterburg et al., 1983; Miners et al., 1984; Roberts et al., 1987). As *S*-allylmercaptocysteine possesses part of the cysteine structure, we assumed that depletion of the hepatic GSH level after acetaminophen overdose would be suppressed by *S*-allylmercaptocysteine treatment. We, therefore, examined whether *S*-allylmercaptocysteine treatment affects hepatic GSH synthesis after acetaminophen administration. *N*-acetylcysteine treatment significantly suppressed the reduction in hepatic GSH and GSSG levels, but *S*-allylmercaptocysteine treatment did not suppress these reductions any more than did in the vehicle group, suggesting that *S*-allylmercaptocysteine treatment does not affect hepatic GSH synthesis. In other words, it is not likely that *S*-allylmercaptocysteine exerts its protective power by supplying its cysteine component to the liver. Concerning the changes in hepatic GSH level after acetaminophen administration, we previously found that *S*-allylmercaptocysteine treatment suppressed the reduction in hepatic GSH level after acetaminophen administration (Sumioka et al., 1998), whereas we now showed that *S*-allylmercaptocysteine treatment did not suppress the reduction at all. In the previous study, CYP2E1 activity had already been suppressed when acetaminophen was administered, because *S*-allylmercaptocysteine was given 2 and 24 h before acetaminophen administration. Therefore, it is thought that the production of NAPQI, a CYP2E1-mediated reactive intermediate metabolite, was suppressed, and that consumption of hepatic GSH for NAPQI detoxification was also suppressed. This was the reason why our previous data showed that reduction of hepatic GSH was suppressed by *S*-allylmercaptocysteine. On the other hand, in the present study, *S*-allylmercaptocysteine was given 0.5 h after acetaminophen administration. We had demonstrated that the hepatic GSH level was rapidly reduced after acetaminophen administration; the hepatic GSH level was 13% of that in normal mice 0.5 h after acetaminophen administration (data not shown), which was the time point at which *S*-allylmercaptocysteine was administered in the present study. Since *S*-allylmercaptocysteine did not affect hepatic GSH synthesis, it is thought that *S*-allylmercaptocysteine treatment 0.5 h after acetaminophen administration did not suppress the reduction of hepatic GSH level in the present study (Fig. 3).

It has been demonstrated that 25kDa HSP (HSP25) and HSP70i in the liver in vivo are induced by several hepatotoxicants, including acetaminophen, carbon tetrachloride, bromobenzene and cocaine (Roberts et al., 1996; Salminen et al., 1997a,b). Each of these hepatotoxicants is metabolized to a reactive metabolite, and the adduction of proteins by reactive metabolites could lead to denaturation of the protein required to trigger HSP induction. It is demonstrated that a necrogenic dose of acetaminophen induces HSP25 and HSP70i in mouse liver, whereas a non-necrogenic dose fails to alter the level of either HSP, and that hepatocytes with increased HSP25 and HSP70i levels can also detect reactive metabolite (NAPQI) binding from acetaminophen. (Salminen et al., 1997a). Moreover, it has been reported that acetaminophen arylation of proteins plays a key role in triggering HSP induction (Salminen et al., 1998). These findings indicate that there was a strong correlation between the hepatic intralobular sites of acetaminophen arylation of protein (NAPQI binding to protein) and HSP induction, and that the induction of these hepatic HSPs can be used as a marker of acetaminophen arylation of protein. Since *S*-allylmercaptocysteine treatment suppressed CYP2E1 activity, we assumed that formation of the CYP-mediated reactive metabolite, NAPQI, and subsequent acetaminophen arylation of protein would also be reduced by *S*-allylmercaptocysteine treatment. To test our assumption, we used Western blotting to examine whether *S*-allylmercaptocysteine treatment affected the induction of acetaminophen-induced hepatic HSP70i, a marker of acetaminophen arylation of protein. Our results showed that acetaminophen administration induced hepatic HSP70i, but *S*-allylmercaptocysteine treatment 0.5 h after acetaminophen administration suppressed it significantly. These data suggest that acetaminophen arylation of protein is reduced by *S*-allylmercaptocysteine treatment. Therefore, it is thought that the mechanism underlying this therapeutic effect of *S*-allylmercaptocysteine is inhibition of CYP2E1 activity, which leads to the suppression of acetaminophen arylation of protein.

Conjugation with glucuronic acid and sulfate is another pathway that metabolizes acetaminophen. Therefore, we investigated whether a single dose of *S*-allylmercaptocysteine affected uridine diphosphate glucuronyltransferase and sulfotransferase activity. A single dose of *S*-allylmercaptocysteine did not affect the activity of either enzyme (data not shown), indicating that the mechanism responsible for this therapeutic effect does not involve the enhancement of glucuronidation and sulfation. We also examined the effect of *S*-allylmercaptocysteine treatment on the plasma acetaminophen level. The plasma acetaminophen level of the vehicle-treated group increased rapidly to a maximum level 0.5 h after acetaminophen administration, and decreased from 1 h onward. *S*-allylmercaptocysteine treatment significantly suppressed the reduction of plasma acetaminophen level 1 and 3 h after acetaminophen administration (data not shown). These data indicate that *S*-allylmercaptocysteine treatment 0.5 h after acetaminophen tends to delay the reduction of the plasma acetaminophen level. We, therefore, inferred that *S*-allylmercaptocysteine treatment prevented a fulminating production of NAPQI by slowing the metabolism of acetaminophen, associated with suppression of CYP2E1 activity. But we think that further studies are required to clarify the process of acetaminophen metabolism after *S*-allylmercaptocysteine treatment.

In summary and conclusion, we have shown that *S*-allylmercaptocysteine treatment, not only before but also after acetaminophen administration, can protect mice against acetaminophen-induced liver injury. *S*-allylmercaptocysteine treatment 0.5 h after acetaminophen administration was the regimen that completely blocked acetaminophen-induced hepatotoxicity. The data presented here indicate that *S*-allylmercaptocysteine has a moderate but significant effect as an antidote for acetaminophen overdose. The mechanism underlying the therapeutic effect of *S*-allylmercaptocysteine against acetaminophen-induced liver injury is inhibition of CYP2E1 activity, which leads to the suppression of acetaminophen arylation of protein.

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