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Mechanisms of selenium methylation and toxicity in mice treated with selenocystine

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Abstract Mechanisms of selenium methylation and toxicity were investigated in the liver of ICR male mice treated with selenocystine. To elucidate the selenium methylation mechanism, animals received a single oral administration of selenocystine (Se-Cys; 5, 10, 20, 30, 40, or 50 mg/kg). In the liver, both accumulation of total selenium and production of trimethylselenonium (TMSe) as the end-product of methylation were increased by the dose of Se-Cys. A negative correlation was found between production of TMSe and level of S-adenosylmethionine (SAM) as methyl donor. The relationship between Se-Cys toxicity and selenium methylation was determined by giving mice repeated oral administration of Se-Cys (10 or 20 mg/kg) for 10 days. The animals exposed only to the high dose showed a significant rise of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities in plasma. Urinary total selenium increased with Se-Cys dose. TMSe content in urine represented 85% of total selenium at the low dose and 25% at the high dose. The potential of Se-methylation and activity of methionine adenosyltransferase, the enzyme responsible for SAM synthesis, and the level of SAM in the liver were determined. The high dose resulted in inactivation of Se-methylation and decrease in SAM level due to the inhibition of methionine adenosyltransferase activity. To learn whether hepatic toxicity is induced by depressing selenium methylation ability, mice were injected intraperitoneally with periodate-oxidized adenosine (100 μmol/kg), a known potent inhibitor of the SAM-dependent methyltransferase, at 30 min before oral treatment of Se-Cys (10, 20, or 50 mg/kg). Liver toxicity induced by selenocystine was enhanced by inhibition of selenium methylation. These results suggest that TMSe was produced by SAM-dependent methyltransferases, which are identical with those involved in the methylation of inorganic selenium compounds such as selenite, in the liver of mice orally administered Se-Cys. Depression of selenium methylation ability resulting from inactivation of methionine adenosyltransferase and Se-methylation via enzymic reaction was also found in mice following repeated oral administration of a toxic dose of Se-Cys. The excess selenides accumulating during the depression of selenium methylation ability may be involved in the liver toxicity caused by Se-Cys.

Key words Selenium · Selenocystine · S-Adenosylmethionine · Methyltransferase · Methionine adenosyltransferase

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Introduction

Selenium is an essential dietary element for health, but is also toxic at relatively low levels. A wide variety of selenium compounds exist, both organically such as selenocystine and selenomethionine, and inorganically such as selenite and selenate. Inorganic selenium compounds like selenite are metabolized by reduced glutathione and/or glutathione reductase to hydrogen selenide via selenodiglutathione and glutathionylselenol intermediates (Ganther 1986). The hydrogen selenide is subject to sequential methylation by S-adenosylmethionine, resulting in the formation of mono-, di- and tri-methylated derivatives (Ganther 1966; Hsieh and Ganther 1977; Hoffman and McConnell 1987; Mozier et al. 1988). The trimethylselenonium ion is excreted in urine, whereas dimethyl selenide is a volatile product and is exhaled via the lungs (Ganther and Kraus 1984).

These methylated selenium metabolites are generally considered much less toxic than the parent compound (Olson 1986); thus the methylation process has been regarded largely as a detoxification mechanism.

However, there have been relatively few studies on the selenium methylation pathway of a selenoamino acid such as selenocystine. Jiang et al. (1983) reported that dimethyl selenide was identified in the breath of mice after administration of selenocystine; trimethylselenonium ion was found in urine from rats treated with selenocystine (Palmer et al. 1970; Nahapetian et al. 1983). Our recent report on the selenium-containing metabolite in mice orally administered selenocystine showed the possibility of hydrogen selenide formation from that metabolite (Hasegawa et al. 1995). Therefore, selenocystine is probably converted by methylation to a dimethyl selenide and trimethylselenonium ion via hydrogen selenide by a similar pathway to the inorganic selenium compound, although there is no direct substantiating evidence.

