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A suppressive effect of selenium on amyloid-β plaque deposition in Tg2576 transgenic mice brain

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Abstract

Alzheimer's disease (AD) is a chronic neurodegenerative disease and characterized by deposition of the amyloid-β (Aβ) peptide in the brain. Reactive oxygen species (ROS) are thought to be associated with the onset and/or progression of AD. Selenium-dependent glutathione peroxidases (GPxs) play a critical role in the brain in the extinction of ROS. The selenium concentration in the brain is kept higher than those of other organs/tissues even when dietary selenium is limited, which suggests the importance of this element in the brain. We previously reported that a dietary selenium deficiency caused the elevated deposition of Aβ plaques in the brain of Tg2576 transgenic mice, which is frequently used as a model of AD. In this study, we analyzed the GPx activity and lipid peroxidation of brain tissues after the feeding of a selenium-deficient diet to Tg2576 transgenic mice. We also investigated the effect of seleno-L-methionine (SeMet) supplementation on the Aβ plaque deposition in the brain. After feeding for 72 weeks, the selenium concentration and GPx activity in the brain of the selenium-deficient diet-fed mice was lower than those in the selenium-adequate diet-fed mice and SeMet-supplemented diet-fed mice. The deposition of Aβ plaques and lipid peroxidation in the SeMet-supplemented diet-fed mice brain appeared to decrease compared to those in the selenium-deficient diet-fed mice. Supplementation of SeMet might have a suppressive effect on the brain Aβ plaque deposition in theTg2576 transgenic mice.

Key words: selenium, Alzheimer's disease, amyloid-β, seleno-L-methionine, Tg2576 mouse

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disease and the most common type of dementia in elderly people. Currently, about 50 million people are suffering from AD around the world and the number is estimated to triple by 2050 [1].

The deposition of amyloid β (Aβ) peptides and aggregation of tau proteins in a patient's brain are the pathological hallmarks of AD, and these are supposed to impair multiple cellular functions [2, 3]. However, the detailed underlying etiological mechanisms causing the AD clinical symptoms, such as memorial damage and cognitive decline, have still not been completely understood. Despite extensive research, few treatments are currently available for AD [4]. Colinesterase inhibitors and antagonist of the *N*-methyl-D-aspartate receptor are commonly used for the treatment of AD patients, although these medicines only slow down the development of the AD symptoms. Recently, the monoclonal antibody against Aβ, aducanumab and lecanemab, has been developed and used for the treatment of AD [5, 6]. These Aβ-targeting drugs has successively reduced the Aβ plaque deposition in AD patients' brain, however, improvement of the cognitive function by these reagents is limited and sometimes caused severe side effects [7, 8].

Aβ peptide deposition is predicted to cause a neurotoxic effect such as synaptic disfunction and neuronal loss in the brain [2, 9]. Understanding of the molecular pathogenesis underlying the onset and progression of AD is necessary for the development of an effective strategy of prevention or treatment of the disease. Increasing evidence indicates that various factors, such as genetic background, environmental pollution, lifestyles and accumulation of heavy metals, are involved in the cause of AD [4, 10, 11].

The accumulation of reactive oxygen species (ROS) was reported to cause cytotoxic oxidation and was thought to be related to the onset and progression of AD [3, 10, 12]. Because selenium is a crucial factor of several antioxidative enzymes, such as selenium dependent glutathione peroxidases (GPxs), which reduces peroxides inevitably generated in organisms, selenium has been suggested to be related to the AD pathogenesis [13, 14]. However, the biological effect of selenium on AD pathology is currently complicated [15, 16]. Some studies have shown a lower selenium status associated with the decreasing cognitive function [17, 18]. On the other hand, a large scale placebo-controlled, randomized clinical trial, PREADViSE, showed no effect of selenium supplementation on the prevention of AD [19]. Several selenoproteins, proteins containing one or more seleno-L-cysteine in their amino acid sequence, including GPxs, selenoprotein P, selenoprotein K and selenoprotein W are reported to be involved in the AD pathology, Aβ metabolism and toxicity caused by Aβ fibrils [20–24]. In our previous research, a selenium deficient diet was fed to Tg2576 transgenic mice for 76 weeks which resulted in a more than 2 times increase of the Aβ plaque deposition in the brains of selenium-deficient diet-fed mice compared to the control selenium-adequate diet-fed mice [25]. This result suggested that selenium in the brain is involved in the metabolism of brain Aβ in Tg2576 transgenic mice. In this study, we evaluated the suppressive effect of dietary selenium against the deposition of Aβ plaques in the Tg2576 transgenic mice brain. We compared the effect of dietary selenium on the Aβ plaque deposition in brains by feeding 3 different diets to Tg2576 transgenic mice, i.e., a selenium-adequate normal diet, selenium-deficient diet, or seleno-L-methionine (SeMet)-supplemented diet.

