Penicillamine Selenotrisulfide as a Selenium-Source in Mice

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(Received February 27, 2004; Accepted April 2, 2004; Published online April 2, 2004)

In this paper, we compared the bioavailability of penicillamine selenotrisulfide (PenSSeSPen) with the authentic selenium-source compound, selenite. When PenSSeSPen was orally administered to selenium-deficient mice for 7 days, selenium contents of blood, liver and heart significantly increased as well as selenite. In addition, when PenSSeSPen-supplemented diet was fed to 3-week old mice for 7 weeks, selenium contents of selected organs were almost the same as those of selenite, and hepatic cellular glutathione peroxidase (GPx) activity was also observed. These facts evidently indicate that PenSSeSPen, a totally foreign selenium compound, serves as a selenium-source compound.

Key words ——— bioavailability, selenotrisulfide, selenium, mice

INTRODUCTION

Selenium is an essential micronutrient that is important for a number of physiological processes and for human health.[1,2] Its essentiality is based on the fact that selenium is a specific component of selenium-dependent enzymes.[3–5] In selenium-dependent enzymes such as glutathione peroxidases (GPx), selenium occurs as an active center amino acid residue, selenocysteine (SeCys). Selenium is known to be taken up in inorganic forms, *e.g.*, selenite (SeO_3^{2-}), or organic forms such as SeCys and selenomethionine (SeMet) mainly from food supply.[1,6]

Selenotrisulfide (RSSeSR) is thought to be an important metabolic intermediate of selenite that is the authentic selenium-source compound.[7,8] Painter first described the reaction of thiols with selenite to yield RSSeSR *in vitro*.[9] Later stoichiometry of reaction of reduced glutathione (GSH) with selenite in an acidic medium was well characterized by Ganther.[10] Definitely it is very difficult to obtain a chemically stable RSSeSR with low molecular weight thiols such as glutathione at the physiological pH *in vitro*. Although the chemical character of RSSeSR in an acidic medium is well established, this may not be true at the physiological pH where actual interactions of RSSeSR with biological systems occur. While penicillamine (Pen) is structurally quite similar to Cys but is capable of forming a chemically stable and isolatable Pen selenotrisulfide (PenSSeSPen). It was already reported that thiol exchange reaction occurs extensively between PenSSeSPen and glutathione *in vitro*, as well as the naturally occurring glutathione selenotrisulfide (GSSeSG).[11,12] Pen has been used in the treatment of detoxification of heavy metals such as mercury and lead in human. PenSSeSPen would be less toxic than selenite, since in general organic selenium compounds are less toxic than inorganic ones.[6] In the present study, we studied whether PenSSeSPen, a totally foreign selenium compound, is nutritionally bioavailable or not in mice.

MATERIALS AND METHODS

Materials ——— L-Penicillamine and selenious acid were purchased, respectively, from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan) and Kanto Chemical Co. Inc. (Tokyo, Japan) and were used without further purification. A selenium-deficient diet and a regular diet CE-2 were from Oriental Yeast Co. Ltd. (Tokyo, Japan) and Clea Japan Inc. (Tokyo, Japan), respectively. Glutathione peroxidase activity assay kit was obtained from Sigma Co. Ltd. (St. Louis, MO, U.S.A.) All other chemicals used

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were of commercial reagent grades. PenSSeSPen was prepared according to the procedure reported previously.[12] Elemental analysis (%); Found C: 31.13, H: 5.86, N: 7.24, Se: 21.89, Calcd C: 31.97, H: 5.33, N: 7.46, Se: 21.04.

Mice ——— Three-week old male ICR mice (weighing 8–12 g, specific pathogen free) were purchased from Clea Japan Inc. Mice were cared for in accordance with the guidelines of Nagasaki University on Animal Care. Mice were housed 10 or less per cage on a 12 hr light/12 hr dark schedule at 23 ± 2°C and 60% relative humidity, and were freely given deionized water in all experiments. Mice were randomly divided into vehicle and selenium compound fed groups.

Determination of PenSSeSPen Concentration ——— The PenSSeSPen concentrations in the sample solutions were determined by reversed-phase liquid chromatography (RPLC). The employed chromatographic conditions were as follows : column: a YMC-Pack ODS-A (150 × 4.6 mm i.d.), column temperature: ambient, mobile phase: 0.05 mol/l acetate buffer (pH 4)-methanol (9 : 1 by volume), flow rate: 1 ml/min, detection: UV 210 nm.