We reported earlier that an acid-volatile selenium existed in the liver cytosol of mice treated with a toxic dose of selenocystine (Hasegawa et al. 1994a). The acid-volatile selenium was assumed to be a source of hydrogen selenide (Diplock et al. 1971), which is one of the most toxic selenium compounds (Ganther 1979). Thus hydrogen selenide, as a precursor of methylated selenium metabolites, may be involved in the toxicity of selenocystine. The present study was conducted to determine the selenium methylation pathway of mice treated with selenocystine and the mechanism of liver toxicity caused by this treatment.

Materials and methods

Animals

Specific pathogen-free male ICR mice (4 weeks old) were obtained from Japan SLC Co., (Hamamatsu, Japan). Animals were randomly assigned to treatment groups and housed in plastic cages containing soft wood-chip bedding with free access to food (NMF, Oriental Yeast Co., Tokyo, Japan) and tap water. Animals were kept in a temperature controlled room at 23°C, with a 12 h light/dark cycle (0700–1900 hours). Mice were allowed to acclimate to this environment for 7 days prior to the start of the experiment.

Chemicals

Seleno-DL-cystine (Se-Cys), S-adenosyl-L-methionine (SAM), S-adenosyl-L-homocysteine (SAH), and S-adenosyl-L-ethionine (SAE) were obtained from Sigma (St. Louis., Mo., USA). Trimethylselenonium (TMSe) iodide was obtained from Tri Chemical Laboratory (Kanagawa, Japan). Dimethyl selenide (DMSe) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Monomethylselenol (MMSe) was purchased from Acros Organics USA (Pittsburgh, USA). Periodate-oxidized adenosine (PAD) was prepared by oxidation of adenosine according to the method of Hoffman (1980). All other chemicals used were of reagent grade or higher.

Preparation and administration of Se-Cys suspensions

Se-Cys was suspended in 0.5% sodium carboxymethyl cellulose (CMC-Na; Kanto Chemical Co., Tokyo, Japan) in physiological saline and administered orally to animals by intragastric tube. The dosing volume was 20 ml/kg.

Single administration experiments

In the time-course study, mice received a single oral administration of either 50 mg/kg Se-Cys or 0.5% CMC-Na solution alone. Five animals per group were killed 5, 15, 30, 60, or 120 min after dosing. In the dose-response study, animals received a single oral administration of either 5, 10, 20, 30, 40, or 50 mg/kg Se-Cys or 0.5% CMC-Na solution alone. Five animals per group were euthanized 15 min after dosing.

Repeated administration experiment

Mice were randomly divided into groups of ten animals each, and received repeated oral administration for 10 days with 10 or 20 mg/kg per day Se-Cys or 0.5% CMC-Na solution alone. Immediately after the last treatment, five animals from each group were placed individually in glass metabolism cages (Sugiyama-gen Ltd., Tokyo, Japan) and their urine collected for 24 h. The other five animals from each group were used for measurement of methionine adenosyltransferase activity 24 h after the final dosing.

Effect of PAD on methylation and toxicity of Se-Cys

Mice were injected intraperitoneally with 100 μmol/kg PAD or physiological saline 30 min before oral treatment of Se-Cys (10, 20, or 50 mg/kg). Animals were killed 1, 6, or 24 h after administration of Se-Cys.

Selenium determination

Total selenium content was measured by a fluorometric method using diaminonaphthalene (Watkinson 1966) after wet digestion of the tissue (0.98–1.02 g) or urine (1.0 ml) sample with 2 ml of mixed acid solution ($HNO_3/HClO_4$, 2 : 1).

TMSe determination

TMSe content in the liver or urine was measured by a modification of the method of Oyamada and Ishizaki (1986). Separation of TMSe was carried out by cation-exchange column chromatography. The supernatant (0.5 ml) of 26% liver homogenate in 0.4 M $HClO_4$ or the urine sample, adjusted to pH 1.2–1.4 with 6 N HCl, was applied to a Toyopac IC-SP M column (H^+ form, 1 ml gel volume: Tosoh Co., Tokyo, Japan). The column was washed stepwise with 3 ml each of water, 1 N ammonia solution, water, and 0.05 N HCl and, finally with 3 ml of 4 N HCl. TMSe was eluted with 4 N HCl and total selenium in the 4 N HCl fraction measured as described above. TMSe content was calculated from the total selenium concentration.