Materials and Methods

Materials

Seleno-L-methionine (SeMet), glutathione in the reduced form (GSH), hydrogen peroxide, 2-thiobarbituric acid (TBA) and thioflavin T (Th-T) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). 2,3-Diaminonaphthalene (DAN) was from Dojindo Molecular Technologies, Inc. (Kumamoto, Japan). Nicotinamide adenine dinucleotide phosphate in the reduced form (NADPH) and glutathione reductase (GR) were from the FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). The water used throughout this study (>18 MΩ cm) was generated by a Milli-Q Reference system (Millipore Corp., Billerica, MA, USA). All other chemicals were of commercial reagent or special grade and used as received.

Animals and diets

Female 16-week-old Tg2576 transgenic mice were purchased from Taconic Farms, Inc. (Germantown, NY, USA). They were cared for in accordance with the guidelines of the Nagasaki University on Animal Care. Animals were randomly assigned to 3 groups (5 in each) and kept one per cage. The mice were housed on a 12 h light-12 h dark schedule at 23 ± 2 °C and 60% relative humidity and freely fed the selenium-adequate regular breeding diet (CE-2, Clea Japan, Inc., Tokyo, Japan), selenium-deficient diet (F2SeDD, Oriental Yeast Co., Ltd., Tokyo, Japan), or SeMet-supplemented diet for 72 weeks. The Torula yeast-based selenium deficient diet was prepared to contain 0.4 μgSe/g or less by the manufacturer and the actual selenium content was 0.007 μgSe/g. The CE-2 regular diet was reported to contain 0.4-0.6 μgSe/g. The SeMet-supplemented diet was prepared every 30 days by the addition of SeMet to the selenium-deficient diet to make the selenium concentration 2 μgSe/g. SeMet was dissolved in Milli-Q water and sprayed on the selenium-deficient diet.

After feeding for 72 weeks, the selected organs/tissues were obtained from the mice under isoflurane anesthesia. The harvested tissues were thoroughly rinsed with 66 mM phosphate buffer (pH 7.4) and stored at −80 °C until used. Blood was collected into heparin-coated tubes and centrifuged at 1,000 *g* for 10 min to separate the plasma and hemocytes. The left hemisphere of the mice brain was immersed in an optimal cutting temperature (OCT) compound (Tissue-Tek O. C. T. Compound, Sakura Finetek USA, Inc., CA, USA) and frozen on dry ice for preparation of the brain slices.

Preparation of tissue lysate

Tissues were homogenized in 66 mM phosphate buffer using a Polytron PT1200E (Kinematica AG, Luzern, Switzerland) on ice. The homogenate was then sonicated at an acoustic power level of 20 W using a probe-type sonicator 250D (Branson Ultrasonic Corp., Danbury, CT, USA). The suspension was subsequently centrifuged in a rotor 70.1Ti using an Optima L-80 Ultracentrifuge (Beckman Coulter, Inc., Indianapolis, IN, USA) at 30,200 *g*, 4 °C for 1 h. The obtained supernatant was used for the determination of the protein concentration and measurement of the GPx activity.

Preparation of brain slice and its staining with thioflavin T

The OCT compound embedded brain was cut into 10-μm thick slices by a cryostat CM1950 (Leica Microsystems, Wetzlar, Germany). For staining of the Aβ plaque, the Tg2576 mice brain slices were covered with a 10 μM Th-T in 50% ethanol solution for 5 min at room temperature, then washed 5 times with 50% ethanol for 1 min. The fluorescence image was captured by an inverted microscope BZ-8100 (KEYENCE Corporation, Osaka, Japan) using a filter set with 450-470 nm excitation and 515-565 nm emission. MetaMorph (Premier) software (Molecular Devices, LLC, Sunnyvale, CA, USA) was used for the qualitative analysis of the obtained fluorescence images of the mice brain slices.