Determination of Selenium Content ——— Selenium contents of diets and organs were determined fluorometrically using 2,3-diaminonaphtharene (DAN) after digestion using a one to five mixture of perchloric acid and nitric acid.[13]

Preparation of Selenium-Deficient Mice and Oral Administration of Selenium Compounds ——— Three-week old male ICR mice were fed freely a selenium deficient diet (selenium content: 4 ng/g-diet) for 8 weeks. PenSSeSPen or selenious acid dissolved in saline was orally administered to selenium-deficient mice once a day for 7 days at 5 μgSe/kg of body weight.

Preparation of Selenium Compound-Supplemented Diets and their Feeding to Mice ——— PenSSeSPen or selenious acid was dissolved in saline at a concentration of 40 μM. Ten milliliters of the solution were sprayed for 1 kg of the selenium-deficient diet spread out on a plastic vat with vigorous agitation. These selenium compound-supplemented diets were newly prepared for every 7-day and were subjected to selenium content determination. Three-week old male ICR mice were given the selenium compound-supplemented diets *ad libitum* for 7-week.

Determination of Cytosolic Glutathione Peroxidase Activity ——— The liver cytosolic GPx activity was determined as follows using a Glutathione peroxidase activity assay kit. Liver samples were rinsed three times with saline and were homogenized by a probe type sonicator (BRANSON MODEL 250D) in 5 ml of saline. Supernatant fractions were prepared by centrifugation at 105000 × g and 4°C for 1 hr on a L-80 (Beckman Coulter, Fullerton, CA, U.S.A.). Glutathione peroxidase activity was measured using hydrogen peroxide as a substrate after appropriate dilution with 0.05 M phosphate buffer (pH 7). Absorbance at 340 nm responsible for nicotinamide adenine dinucleotide phosphate in reduced form (NADPH) was recorded at every 10 sec just after mixing well by inversion. The GPx activity was calculated from the following equation as μmoles NADPH oxidized per minute.[14,15]

$$\text{Activity } (\mu\text{mol/min/ml}) = -(DA_{\text{Sample}} - DA_{\text{Reagent Blank}}) \times 5/\varepsilon_{\text{mM}}$$

DA: difference in absorbance at 340 nm between 15 and 75 sec after addition of substrates, 5: dilution factor, ε_{mM}: extinction coefficient for NADPH, 6.22

Statistical Analysis ——— All data were presented as the mean ± standard deviation. Statistically significant differences between selenite and PenSSeSPen-fed groups were determined by two-tailed Student's t-test. Comparisons were considered statistically significant at $p < 0.05$.

RESULTS

Preparation of PenSSeSPen

The results of elemental analysis for PenSSeSPen were in good agreement with calculated values (data shown in Materials). Purified PenSSeSPen were also analyzed by RPLC. Under the chromatographic conditions used in this study, diastereomeric PenSSeSPen (L-Pen-SSeS-L-Pen and D-Pen-SSeS-L-Pen) are completely separable. The purified PenSSeSPen gave a sharp single peak, and residual Pen and oxidized Pen (PenSSPen, a major by-product) were not detected in the chromatogram (Fig. 1). An enantiomeric PenSSeSPen (D-Pen-SSeS-D-Pen) also did not produce during the PenSSeSPen preparation, as PenSSeSPen gave $[\alpha]_D$ values (specific rotation) of +7.07 degrees.

Absorbability of PenSSeSPen in Selenium-Deficient Mice

First, we examined the absorbability of PenSSeSPen using selenium-deficient mice in com-