High performance liquid chromatography (HPLC) analysis

SAM, SAH, and SAE in the liver were analysed by HPLC using octanesulfonic acid as ion-pair reagent according to the method of Wagner et al. (1984). The liver (0.2 g) of animals treated with Se-Cys was homogenized in 0.4 M $HClO_4$ containing 0.05% EDTA and 0.15% $Na_2S_2O_5$ at a rate of 1 : 4 (w/v). After centrifugation, the clear

supernatant was filtered (0.45 μm pore size). This extract (20 μl) was chromatographed directly on an ODS column (Capcell PAC C_{18} AG120, 4.6 × 150 mm; Shiseido, Tokyo, Japan). SAM and analogues of SAM were detected by monitoring the effluent at 254 nm.

Measurement of activities of selenium (Se)-methylation and thioether S-methyltransferase in liver

Se-methylation activity or thioether S-methyltransferase activity was determined by measurement of TMSe synthesized from MMSe or DMSe, respectively, according to the method of Hsieh and Ganther (1977) and Mozier et al. (1988) with some modifications. The liver (0.4 g) of animals treated with Se-Cys was homogenized in 1.15% KCl at a volume ratio of 1 : 4 (w/v). Hepatic soluble fractions were obtained by differential centrifugation at 4°C, and the resultant supernatants identified as crude microsomal fraction (9000 *g*, 20 min) and cytosol fraction (105 000 *g*, 60 min). For Se-methylation activity, the reaction mixture contained 1.0 ml of the crude microsomal fraction, 200 μM MMSe, 100 μM SAM, 200 μM dithiothreitol, and 25 mM TRIS-HCl buffer (pH 6.3) containing 1 mM EDTA in a total volume of 3.0 ml. For thioether S-methyltransferase activity, the reaction mixture contained 1.0 ml of the cytosol fraction, 100 μM DMSe, 40 μM SAM, 200 μM dithiothreitol, and 25 mM TRIS-HCl buffer (pH 6.3) containing 1 mM EDTA in a total volume of 3.0 ml. The reaction mixtures were incubated for 30 min at 37°C; the reactions were stopped by addition of 500 μl 30% $HClO_4$ and deproteinized by centrifugation at 1500 *g* for 10 min. TMSe content in the acid-soluble supernatant was analysed using a cation-exchange column as described above. Protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Measurement of methionine adenosyltransferase activity

Methionine adenosyltransferase activity was determined by the method of Hoffman (1977). Mice treated with Se-Cys for 10 days were injected intraperitoneally with 250 mg/kg ethionine. After 45 min, livers were extracted with 5% sulfosalicylic acid at a rate of 1 : 4 (w/v) and SAE in the acid-soluble fractions analysed by HPLC as described above.

Biochemical measurements

Blood was collected with heparinized syringes from the abdominal aorta of animals under ethyl ether anesthesia, and plasma was separated from the blood cells by centrifugation. Activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the plasma were measured using a commercially available Wako test kit (transaminase CII-test Wako, Wako Pure Chemical Industries Ltd., Osaka, Japan).

Statistical analysis

Data were analysed using one-way analysis of variance (Anova); Duncan's multiple range test was used to pinpoint significant differences. Student's *t*-test was used to determine the significance of the differences in TMSe content and transaminase activities between PAD (−) and PAD (+) groups given the same dose of Se-Cys. A *P*-value of 0.01 was assumed to indicate a statistically significant difference.

Results

Selenium methylation in liver of mice treated with Se-Cys

The time course of changes in total selenium accumulation and TMSe production in the liver of mice receiving a single oral administration of Se-Cys is shown in Fig. 1. The accumulation of total selenium displayed an initial phase of rapid accumulation, which was linear for the first 30 min and reached a value of 20 μg Se/g liver. Following this initial rate of accumulation, a slower phase continued for an additional 90 min. Production of TMSe was found as early as 5 min after Se-Cys administration with a concentration of approximately 1.0 μg Se/g liver after 15 min, and this level was maintained over 120 min following administration.