Determination of selenium

After acid digestion with a 1 : 4 mixture by volume of perchloric acid and nitric acid, the selenium concentrations in the specimens were fluorometrically determined using DAN [26]. The digested samples were reacted with DAN in a 0.1 M HCl solution containing 0.1 M EDTA and 0.1 M NaF at 60 °C for 20 min with 120 str/min followed by extraction with cyclohexane. The fluorescent intensity (Ex: 375 nm, Em: 520 nm) was measured by a FP-6600 fluorometer (Jasco Corporation, Tokyo, Japan). The selenium standard solution for the atomic absorption spectrometry (1000 mg/L in 0.1 mol/L nitric acid, Kanto Chemical Co., Inc., Tokyo, Japan) was used as the standard material for preparation of the calibration curve (0.1-1000 ng Se/mL).

Determination of glutathione peroxidase activity and protein concentrations

The protein concentrations in the specimens were measured by the Lowry method using bovine serum albumin (Nacalai Tesque, Inc.) as the standard [27].

The tissue GPx activity was measured by monitoring the absorbance at 340 nm due to the NADPH [28]. The tissue lysate was combined with sodium azide (1 mM), a GR solution (1 unit/mL), a reduced GSH solution (1 mM) and an NADPH solution (0.2 mM) in 66 mM phosphate buffer (pH 7.4). The reaction was initiated by the addition of a hydrogen peroxide solution (0.25 mM). The absorbance at 340 nm was recorded every 1 min just after mixing by inversion using a V-660 spectrometer (JASCO Corp.). The GPx activity was calculated using the following equation (1) as μmoles of NADPH oxidized per minute, where ΔA_{SAM} is the decrease in the absorbance at 340 nm of the sample solutions between 15 and 75 s after the addition of the substrates, ΔA_{BLK} is the decrease in absorbance at 340 nm per minute of the solutions using the 66 mM phosphate buffer instead of the sample solutions, 10 is the dilution factor, ε_{mM} is the extinction coefficient for the 1 mM NADPH solution (6.22 mM^{-1} cm^{-1}), and c is the protein content (mg/mL).

$$\text{GPx activity} = (\Delta A_{SAM} - \Delta A_{BLK}) \times 10/\varepsilon_{mM}/c \quad (1)$$

Determination of TBA reactive substance (TBARS)

The TBARS was measured according to the method of Tien & Aust [29]. The 2× amount of TBA reagent solution (0.375% TBA, 15% butyl hydroxytoluene, 0.4% trichloroacetic acid in 0.25 M HCl) was mixed with the brain cell lysate and boiled for 15 min. After centrifugation at 3,000 rpm for 10 min at room temperature, the absorbance at 535 nm of the supernatant was measured by a V-660 spectrometer (JASCO Corp.). The TBARS was calculated by equation (2) as moles of red pigment produced by the reaction, where A_{SAM} is the absorbance at 535 nm of the sample solutions, A_{BLK} is the absorbance at 535 nm of the solutions using the 66 mM phosphate buffer instead of the sample solutions, 6 is the dilution factor, and 1.56×10^5 is the molar extinction coefficient of the produced red pigment after the reaction.

$$\text{TBARS (mol/g)} = (A_{SAM} - A_{BLK}) \times 6/(1.65 \times 10^5 \times \text{brain tissue weight}) \quad (2)$$

Statistical analysis

All the data are presented as the mean and standard error (SE). Statistical analyses were performed using PRISM 10 (GraphPad Software, Inc., La Jolla, CA, USA). Statistically significant differences between the feeding groups were determined by a one-way ANOVA using the Tukey method. Comparisons were considered to be statistically significant at $P < 0.05$.

