Univ. Bull. Chiba Inst. Sci. 15. 1-5. 2022
[Original Article]

Water-soluble Organosulfur Compounds in Onion Influence the Induction of Glutathione *S*-Transferase

Keiko SASAKI[1,2], Keiji WADA[3] and Koozi MATUOKA[2]

The effects of water-soluble organosulfur compounds, S-methyl-L-cysteine (SMC) and S-methyl-L-cysteine sulfoxide (SMCS) and cycloalliin (Cyc) on the activities of xenobiotic-metabolizing enzyme, glutathione S-transferase (GST) were investigated. Mice were treated orally with SMC (50-200 mg/kg), SMCS (50-200 mg/kg), Cyc (50-200 mg/kg) once a day for 5 days, and then the hepatic and extrahepatic enzyme activities were analyzed. SMC treatment resulted in significantly higher GST activities in liver, small intestine, kidney and brain by 1.1-1,4-fold, and SMCS treatment caused significantly higher GST activities in liver and small intestine and kidney except brain by 1.1-1.3-fold, and Cyc treatment caused higher hepatic and kidney GST activities by 1.3-1.4-fold. The present study has suggested that water-soluble organosulfur compounds SMC, SMCS and Cyc might be partly responsible for the biological effects of onion, such as a reduction in the risk of cancer, and deserves more in-depth research in order to improve human health.

Ⅰ. INTRODUCTION

Dietary factors play a major role in human health and reduce the risk of cancer by ability to modulate the detoxification systems[1]. Several epidemiological studies suggest that an inverse relation between Alliums vegetables intake such as onion (*Allium cepa* L.) and the risk of some cancers[2]. As to the biological activities of antitumor chemicals, either synthetic or naturally occurring, they have been strongly suggested to act through modulations of drug-metabolizing enzymes composed of phase I (cytochrome P_{450} enzymes) and phase II enzymes, affecting their expression or activation[3]. A widely accepted mechanism in cancer chemoprevention by dietary phytochemicals is through the induction of antioxidant and cytoprotective systems, such as the activity of the phase II enzymes GST （EC 2.5.1.18） and quinone reductase (QR; EC 1.6.5.2) [4]. SMCS and its biochemical precursor SMC, and Cyc are water-soluble organosulfur compounds found in onion that are well known to have various health functions such as antioxidative activity and antidiabetic effect[5] (Figure 1). However, it is not known with certainty whether SMC and other organosulfur compounds in onion are the components that modulate various phase II enzymes in an animal model under basal conditions (i.e., not following pharmacologic or carcinogen-mediated induction). Therefore, the objective of this study was to test the hypothesis that dietary SMC and other organosulfur compounds increase the phase II enzymes in various tissues of mice under basal conditions.

Correspondance：Keiko Sasaki ksasaki@cis.ac.jp
1) SSC, Chiba Institute of Science
2) Faculty of Pharmacy, Chiba Institute of Science
3) Health Sciences University of Hokkaido
(Received September 30 2021., Accept January 27 2022)

Ⅱ. MATERIALS AND METHODS

1. Materials

SMC and SMCS were obtained commercially from Sigma-Aldrich (St. Louis, MO, USA) and Research Organics, Inc., (Cleveland, OH, USA), respectively. Cyc was gifted kindly from Nippon Shinyaku Co., Ltd (Kyoto, JAPAN). Chemicals and reagents were of analytical grade and obtained commercially from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless otherwise stated. Chemicals and reagents were of analytical grade and obtained commercially

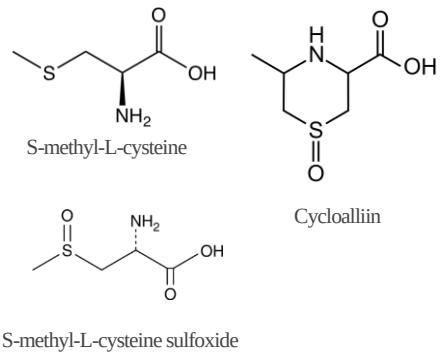

Fig. 1. Chemical structures of organosulfur compounds used in this study

from Kanto Chemical Co., Inc. (Tokyo, Japan) unless otherwise stated.