The time course of changes in SAM and SAH levels in the liver of mice treated with Se-Cys is shown in Fig. 2. SAH is mainly formed in transmethylation reactions which use SAM as a methyl donor. No significant changes were recognized in the control group. However, in the Se-Cys treated group, there were significant transient changes in SAM and SAH levels ($P < 0.01$).

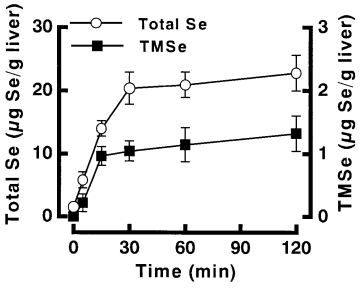

Fig. 1 Accumulation of total selenium and production of TMSe in liver of mice receiving a single oral administration of selenocystine (50 mg/kg). (*TMSe* Trimethylselenonium ion)

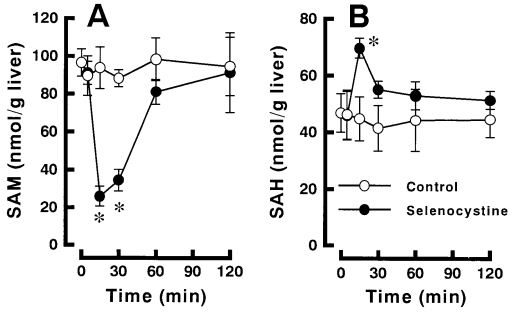

Fig. 2 Changes in SAM (**A**) and SAH (**B**) levels in liver of mice receiving a single oral administration of selenocystine (50 mg/kg). Control animals were treated with 0.5% CMC-Na solution alone. *Significantly different from control mice ($P < 0.01$). (*SAM* S-adenosyl-L-methionine, *SAH* S-adenosyl-L-homocysteine, *CMC-Na* sodium carboxymethyl cellulose)

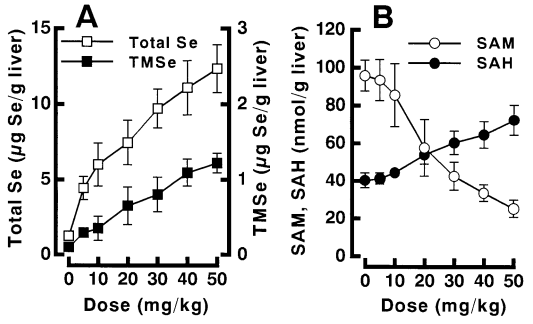

Fig. 3 Dose-dependent changes of total selenium, TMSe (**A**), SAM and SAH (**B**) levels in liver of mice receiving a single oral administration of selenocystine (50 mg/kg). Mice were killed 15 min after the administration

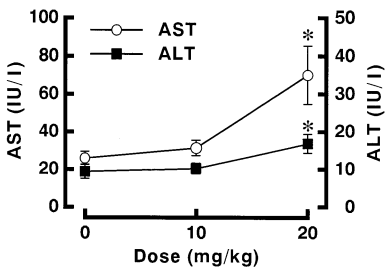

Fig. 4 AST and ALT activities in plasma of mice following repeated oral administration of selenocystine (10 or 20 mg/kg) for 10 days. Control animals were treated with 0.5% CMC-Na solution alone. Mice were killed 24 h after the last administration. *Significantly different from control mice ($P<0.01$). (*AST* Aspartate aminotransferase, *ALT* alanine aminotransferase)

The level of SAM between 15 and 30 min after Se-Cys administration dropped to about 30% of that in the control group, then recovered to the control level after 60 min. In contrast, the SAH level was significantly increased 15 min after administration of Se-Cys.

A dose-response study on total selenium accumulation, TMSe production (Fig. 3A) and SAM and SAH levels (Fig. 3B) in the liver of mice treated with Se-Cys showed that the former two were increased by the treatment (Fig. 3A). The increase in SAH level in the liver was concerned with a decrease in SAM level (Fig. 3B); there was a negative correlation between the production of TMSe and the level of SAM. These results suggest that selenium is transformed by SAM-dependent methylation to TMSe in the liver of mice treated with Se-Cys.