Results and discussion

Selenium concentrations in the organs/tissues of selenium-deficient diet-fed Tg2576 mice substantially decreased compared to those of the selenium-adequate diet-fed mice after feeding for 72 weeks (**Figure 1**). Selenium concentrations of the livers, kidneys, hearts and muscles in the selenium-deficient diet-fed mice were 8.2%, 20.0%, 10.7% and 9.3% of those in the selenium-adequate diet-fed mice, respectively. On the other hand, the brain selenium concentration in the selenium-deficient diet-fed mice decreased to 50% of that in the selenium-adequate diet-fed mice. A similar selenium status in the organs/tissues after selenium-deficient diet feeding was observed in our previous research using the ICR and Tg2576 transgenic mice [25, 30]. The selenium concentration in the brain is far less variable than those in other peripheral organs/tissues when the selenium intake from the diets is limited [31, 32]. This brain specific selenium retention suggested the vital role of selenium in the brain. Feeding of the SeMet-supplemented diet increased the selenium concentrations in the organs/tissues of the Tg2576 mice 1.5-5.6 times in comparison to the selenium-adequate diet feeding. Selenium concentrations of the brains, livers, kidneys, hearts and muscles in the SeMet-supplemented diet-fed mice were 3.7, 2.1, 1.6, 4.3 and 5.6 times higher than those in selenium-adequate diet-fed mice, respectively. Because SeMet is non-specifically incorporated into proteins in the position of Met, it is known to be incorporated into organs/tissues with high rates of protein synthesis such as the skeletal muscles, pancreas, erythrocytes, livers and kidneys [33, 34]. SeMet was also reported to be retained

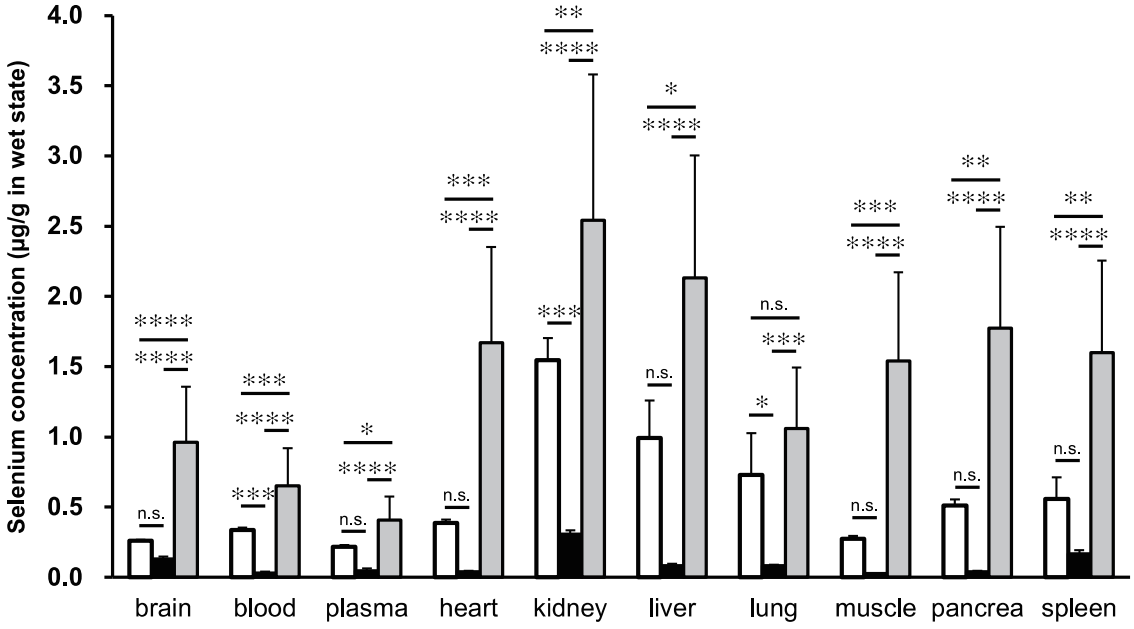

Figure 1. Selenium concentration in organs/tissues of Tg2576 transgenic mice after the feeding. White column, selenium-adequate diet-fed mice; black column, selenium-deficient diet-fed mice; gray column, SeMet-supplemented diet-fed mice. Data express mean ± SE (n = 3-6). *, **, ***, ****: significantly different between the diets with P < 0.05, < 0.01, < 0.001, < 0.0001, respectively (ANOVA with a Tukey test), n. s.: not significantly different from each other.

by the brain.