2. Animal care and treatment

Four-week-old mice (ddY strain, male) were obtained from Japan SLC, Inc. (Shizuoka, Japan). Principles in good laboratory animal care were followed and animal experimentation was in compliance with the Guidelines for the Care and Use of Laboratory Animals in the Health Sciences University of Hokkaido. Mice were maintained under a controlled environment (22 ± 2°C with constant humidity of 55 ± 10% and a 12 h light/dark cycle) and provided with water and diet *ad lib*. SMC, SMCS, Cyc (50, 100 and 200 mg/kg/day, respectively) or a vehicle (2% gum Arabic solution) was administered orally to mice once a day for 5 successive days.

Twenty-four hours after the last treatment the mice were sacrificed by decapitation. Tissues were removed, washed with ice-cold 1.15% potassium chloride and blotted briefly. They were then weighed and subjected to the preparation of cytosolic fractions as essentially described before[6]. Briefly, livers, kidneys, and brains were homogenized with Potter-Elvehjem homogenizers in 7 volumes of ice-cold physiological saline. The homogenates were first centrifuged at 9,000 x g for 20 minutes and the resultant supernatants were centrifuged at 105,000 x g for 60 minutes at 4°C. The supernatants from the second centrifugation were referred to as cytosolic fractions and stored at -80°C until use. Protein contents in the samples were determined by the Lowry-Folin method.

3. Measurement of GST activities

Measurement of enzyme activities were determined as described[6]. Cytosolic GST activity with 1-chloro-2,4-dinitrobenzene (CDNB; Kanto Kagaku, Tokyo, Japan) as a substrate were determined by the method of Habig *et al*. Briefly, 3 ml reaction mixture consisted of 1.2 ml of phosphate buffer (0.25 M, pH 6.5), 0.15 mL of GSH (20 mM), 0.15 mL of CDNB (20 mM, prepared in ethanol) and 0.15 mL of sample. Changes of absorbance at 340 nm for 1 min were recorded. GST activity was calculated using extinction coefficient of 9.6 $mM^{-1}cm^{-1}$for CDNB-GSH conjugate and expressed as U/g for liver and other tissues.

4. Western blot analysis of GST isozymes

Western blot analysis was performed as described previously[6]. Briefly, the cytosolic fraction from the liver was dissolved, blotted and immunoreacted by using polyclonal rabbit antiserum against GST α (PCF 403), μ (PCF408) and π (PCF401) from YLEM (Rome, Italy). Signals were quantified by densitometry with a densitograph (Lumino CCD Model AE-6930, ATTO, Tokyo, Japan).

5. Statistical analysis

Statistical analyses were performed by Dunnett test. Differences with P values < 0.05 were considered statistically significant.

Ⅲ. RESULTS

1. Effects of SMC, SMCS, Cyc on the activities of GST in liver and the other organs

In the liver, oral administration of SMC (50-200 mg/kg) potentiated GST activity in a dose-dependent manner as 100 and 200 mg/kg SMC elevated the activity 1.3-fold and 1.4-fold, respectively, compared with the control value. A similar enhancement in GST activity was observed in the livers of SMCS-treated and Cyc-treated mice; 200 mg/kg SMCS and 100 mg/kg Cyc caused 1.3- fold increase as compared with the control values, although the elevation observed at 200 mg/kg Cyc did not reach statistical significance. Both SMC and SMCS significantly enhanced GST activity in the small intestine at a dose of 50 mg/kg or higher as compared with the control. In contrast, there were no significant differences found in small intestine GST activity between the Cys-treated and the control mice. In kidney, SMC and Cyc significantly increased GST activity in a dose-dependent manner. SMC (200 mg/kg) and Cyc (200 mg/kg) enhanced GST activity 1.2-fold and 1.4-fold, respectively. SMCS enhanced GST activity at a dose of 50 mg/kg or higher 1.2-fold as compared with the control, although the elevation observed at 200 mg/kg SMCS did not reach statistical significance. SMC enhanced brain GST activity at a