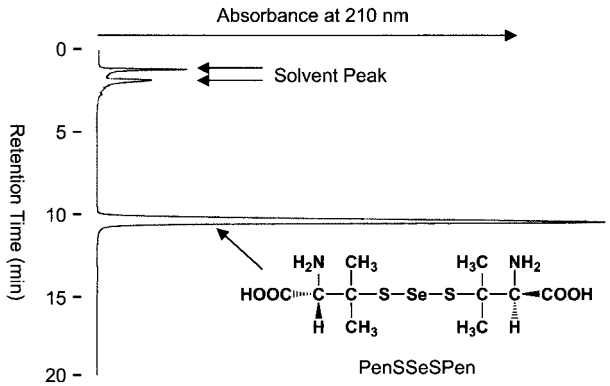

Fig. 1. Chemical Structure and Chromatogram of PenSSeSPen

parison with selenite. Selenium contents of the selected organs in selenium-deficient mice were apparently lower than those in non-treated group (11-week old mice which were fed a selenium-adequate regular diet CE-2 containing 0.39 μgSe/g-diet.). There were not significant differences in the brain, heart and body weights among PenSSeSPen-, selenite- and vehicle-administered groups (Fig. 2). These values were also similar to those for non-treated group. In contrast, an increase in the liver weight due to the selenium-deficiency was observed, as compared with that of non-treated mice. When PenSSeSPen was administered, the liver weight decreased more effectively to the normal value than the selenite administration. Selenium contents of the blood, liver and heart significantly increased after the oral administration of PenSSeSPen, while selenium content of the brain did not change (Fig. 3). These results were similar to those observed for selenite-administered mice.

Feeding of PenSSeSPen to Mice and Selenium Distribution

PenSSeSPen or selenite-supplemented diet was fed to 3-week old mice for 7 weeks, and the distribution of selenium of the two groups was compared. During this experiment, PenSSeSPen-supplemented diet was freshly prepared for each 7-day. When the saline solution of PenSSeSPen at 7 days after the diet preparation was analyzed by RPLC, more than 95% PenSSeSPen still remained without chemical degradation. Therefore, most of PenSSeSPen in the diet is thought to keep its chemical structure over the period of 7-day. When determined by DAN method, selenium contents of both PenSSeSPen- and selenite-supplemented diets were 0.25 μg/g-diet.

During the feeding of PenSSeSPen-supplemented diet, no remarkable changes of the appearance and behavior of mice were observed. PenSSeSPen-fed mice were favorably gained the body weight, which was almost identical to that of selenite-fed mice (Fig. 4). The weights of liver and

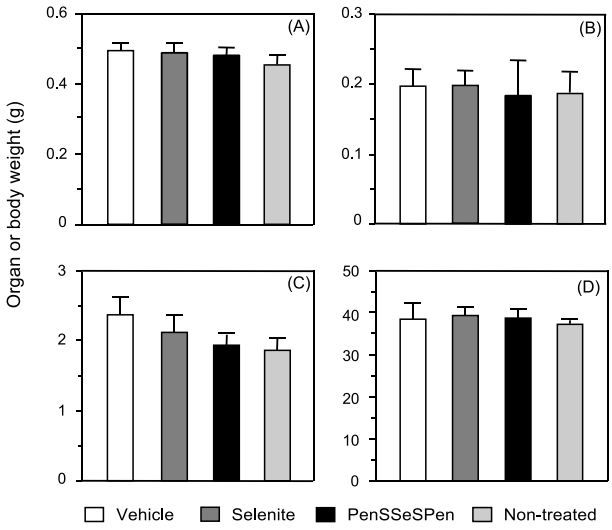

Fig. 2. Comparison of Organ and Body Weight after Oral Administration of PenSSeSPen and Selenite for 7 days to Selenium-Deficient Mice

(A), Brain; (B), Heart; (C), Liver; (D), Body Weight. Liver weights were smaller in PenSSeSPen-administered and non-treated groups compared with vehicle-administered group ($p < 0.1$). Non-treated group: Eleven-week old mice that were fed a selenium-adequate regular diet. Data are mean ± standard deviation ($n = 10$/group).

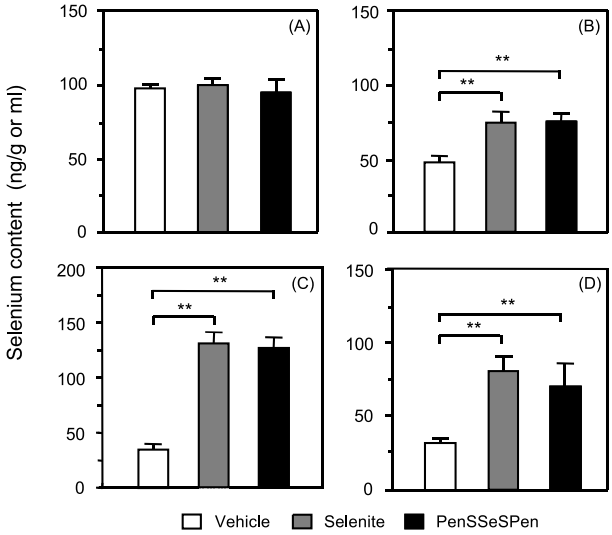

Fig. 3. Organ Selenium Concentrations after Oral Administration of PenSSeSPen and Selenite to Selenium-Deficient Mice

