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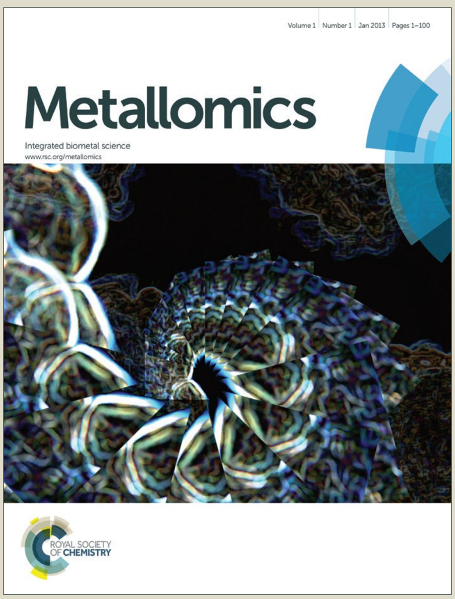

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Selenomethionine reduces the deposition of beta-amyloid plaques by modulating β-secretase and enhancing selenoenzymatic activity in a mouse model of Alzheimer's disease

Zhong-Hao Zhang[1], Chen Chen[2], Qiu-Yan Wu[2], Rui Zheng[2], Qiong Liu[2], Jia-Zuan Ni[1,2], Peter R Hoffmann[3] and Guo-Li Song[2*]

[1]Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, University of Chinese Academy of Sciences, Changchun, China.

[2] Shenzhen Key Laboratory of Marine Bioresources and Ecology, College of Life Sciences and Oceanography, Shenzhen University, Shenzhen, China.

[3] Department of Cell and Molecular Biology, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA.

* Corresponding author.

E-mail: lilys@szu.edu.cn (GLS)

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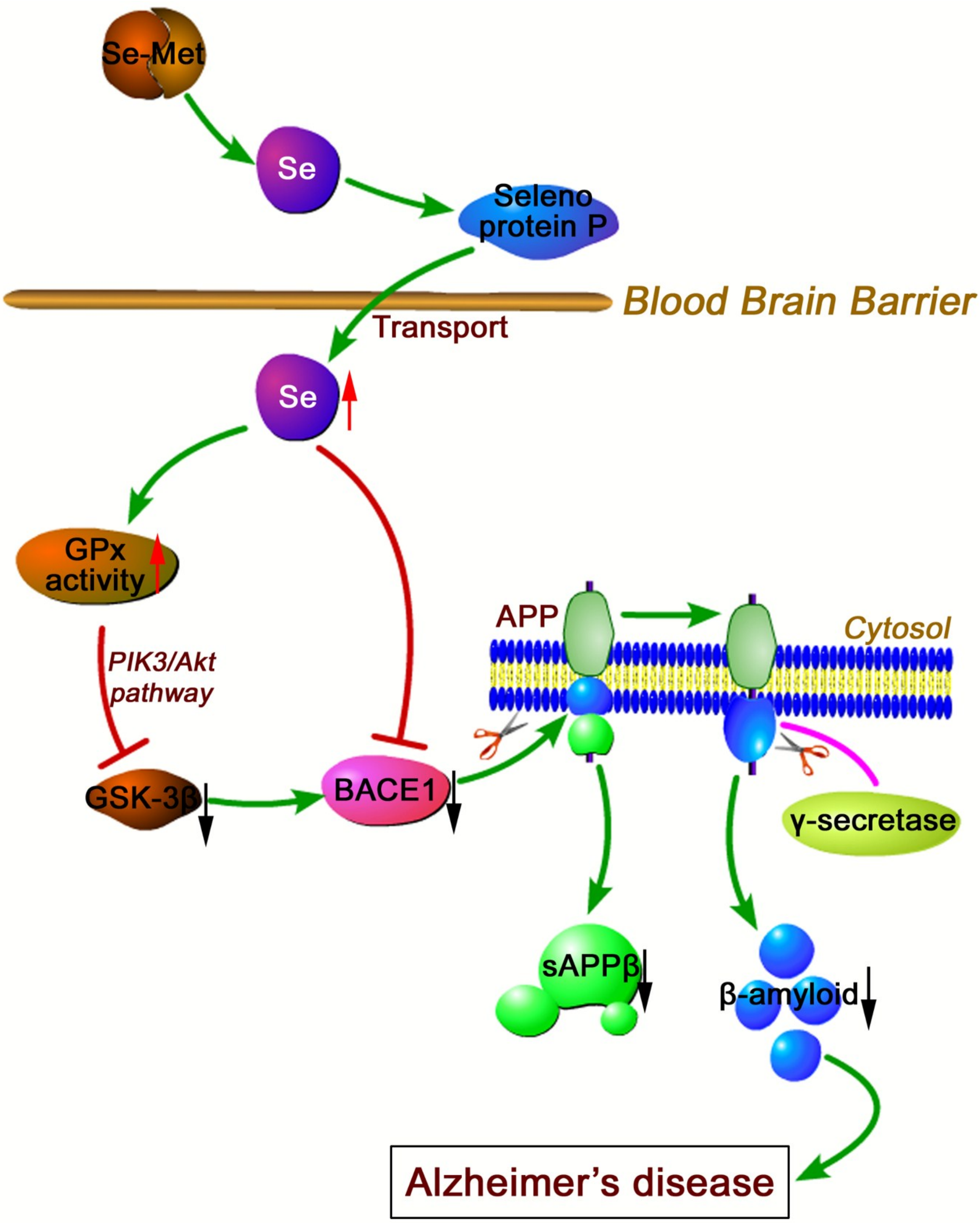