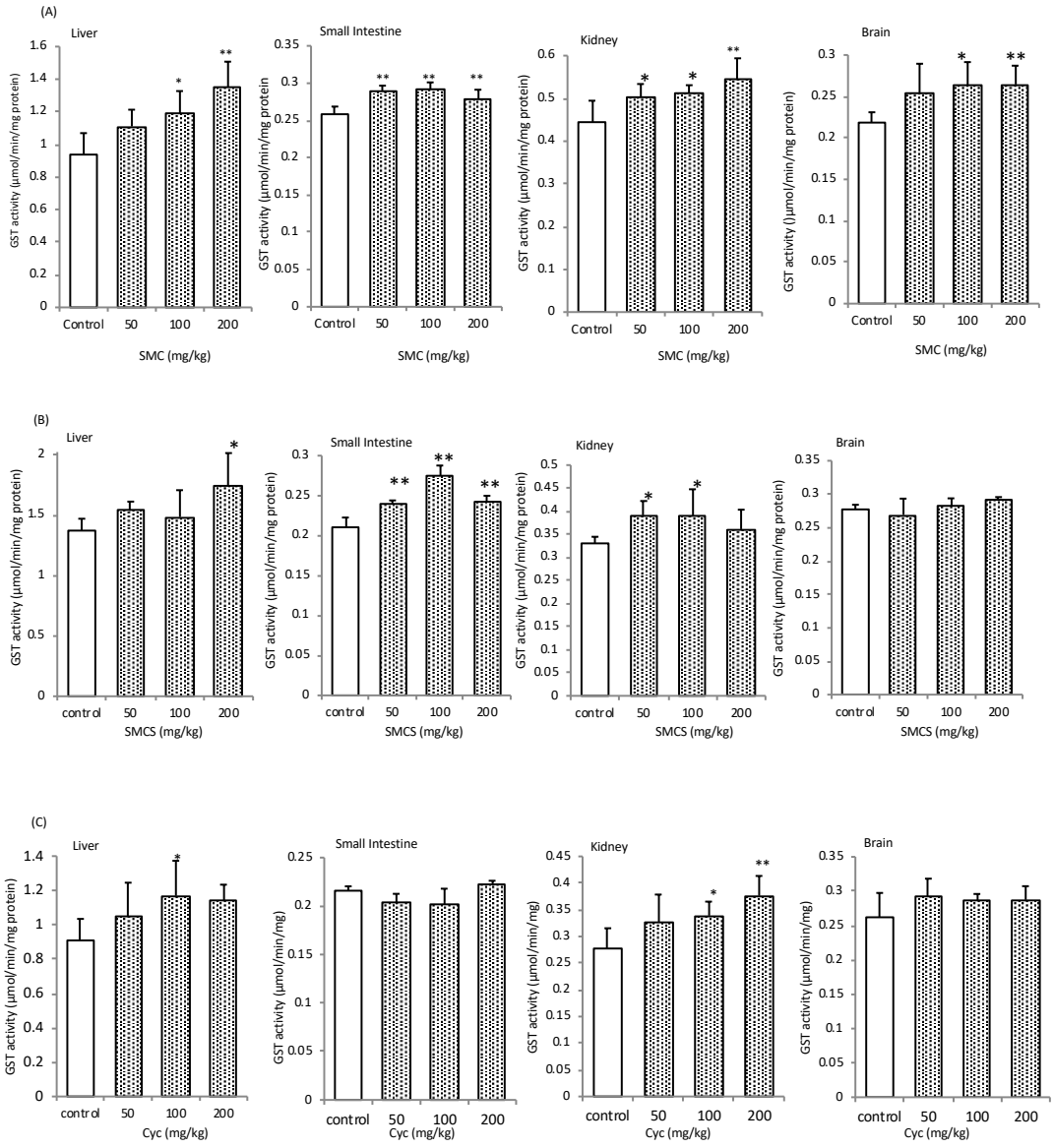

Fig. 2. Effects of S-methyl-cysteine (A), S-methyl-cysteine sulfoxide (B) or cysteine (C) on the activities of GST in mouse liver, small intestine, kidney and brain.