(A), Brain; (B), Heart; (C), Liver; (D), Blood. Data are mean ± standard deviation ($n = 10$/group). **Different from the vehicle-administered group, $p < 0.01$.

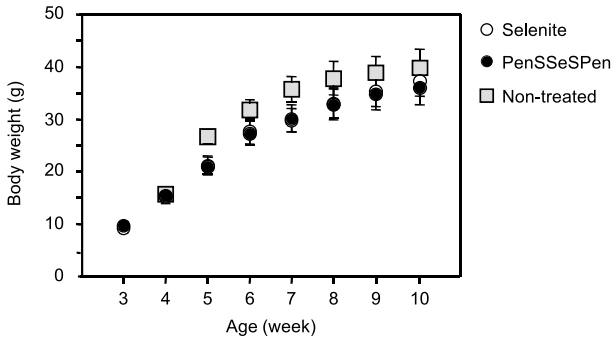

Fig. 4. Body Weight of Mice during Feeding of PenSSeSPen and Selenite-Supplemented Diets
Data are mean ± standard deviation (n = 7/group).

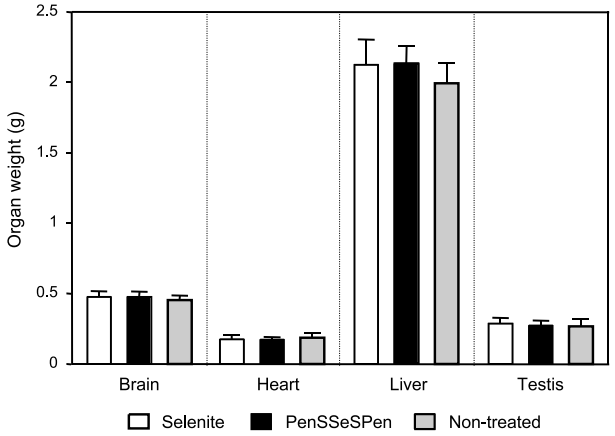

Fig. 5. Organ Weight of Mice after Feeding of PenSSeSPen and Selenite-Supplemented Diets
Data are mean ± standard deviation (n = 7/group).

testis that are relatively selenium-rich organs were also quite similar between the two groups. In the testis, liver, blood and thyroid that involve selenium-containing proteins, no significant differences in the selenium contents between the two groups were observed. There were also no differences in weights and selenium contents of the brain and heart between the two groups (Figs. 5 and 6). These results of the PenSSeSPen- and selenite-fed groups were comparable to that of the non-treated (a regular diet CE-2-fed) group, except for the brain selenium content.

GPx Activity after PenSSeSPen Feeding in Mice

The liver has a central role of the selenium metabolism. The hepatic cellular GPx activity was measured in order to check whether selenium in PenSSeSPen delivers to the selenium-containing enzymes and then is utilized or not, as well as selenite. Most common inorganic hydrogen peroxide was

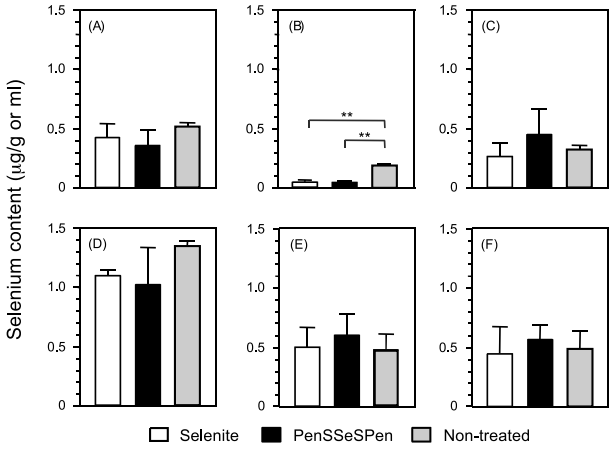

Fig. 6. Organ Selenium Concentrations in Mice after Feeding of PenSSeSPen and Selenite-Supplemented Diets for 7 weeks
(A), Blood; (B), Brain; (C), Heart; (D), Liver; (E), Testis; (F), Thyroid. Data are mean ± standard deviation (n = 7/group). $^{**}p < 0.05$: significantly different with 0.5% probability.