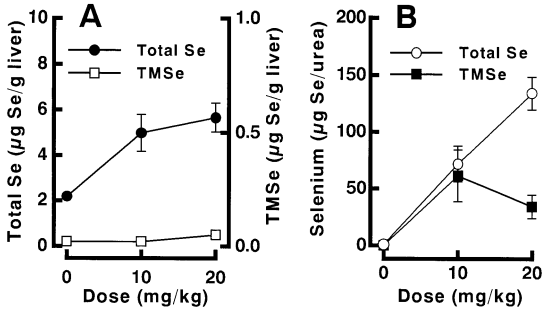

Fig. 5 Total selenium and TMSe contents in liver (**A**) and urine (**B**) of mice following repeated oral administration of selenocystine (10 or 20 mg/kg) for 10 days. Control animals were treated with 0.5% CMC-Na solution alone. Urine was collected for 24 h after last administration, and mice were then killed

Relationship between Se-Cys toxicity and selenium methylation

We have already reported that the liver was damaged in mice after repeated oral administration of Se-Cys (Sayato et al. 1993). To elucidate the relationship between hepatic toxicity and selenium methylation, a 10-day repeated dose study was designed. The AST and ALT activities in plasma of animals following repeated oral administration of Se-Cys are shown in Fig. 4. Animals exposed to 20 mg/kg showed a significant rise in the activities of those enzymes ($P<0.01$), although levels were not increased in mice treated with 10 mg/kg. These results are consistent with our previous study (Sayato et al. 1993).

Total selenium and TMSe contents in the liver and urine of mice following repeated oral administration of Se-Cys are shown in Fig. 5. Treatment of Se-Cys at doses of 10 and 20 mg/kg markedly elevated the total selenium content in the liver 24 h after the last administration (Fig. 5A). TMSe contents were not increased by either dosage, however, and remained similar to the control level, indicating that 24 h after the administration of Se-Cys, the TMSe produced in the liver was transported to other organs such as kidney. In contrast, urinary total selenium increased with increase of the Se-Cys dose: levels of 72 μg Se/urea (at 10 mg/kg) or 134 μg Se/urea (at 20 mg/kg) were obtained during a 24 h period after the last administration (Fig. 5B). The TMSe content at 10 mg/kg Se-Cys was 61 μg Se/urea representing 85% of total selenium at the same dosage; at 20 mg/kg Se-Cys the TMSe content was 31 μg Se/urea or 25% of the same dose of total selenium. Thus selenium methylation ability appeared depressed in the mice treated with 20 mg/kg Se-Cys.

To gain some insight into the mechanism of this Se-methylation depression, Se-methylation activity and SAM and SAH levels in the liver of mice 24 h after the last administration of Se-Cys were measured (Fig. 6). The Se-methylation activity measured by formation of TMSe from MMSe, an intermediate produced from hydrogen selenide to form DMSe, was significantly decreased with treatment of 20 mg/kg Se-Cys (Fig. 6A). Although the SAH level was not changed, the SAM level in the liver of mice treated with 20 mg/kg was also significantly lowered (Fig. 6B). In mice receiving a single oral administration of Se-Cys, SAM level in the

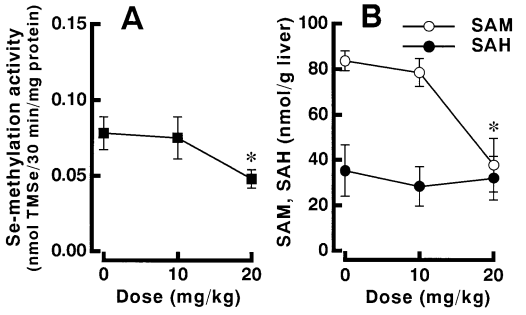

Fig. 6 Selenium (Se)-methylation activity (**A**) and SAH and SAH levels (**B**) in liver of mice following repeated oral administration of selenocystine (10 or 20 mg/kg) for 10 days. Control animals were treated with 0.5% CMC-Na solution alone. Mice were killed 24 h after the last administration. *Significantly different from control mice ($P<0.01$)