Data are mean ± standard deviation values of three to six animals. Significant differences are indicated as follows: **P* < 0.05 and ***P* < 0.01 compared with control.

dose of 100 mg/kg or higher 1.2-fold as compared with the control, whereas there was no significant increase of the enzyme activity in the brains of SMCS-treated and Cys-treated mice.

2. SDS-PAGE and western blot analysis

Effects of SMC, SMCS and Cyc (50-200 mg/kg, respectively) on the protein levels of GST isozymes in the liver were examined by western blot analysis. Figure 3 shows representative immunoblots of three classes of GST isozymes in the cytosol of liver. The immunoblot assay showed that 50-200 mg/kg of SMC concentration dependently increased GST protein levels. Class α was the main GST isozyme that increased in the protein amount upon treatment of 200 mg/kg SMCS, whereas treatment of Cyc induced class π.

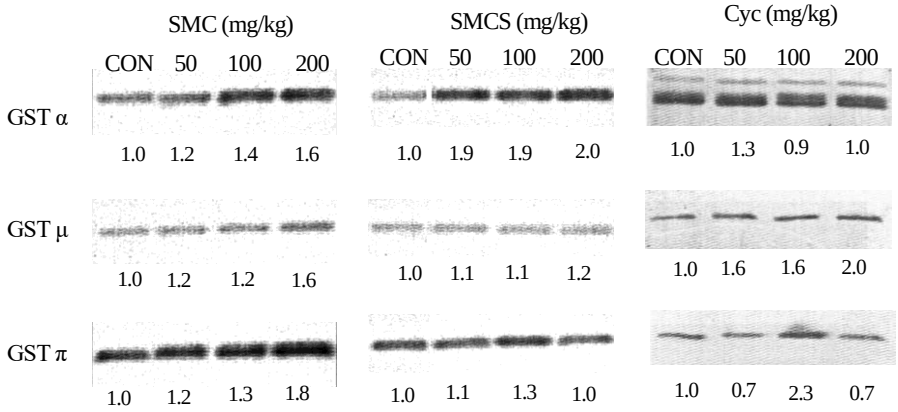

Fig. 3. Western blot analysis of the protein levels of GST isozymes. Cytosol fractions were prepared from the liver of mice treated with SMC (50-200 mg/kg, p.o. for 5 days), SMCS (50-200 mg/kg, p.o. for 5 days) and Cyc (50-200 mg/kg, p.o. for 5 days) and analyzed by immnoblotting.

Ⅳ. DISCUSSION

The present study demonstrates that oral administration of water-soluble organosulfur compound found in onion, SMC, SMCS and Cyc increase the activities of GST, in mouse organs. GST activity was increased by SMC, SMCS, and Cyc in the liver and kidney, by SMC and SMCS in the small intestine, and by SMC in brain. CDNB used in this study is a non-specific substrate, and collectively measures α, μ and π class of GST[7].

The results of the present study indicated that there may be some kind of regularities on chemical structure because SMCS exhibited higher activity in small intestine than SMC. Furthermore, it has been reported that orally administered Cyc appeared rapidly in plasma and was distributed to the tissues of the heart, lung, liver, spleen and especially kidney. This might be helpful for understanding the higher activity of Cyc in kidney[8]. Further studies are needed to determine the fate of OSC metabolite to elucidate their precise role in human health.