Effects on Aβ production and the probable connection among selenoenzymes, GSK3β and Aβ pathology by selenomethionine treatment in AD mice.

Abstract

Alzheimer's disease (AD) is characterized by the production of large amounts of beta-amyloid (Aβ) and the accumulation of extracellular senile plaques, which have been considered to be potential targets in the treatment of AD. Selenium (Se) is a nutritionally essential trace element with known antioxidant potential and Se status has been shown to decrease with age and has a close relationship with cognitive competence in AD. Selenomethionine (Se-Met), a major reserve form of Se in organisms, has been shown in our previous study to ameliorate the decline in cognitive function, increase oxidation resistance, and reduce tau hyperphosphorylation in a triple transgenic mouse model of AD. However, it has not been reported whether Se-Met has any effects on Aβ pathology in AD mice. To study the effect of Se-Met on Aβ pathology and the function of selenoproteins/selenoenzymes in 3×Tg-AD mice. 3×Tg-AD mice at 8 months of age were treated with Se-Met for 3 months. Se-Met led to significantly reduced production and deposition of Aβ, down-regulation of β-secretase levels and enhanced activity of selenoenzymes as well as increased levels of Se in the hippocampus and cortex. Se-Met reduces amyloidogenic processing of amyloid precursor protein while modulates β-secretase and selenoenzymatic activity in the AD mice. These results indicate that Se-Met might exert its therapeutic effect through

multiple pathways in AD.

Keywords: Alzheimer's disease, selenomethionine, Aβ, β-secretase, selenoenzyme

Significance to metallomics

Selenium has been widely recognized as a vital trace element abundant in the brain with effects of antioxidative, anticancer, and anti-inflammatory as well as robust immunity. Understanding how selenomethionine (possessing relatively higher bioavailability and lower toxicity compare to those inorganic forms of selenium) exerts its effect to improve the cognitive deficit is important in the research of Alzheimer's Disease. This study suggested the role and underlying mechanism of selenomethionine on Aβ pathology and selenoenzymes to protect against Alzheimer's Disease.

Introduction

Alzheimer's disease (AD) is an irreversible age-associated neurodegenerative disorder with progressive decline in cognitive function and loss of memory. AD pathology is characterized by the accumulation of extracellular senile plaques (SPs) and intracellular neurofibrillary tangles (NFTs) in the brain [1-3]. Beta-amyloid (Aβ) peptide, the main component of SP, is generally considered to act directly as a trigger for the death of neuronal cells in AD. Numerous studies have suggested that amyloid plaque build-up occurs primarily before the onset of cognitive deficits and the aggregation and accumulation of Aβ causes synaptic and neuronal dysfunction and aggravates memory impairments [4]. Emerging evidence supports the notion that NFTs, which are mainly composed of hyperphosphorylated tau, may be as important as Aβ in AD [5]. Interestingly, Aβ oligomers could also induce mislocalization and hyperphosphorylation of tau in vitro, and Aβ1-42 fibrils significantly promote the formation of NFTs in P301L tau transgenic mice [6, 7]. Most studies have demonstrated that Aβ might have an important role in the early stage of the pathological process of AD and reducing Aβ is regarded as a crucial therapy for the intervention of AD progression. Therefore, most current efforts to find therapies for AD treatment are directed at the inhibition of Aβ production by modulating the amyloidogenic pathway or promoting Aβ clearance through autophagy and/or ubiquitination pathway[8]. As the Aβ pathology is the major cause of AD, reducing the overproduction and deposition of Aβ is still regarded as a useful therapeutic strategy for AD.