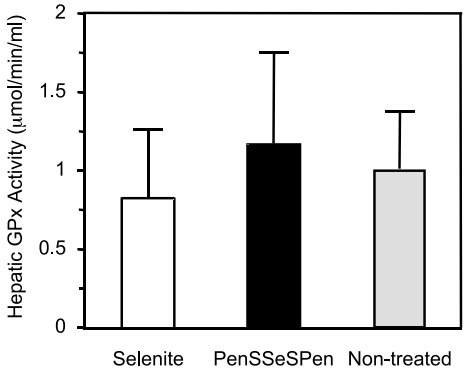

Fig. 7. Comparison of Hepatic Cellular GPx Activity for Hydrogen Peroxide
Data are mean ± standard deviation (n = 7/group).

employed as a substrate for the measurement of GPx activity. The observed GPx activity of PenSSeSPen-fed mice was almost comparable with those of selenite- and CE-2-fed mice (Fig. 7).

DISCUSSION

From the data of PenSSeSPen absorption in the selenium-deficient mice, it was found that PenSSeSPen in the diet can be absorbed through the gastrointestinal (GI) tract and then selenium in PenSSeSPen is distributed to the blood, liver and heart. Selenium supplied by PenSSeSPen seems to behave as well as that by selenite after the absorp-

tion through the GI tract. In addition, the observed GPx activities in the PenSSeSPen-fed mice demonstrate that selenium in PenSSeSPen is inserted to cellular GPx, one of the major selenium-containing enzymes. These facts mean that PenSSeSPen can serve as a selenium-source compound in mice, as well as the authentic selenite. Oral administration of PenSSeSPen for 7 days to the selenium-deficient mice did not affect the selenium content of the brain at all, although selenium content of the brain in the deficient state was lower than that in normal state. The uptake behavior of selenium in PenSSeSPen by the brain was also different from that by the liver and heart. A longer period of administration may require for the recovery of selenium of the brain in the selenium-deficient mice.[16)]

Organ and body weight and organ selenium contents were not significantly different between the two groups that were fed PenSSeSPen- and selenite-supplemented diets for 7 weeks. In general, cellular GPx activity correlates to the selenium contents of organs (or cells).[17)] In this experiment, judging from the organ selenium contents of the non-treated group, all mice in PenSSeSPen- and selenite-fed groups are in a selenium-adequate state.[1,18)] Hepatic cellular GPx activity for hydrogen peroxide in the PenSSeSPen-fed group was not significantly different from the other groups.

When a metabolic intermediate of small organic molecules, in general, is administered to the living systems, it takes almost the same metabolic fate as its mother compound and generates similar metabolites. By contrast, the metabolism of selenite is a very complicated process, since selenite and it metabolites suffer from various chemical transformations by the reactions with a variety of endogenous thiol-containing compounds, not only a GSH. Indeed, Self *et al*. recently reported that lipoic acid, a coenzyme of the pyruvate dehydrogenase and the 2-oxoglutarate dehydrogenase, can form an intramolecular selenotrisulfide.[8)] Because RSSeSR possesses a high reactivity with thiol-containing proteins such as rhodanese and thioredoxin reductase,[19,20)] it is likely to react with thiol-containing plasma constituents.

In the present study, we exhibited that PenSSeSPen served as a selenium-source compound in mice. The organ distribution of selenium after the 7-week feeding of this compound was almost the same as that of selenite. However, the PenSSeSPen feeding gave somewhat different actions in the recovery of the liver in a swollen state of the selenium-deficient mice. Now, the characterization of the blood constituents that is reactive with PenSSeSPen is being in progress.

Acknowledgements The authors wish to thank Ms. Akiko Serino and Ms. Mai Fukushima for their skillful technical assistance.

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