GST is a GSH-dependent cytosolic enzyme, which protects cells from the damage caused by ROS and catalyzes the conjugation of glutathione with a variety of electrophilic xenobiotics and facilitates their excretion. Higher tissue levels of phase II detoxification enzymes *viz.,* GST or QR result in lower susceptibility to carcinogenic insult. Recent findings suggest that SMC significantly reduced diethylnitrosamine-induced putative preneoplastic lesions, glutathione S-transferase placental form (GST-P) positive foci and cell proliferation in rat liver[9]. Feeding SMCS isolated from onion showed low cholesterol levels in serum and tissues in alloxan diabetic or cholesterol-fed diet rats[10]. Furthermore, Xiao and Parkin have reported that Cyc was capable of doubling the level of QR in hepa 1c1c7 cells[11]. Therefore, these organosulfur compounds are recognized as potential chemopreventive compounds.

In summsry, water-soluble organosulfur compounds with different structural characteristic were evaluated to understand their detoxification potential. The present study has shown that treatment with water-soluble organosulfur compounds SMC, SMCS and Cyc causes elevation of the activity of GST in mouse liver, kidney, small intestine and brain. Induction of the phase Ⅱ enzyme by these organosulfur compounds might be partly responsible for the biological effects, such as a reduction in the risk of cancer.

Ⅴ. REFERENCES

1) Kotecha R, Takami A, Espinoza JL, : Dietary phytochemicals and cancer chemoprevention: a review of the clinical evidence, Oncotarget, 7, 52517–52529, 2016.

2) Galeone C, Turati F, Zhang ZF, *et al.* : Relation of allium vegetables intake with head and neck cancers: evidence from the INHANCE consortium. Mol Nutr Food Res, 59, 1641-1650, 2015.

3) Lee JH, Khor TO, Shu L, *et al.* : Dietary phytochemicals and cancer prevention: Nrf2 signaling, epigenetics, and cell death mechanisms in blocking cancer initiation and progression. Pharmacol Ther, 137, 153–171, 2013.

4) Damazo-Lima M, Rosas-Pérez G, Reynoso-Camacho R, *et al.* : Chemopreventive Effect of the Germinated Oat and its Phenolic-AVA Extract in Azoxymethane/Dextran Sulfate Sodium (AOM/DSS) Model of Colon Carcinogenesis in Mice. Foods, 9, 169, 2020.

5) Khovarnagh N, Seyedalipour B.: Antioxidant, histopathological and biochemical outcomes of short-term exposure to acetamiprid in liver and brain of rat: The protective role of N-acetylcysteine and S-methylcysteine. Saudi Pharm J, 29, 280-289, 2021.

6) Sasaki K, Wada K, Kobayashi D, Murakami A, Matuoka K : Zerumbone elevates phase Ⅱ, but not phase Ⅰ, xenobiotic-metabolizing enzymes in mouse liver. Univ Bull Chiba Inst Sci, 13, 11-20, 2020.

7) Pushparajah S, Umachandran M, Plant K, *et al.*: Up-regulation of the glutathione S-transferase system in human liver by polycyclic aromatic hydrocarbons; comparison with rat liver and lung. Mutagenesis, 23, 299-308, 2008.

8) Ichikawa M, Mizuno I, Yoshida J, *et al.* : Pharmacokinetics of cycloalliin, an organosulfur compound found in garlic and onion, in rats. J Agric Food Chem.54, 9811-9819, 2006.

9) Nishikawa-Ogawa M, Wanibuchi H, Morimura K, *et al.* : N-acetylcysteine and S-methylcysteine inhibit MeIQx rat hepatocarcinogenesis in the post-initiation stage. Carcinogenesis. 27, 982-988, 2006.

10) Kumari, K.; Augusti, K.T. : Antidiabetic and antioxidant effects of S-methyl cysteine sulfoxide isolated from onions (*Allium cepa* Linn) as compared to standard drugs in alloxan diabetic rats. Indian J. Exp. Biol. 40, 1005–1009, 2002.

11) Xiao H, Parkin KL. : Antioxidant functions of selected allium thiosulfinates and S-alk(en)yl-L-cysteine sulfoxides. J Agric Food Chem. 50, 2488-2493, 2002