The Tg2576 transgenic mice overexpress a human amyloid precursor protein (APP) containing a Lys670Asn and Met671Leu mutation which was found in familial AD patients and significantly elevated amounts of Aβ peptides were detected in the 11–13 month-old Tg2576 mice brain [35]. In this study, we compared the extent of Aβ plaque deposition in the Tg2576 mice brain among the different selenium statuses. Th-T, a commonly used amyloid staining fluorescent dye, was used for the detection of Aβ plaque deposition in the Tg2576 mice brain slices in this study. The obtained fluorescent images of the Th-T-treated mice brain slices were qualitatively analyzed using MetaMorph software (**Figures S1**). Although it is not statistically significant, feeding of both the selenium-deficient diet and SeMet-supplemented diet changed the Aβ plaque deposition in the Tg2576 mice brain (**Figure 2**). In the selenium-deficient diet-fed mice brain, the deposition of Aβ plaques appeared to have increased. On the other hand, a slightly decreased Aβ deposition was detected in the SeMet-supplemented diet-fed Tg2576 mice brain. In our previous research, Aβ plaque deposition in the selenium-deficient diet-fed Tg2576 mice brain significantly increased after the 76 weeks-feeding compared to the selenium-adequate diet-fed Tg2576 mice [25]. This difference between the two studies might be caused by the interindividual difference of the Tg2576 mice used in both studies.

Because the selenium concentration in organs/tissues substantially changed by the feeding of selenium-deficient or SeMet-supplemented diets, the influence of feeding these diets on the selenoprotein activity in the brain was examined (**Figure 3**). GPxs are the major selenoprotein family and play pivotal roles by reducing hydrogen peroxide and lipid peroxides [36]. The GPx activity against both inorganic (H_2O_2) and organic (TBHP) peroxides of the selenium-deficient diet-fed Tg2576 mice brain tissue lysate was lower than that of the selenium-adequate diet-fed mice. On the other hand, the brain GPx activity of the SeMet-supplemented diet-fed mice was at the same

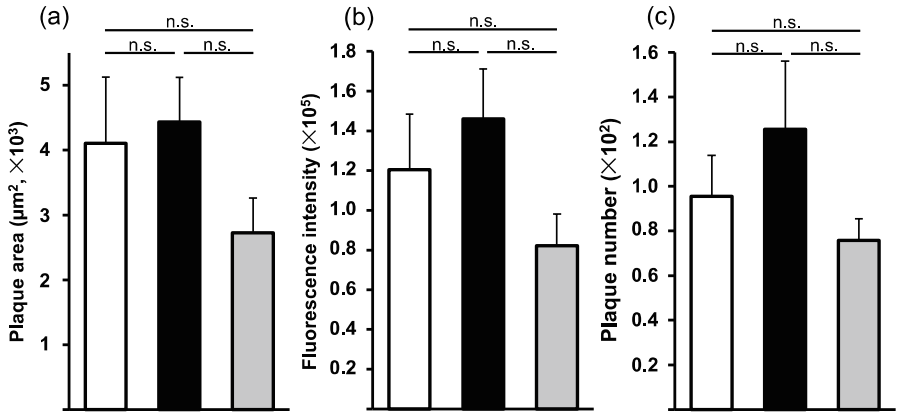

Figure 2. Image analysis of Th-T fluorescence in the brain slices of the Tg2576 transgenic mice. (a) Total area stained with Th-T per brain slice; (b) total fluorescence intensity of stained area; (c) the plaque number. White column, selenium-adequate diet-fed mice; black column, selenium-deficient diet-fed mice; gray column, SeMet-supplemented diet-fed mice. Data express mean ± SE (n = 13-15). n. s.: not significantly different from each other.