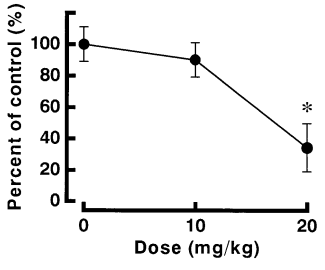

Fig. 7 Methionine adenosyltransferase activity in liver of mice following repeated oral administration of selenocystine (10 or 20 mg/kg) for 10 days. Control animals were treated with 0.5% CMC-Na solution alone. Mice were killed 24 h after the last administration. *Significantly different from control mice ($P<0.01$)

liver was transiently decreased due to the increased utilization of SAM for production of methylated selenium metabolites (Fig. 2A). However, these results indicated that the SAM level was depressed 24 h after the last administration of 20 mg/kg Se-Cys. It is suggested that the SAM supply is limited by the inhibition of its biosynthesis. SAM is synthesized by methionine adenosyltransferase from methionine and adenosine 5′-triphosphate (ATP). The activity of methionine adenosyltransferase in the liver of mice treated with Se-Cys was measured (Fig. 7), and was found to be significantly decreased following treatment of 20 mg/kg Se-Cys ($P<0.01$). These results indicated that a toxic dose of Se-Cys caused depression of selenium methylation ability by inactivating methionine adenosyltransferase and Se-methylation.

Effect of blocking selenium methylation on Se-Cys toxicity

A further experiment was designed to determine whether hepatic toxicity was induced by the depression of selenium methylation. PAD is known to be a potent

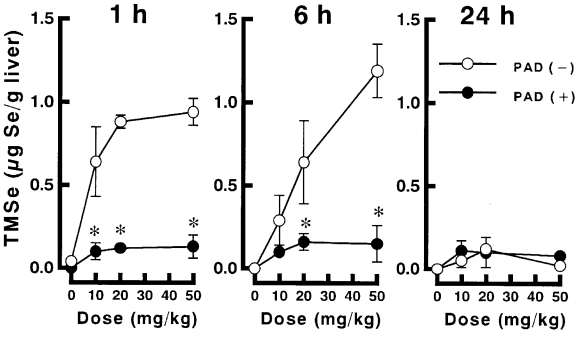

Fig. 8 Effect of PAD on TMSe production in liver of mice at different time intervals following oral administration of selenocystine. PAD (100 μmol/kg, i.p.) was injected 30 min before the administration. *Significant difference between PAD (−) and PAD (+) of the same dose groups ($P<0.01$). (*PAD* Periodate-oxidized adenosine)

inhibitor of the SAM-dependent methyltransferase in vivo (Marafante and Vahter 1984; Tandon et al. 1986; Hoffman and McConnell 1987). The effect of PAD on TMSe production in the liver of mice administered Se-Cys is shown in Fig. 8. At 1 and 6 h after Se-Cys administration, significant levels of TMSe production occurred in animals with no PAD-pretreatment; however, PAD-pretreatment inhibited TMSe production at both time intervals. Twenty four hours after Se-Cys administration, no production of TMSe was recognized either with or without PAD-pretreatment, a result consistent with the data of Fig. 5A measuring TMSe production in liver 24 h after Se-Cys administration. The effect of PAD on liver damage of mice administered Se-Cys is shown in Figs. 9 and 10. At 1 h after Se-Cys treatment, AST and ALT activities were not increased in mice with or without PAD-pretreatment. At 6 and 24 h after Se-Cys administration, the activities of both enzymes were raised in animals without PAD-pretreatment; in those pretreated with PAD, the levels were increased to values even higher than in the former group at both time periods. These results show that hepatic toxicity induced by Se-Cys was enhanced by inhibition of selenium methylation.