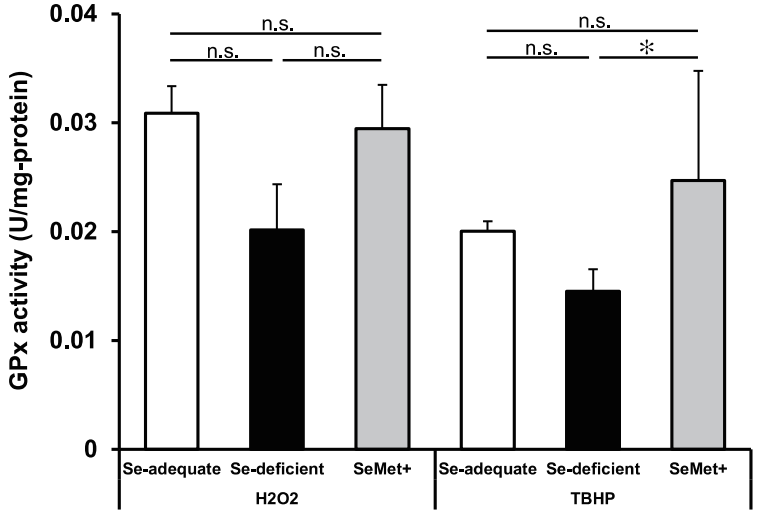

Figure 3. GPx activity of brain tissue lysate of Tg2576 transgenic mice. White column, selenium-adequate diet-fed mice; black column, selenium-deficient diet-fed mice; gray column, SeMet-supplemented diet-fed mice. Data express mean ± SE (n = 3-6). *: significantly different between the diets with $P < 0.05$ (ANOVA with a Tukey test), n. s.: not significantly different from each other.

level as that of the selenium-adequate diet-fed mice. Although the brain selenium concentration of the SeMet-supplemented diet-fed mice was nearly 3 times higher than that of selenium-adequate diet-fed mice, the change in the brain GPx activity was small. This suggested that the GPx activity in the brain was saturated by the feeding of the selenium-adequate diet and the additional supply of selenium did not have an influence on the GPx activity in the SeMet-supplemented diet-fed mice.

Tg2576 transgenic mice are commonly used as a model of familial AD. It was reported that the generation of ROS in the Tg2576 mice brain was increased and this caused cerebrovascular dysfunction [37, 38]. To examine the extent of lipid peroxidation in the brain of the Tg2576 mice, TBARS in the brain tissue lysates was measured (**Figure 4**). The value of TBARS in the selenium-deficient diet-fed Tg2576 mice brain was almost twice that of the selenium-adequate diet-fed Tg2576 mice brain. On the other hand, a decreasing tendency of the TBARS value was observed in the brain tissues of the SeMet-supplemented diet-fed Tg2576 mice. These results suggested that the reduced brain GPx activity in the selenium-deficient diet-fed mice led to the increased lipid peroxidation. The reduced GPx activity and increased lipid peroxidation in the Tg2576 transgenic mice were also reported in previous studies [38]. In this study, about a 5 times higher content of selenium as SeMet was supplied to the SeMet-supplemented diet-fed mice compared to the selenium-adequate diet-fed mice and this treatment resulted in a 3.7 times higher selenium concentration in the brain of the SeMet-supplemented diet-fed mice than that of the selenium-adequate diet-fed mice. However, the brain GPx activity of the SeMet-supplemented diet-fed mice was almost the same as that of the selenium adequate-diet fed mice. These facts suggested that an excess amount of SeMet was randomly incorporated into the Met position of proteins rather than being used to make the brain GPx activity higher than that of the selenium-adequate diet-fed mice. Met substitution by SeMet sometimes changes the protein stability due to the increased hydrophobicity and distinct oxidation susceptibility of SeMet compared to Met [39–43]. Martinez et al. prepared the SeMet substituted Aβ40 peptide in the position of Met35 and it resulted in inhibition of the peptide fibrillation and decrease in the cytotoxicity [44]. The reduced tendency of Aβ plaque deposition in the SeMet-supplemented diet-fed mice might be caused by substitution of Met in APP by SeMet.

In this study, the feeding of the selenium-deficient diet reduced the selenium concentration in the brain of the Tg2576 transgenic mice and it was presumed to cause the reduced GPx activity in the brain tissues. On the other hand, the SeMet-supplemented diet feeding significantly increased the brain selenium concentration of the Tg2576 transgenic mice, and it was effective in maintaining the brain GPx activity. Although it was not significant, the increased lipid peroxidation in the selenium-deficient diet-fed mice brain was observed and the lipid peroxidation

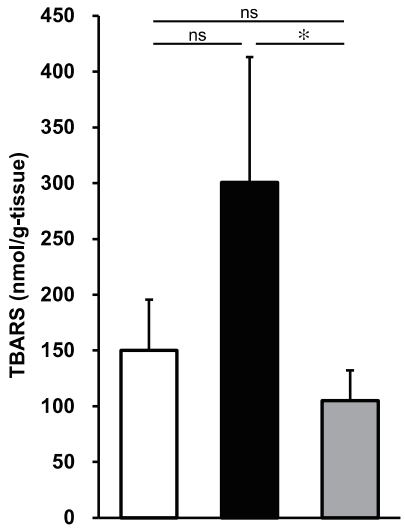

Figure 4. TBARS of brain tissue lysate of Tg2576 transgenic mice. White column, selenium-adequate diet-fed mice; black column, selenium-deficient diet-fed mice; gray column, SeMet-supplemented diet-fed mice. Data express mean ± SE (n = 3). *: significantly different between the diets with $P < 0.05$ (ANOVA with a Tukey test), n. s.: not significantly different from each other.

was reduced in the SeMet-supplemented diet-fed mice brain. These results were presumed to be partially caused by the difference in the brain GPx activity. Additionally, the decreased tendency of the Aβ deposition in the SeMet-supplemented diet-fed Tg2576 mice brain compared to the selenium-deficient diet-fed mice might be the effect of the increased brain GPx activity and reduced lipid peroxidation. Although we need a further investigation, there is the possibility that supplementation of SeMet to selenium deficient mice suppresses the deposition of Aβ in the Tg2576 transgenic mice brain. The analysis of the expression levels of GPxs and the ROS generation in brain tissues after feeding of selenium species into AD model mice would be helpful for elucidation of the mechanisms of the suppressive effect of selenium on Aβ deposition in future experiment.

Considering the average amount of the diets the Tg2576 mice had in this study (5–7 g/day), selenium intakes of the selenium-adequate diet-fed mice and the SeMet-supplemented diet-fed mice were about 200 and 1,000 times higher than that of recommended dietary allowance established in Dietary Reference Intakes for Japanese (25–30 μg/day for adults) [45], respectively. Because feeding C57BL/6J mouse with the excess amount SeMet (8 mgSe/kg diet) did not cause apparent toxic symptom of selenium [46], mouse seemed to have higher tolerance for SeMet compared to human. Selenium concentration in food is highly dependent on the selenium concentration in the soil [47]. Therefore, human dietary selenium intake also geographically varies, which range from 3 to 6,690 μg/day [48]. It is reported that about 1 billion people are affected by insufficient selenium intake worldwide [49]. More detailed research on the relevance of selenium intakes to various disease including AD is needed.

Despite the numerous efforts for developing medications with the aim of the complete cure of AD for many decades, the option and the efficacy of medications currently used for the treatment of AD is limited. This is probably attributed to the complicated pathology of AD [50]. Although the relevance of AD onset/progression and selenium is still controversial, various selenium species have been developed for the prevention and treatment of AD [21, 51, 52]. The administration of inorganic and organic selenium species, including SeMet, have been reported to be effective for the improvement of the cognitive function and the reduced neurotoxicity of Aβ and tau protein in the transgenic AD model mice [53–56]. Zheng et al. administered sodium selenate with drinking water to AD model mice. Treatment with 7.5–10.0 μgSe/day for 10 months significantly reduced the Aβ deposition and neurofibrillary tangles and increased the activity of GPxs and thioredoxin reductases in the mice brain [57]. The results obtained from these research studies will contribute to a further understanding of AD pathology and help us to develop effective medicine for the prevention and treatment of AD [24, 58–62].

In conclusion, reduced GPx activity in the selenium-deficient diet-fed Tg2576 mice brain was predicted to increase lipid peroxidation, which may be related to the enhanced deposition of Aβ plaques in the brain. SeMet supplementation raised the brain selenium concentration and was effective in maintaining the brain GPx activity at the same level as that of the selenium-adequate diet-fed mice which appeared to prevent the production of lipid peroxides.