Discussion

Concerning inorganic selenium compounds, the liver is known to be an important site for selenium methylation reactions and SAM is the methyl donor for methylation (Ganther 1966; Oyamada 1988). Methylated selenium metabolites are formed by successive methylation of hydrogen selenide by SAM-dependent methyltransferases (Ganther 1986). Some investigators found TMSe in urine or DMSe in exhalation of animals treated with Se-Cys, but the production mechanisms of these methylated selenides are not known (Palmer et al. 1970; Jiang et al. 1983; Nahapetian et al. 1983). The

present study showed that TMSe was produced by SAM-dependent methylation in the liver of mice orally treated with Se-Cys. Pretreatment with PAD as specific inhibitor of SAM-dependent methyltransferase inhibited the selenium methylation in mice treated with Se-Cys (Fig. 8), a result consistent with reports on selenium methylation of inorganic selenium compounds (Tandon et al. 1986; Hoffman and McConnell 1987). Although an inorganic selenium compound such as selenite is metabolized to hydrogen selenide via selenodiglutathione (Ganther 1986), Se-Cys is not converted to selenodiglutathione. We very recently recognized that selenocysteine-glutathione selenenyl sulfide (CySeSG) as an initial metabolite is present in the mouse small intestine after oral administration of Se-Cys (Hasegawa et al. 1996). This CySeSG is reduced by excess GSH and/or glutathione reductase to yield selenocysteine, which is decomposed to hydrogen selenide by selenocysteine β-lyase. CySeSG was found in the liver of mice after oral administration of Se-Cys (Hasegawa et al. 1995). Thus, it was suggested that hydrogen selenide generated from Se-Cys via CySeSG is methylated via a SAM-dependent methylation pathway, which is identical to that of inorganic selenium compounds.

The concentration of total selenium in urine of mice with depressed selenium methylation ability was higher than that in the urine of animals having normal selenium methylation ability (Fig. 5B). TMSe is identified as a major urinary metabolite of selenium compounds and is an end-product of selenium methylation (Ganther 1979). A small amount of MMSe as an initial methylated product was recently identified in urine from rats that had received sodium selenite (Vadhanavikit et al. 1993; Suzuki et al. 1995). Thus, when the selenium methylation ability is depressed by the toxic level of Se-Cys, a great amount of MMSe may be excreted in urine. Hydrogen selenide and MMSe are nucleophilic agents, the former being one of the most toxic selenium compounds (Ganther 1979). Although TMSe and DMSe are less toxic, MMSe is nearly as toxic as hydrogen selenide (Drotar et al. 1987). We reported earlier that subacute liver damage of mice receiving repeated oral administration of 15 mg/kg Se-Cys for 90 days was dependent on the level of acid-volatile selenium, such as hydrogen selenide, in the liver cytosol fraction (Hasegawa et al. 1994b). The hepatic toxicity caused by Se-Cys may be related to the excess selenides produced as a result of the inhibited selenium methylation ability.

Bremer and Natori (1960) demonstrated that rat liver microsomes in the presence of SAM can methylate hydrogen selenide and MMSe to produce DMSe. The enzyme methylating hydrogen selenide and MMSe to DMSe may be identical to thiol S-methyltransferase (Hsieh and Ganther 1977; Ganther 1986); however, direct evidence is still lacking of whether the enzyme converts DMSe to TMSe. It was also reported that hydrogen selenide was nonenzymatically methylated by SAM to MMSe (Mozier et al. 1988). The mechanism for the first and second steps of methylation is not yet known.

Foster et al. (1986) examined the third step of methylation and showed that DMSe was a direct precursor of TMSe. Mozier et al. (1988) purified a thioether S-methyltransferase from mouse lung cytosol which is specific for this third methylation reaction. This enzyme is also distributed in liver cytosol, but not in the microsomal fraction. Although the activity of the microsomal enzyme thiol S-methyltransferase is sensitive to inhibition by arsenite, thioether S-methyltransferase is not inhibited by arsenite (Mozier et al. 1988). The present study showed that Se-methylation activity in the crude microsomal fraction which includes thiol S-methyltransferase and thioether S-methyltransferase was inhibited by treatments of Se-Cys at a toxic level (Fig. 6A). However, thioether S-methyltransferase in cytosol was not affected by the Se-Cys treatments (data not shown). These findings suggest that activity of thiol S-methyltransferase, which may have DMSe-synthesizing activity from MMSe, was inactivated by a toxic dose of Se-Cys.