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Supplemental information

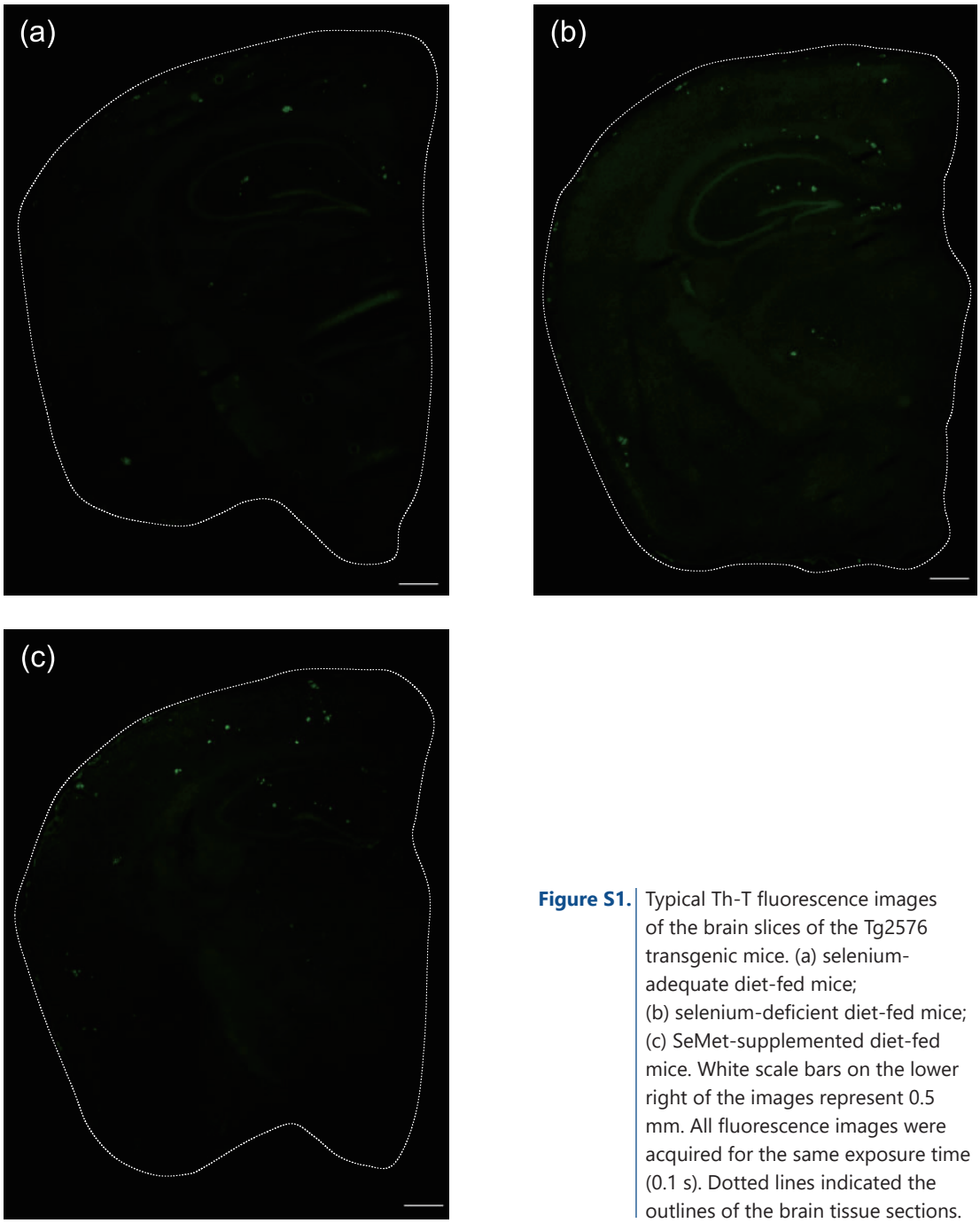

Figure S1. Typical Th-T fluorescence images of the brain slices of the Tg2576 transgenic mice. (a) selenium-adequate diet-fed mice; (b) selenium-deficient diet-fed mice; (c) SeMet-supplemented diet-fed mice. White scale bars on the lower right of the images represent 0.5 mm. All fluorescence images were acquired for the same exposure time (0.1 s). Dotted lines indicated the outlines of the brain tissue sections.