The decrease in SAM by Se-Cys at a toxic level is not only due to the increased utilization of SAM for production of methylated selenides (Fig. 3), but also to the inactivation of methionine adenosyltransferase (Figs. 6B, 7). Hoffman (1977) also reported inactivation of the enzyme and reduction in SAM levels in the liver of mice following intraperitoneal injection of a toxic dose of sodium selenite. The reason for the inhibition of methionine adenosyltransferase in the liver of mice treated with Se-Cys or selenite is not clear; we hypothesize that if this enzyme has an active site sensitive to nucleophilic agents such as selenides, the excess selenides generated from an inorganic or organic selenium compound at a toxic level may inactivate the enzyme. The fact that methionine adenosyltransferase activity is strongly dependent on the presence of sulfhydryl compounds (Mudd and Cantoni 1958) would support this hypothesis.

Hepatic toxicity caused by a single dose of Se-Cys was enhanced by inhibition of selenium methylation (Figs. 9, 10). However, weak hepatic injury was recognized in mice even without inhibition of selenium methylation. Active oxygen species were formed by the reaction of Se-Cys with reduced glutathione GSH (Sayato et al. 1992; Yan and Spallholz 1993). We found a reduction in GSH level in the liver of mice after oral administration of Se-Cys (unpublished data). Kitahara et al. (1993) demonstrated that active oxygen species were involved in the cytotoxicity of selenite. Thus another toxic mechanism, such as active oxygen species, in addition to the depression of selenium methylation ability may also be involved in the hepatic toxicity caused by Se-Cys.

In conclusion, the main findings of this study were that TMSe was produced in mouse liver following oral

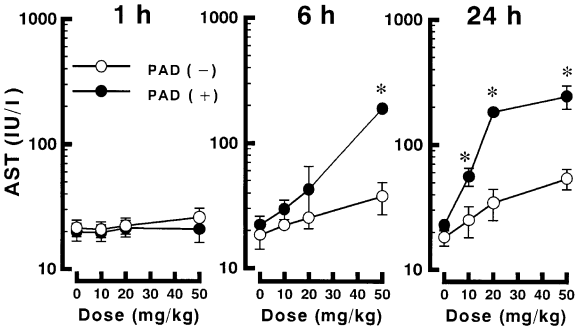

Fig. 9 Effect of PAD on plasma AST activity of mice following oral administration of selenocystine. PAD (100 μmol/kg, i.p.) was injected 30 min before administration of Se-Cys. *Significant difference between PAD (−) and PAD (+) of the same dose groups ($P<0.01$)

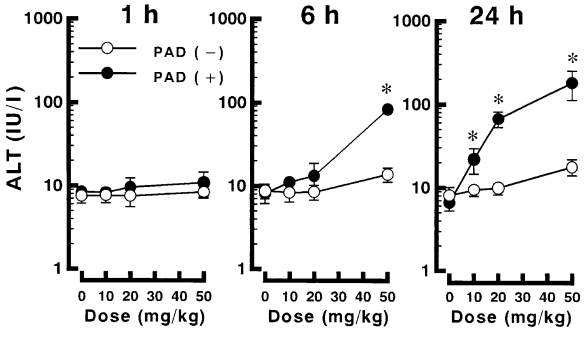

Fig. 10 Effect of PAD on plasma ALT activity of mice following oral administration of selenocystine. PAD (100 μmol/kg, i.p.) was injected 30 min before administration of Se-Cys. *Significant difference between PAD (−) and PAD (+) of the same dose groups ($P<0.01$)

administration of Se-Cys by SAM-dependent methyltransferases, which are identical with those of inorganic selenium compounds. It was further recognized that the repression of selenium methylation ability was strengthened by inactivation of Se-methylation and methionine adenosyltransferase in the animals after repeated oral treatment of Se-Cys. The excess of selenides produced by depression of selenium methylation ability may contribute to the hepatic toxicity caused by Se-Cys.

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