Selenium (Se) has been widely recognized as a vital non-metallic trace element abundant in the brain [9]. Evidence has indicated that Se is also an essential nutrient that possesses a wide range of beneficial biochemical and pharmacological properties including antioxidation, anticancer and anti-inflammation, as well as promotion of efficient protein synthesis and robust immunity [10, 11]. In addition, it has been reported that the level of Se in the brains of dementia patients is negatively correlated with cognitive function, and particularly low levels of Se could increase the risk of AD [12, 13]. Recently, sodium selenite and sodium selenate, the two main inorganic Se compounds, have been shown to protect primary cultured rat hippocampal neurons against Aβ42-induced toxicity and mitigate functional deficits induced by tau pathology in AD models [14, 15].

Our previous study indicated that Selenomethionine (Se-Met), a major bioactive form of Se present in organisms, could ameliorate the decline in cognitive function, reduce tau hyperphosphorylation, and reverse synaptic deficit in a triple transgenic mouse model of AD [16]. It could also facilitate the survival of primary hippocampal neurons treated by Fe^{2+}/H_2O_2 or Aβ [17]. Most of the biological effects of Se in vivo are exerted through anti-oxidant or redox regulating selenoproteins including selenoprotein P (Sel-

P), selenoprotein R (Sel-R), selenoprotein M, glutathione peroxidase (GPx), and thioredoxin reductase (TrxR). Our previous study also found that Se-Met could dramatically elevate the level of glutathione (GSH) in AD mice at 4 months of age [16]. However, it is still unknown what roles selenoproteins and selenoenzymes may play during this process and what effect Se-Met has on amyloid pathology. In this study, the effect of Se-Met on Aβ pathology and the function of selenoproteins/selenoenzymes was studied using a triple transgenic AD mouse (3×Tg-AD).

Materials and methods

Animals and treatment

3×Tg-AD mice were purchased from The Jackson Laboratory (JAX order number 3591206, Bar Harbor, ME, USA), which express human gene mutants APPswe, PS1M146V, and tauP301L. According to the manufacturer's instructions and our previous studies using 2- to 12-month old AD mice, various pathological indices (such as hyperphosphorylation of tau, Aβ deposit, and inflammation) in 3×Tg-AD mice were significantly elevated in the hippocampus and cortex of mice at 8 months of age when compared to mice at two to four months of age [18, 19]. Therefore, 3×Tg-AD mice at 8 months of age (n=12; 6 males and 6 females) were treated with 6 μg/mL Se-Met (Sigma-Aldrich, USA) in drinking water for 12 weeks, while the control group (n=12; 6 males and 6 females) received normal drinking water. The body weight of each mouse was recorded every two weeks.

After treatment with Se-Met for 12 weeks, mice were euthanized with ether anhydrous inhalation, and their brains were rapidly removed. The left hemisphere was immersion-fixed with 4% paraformaldehyde for 24 h followed by dehydration with serial ethanol, clearing with xylene, infiltration with paraffin, and was cut into 5-μm-thick sections. The right hemisphere was further dissected into hippocampal and cortical samples, snap frozen in liquid nitrogen, and stored at -80°C until analysis.

The experiments and procedures described here were performed in strict accordance with institutional guidelines regarding experimental animal use in Shenzhen University. The protocol was approved by the Animal Ethical and Welfare Committee of Shenzhen University (Permit Number: AEWC-20140615-002). All surgeries were performed under ether anhydrous inhalation anesthesia, and all efforts were made to minimize suffering.

Immunohistochemical staining

Sagittal paraffin sections (5-μm thick) of mouse brains were mounted on glass slides. The sections were pretreated by washing with 0.01 mol/L phosphate-buffered saline (PBS) and formic acid (70%), restoring Aβ for 20 min, and then with 3% H_2O_2 in methanol for 10 min to eliminate endogenous peroxidase activity in the tissue. After blocking with 5% goat serum in PBS for 10 min, these sections were further incubated with primary antibodies (1:150, 6E10/39320, Convance, USA) overnight at 4°C, followed by incubation with secondary antibodies (1:500 in PBS) for 1 h at 37°C, and finally developed using the avidin-biotin complex method with 3,3'-diaminobenzidine as the chromogen. Three equidistant sections, including the whole hippocampal and frontal cortical areas, were evaluated for each animal and then imaged with microscopy (Olympus, Japan).

Immunoblot analysis

The hemibrain was homogenized in nine volumes of Tris-buffered saline (TBS) with a protease inhibitor cocktail and phosphatase inhibitors (Roche, Basle Switzerland). The samples were centrifuged at 13,000 x *g* for 1.5h at 4°C. The TBS-soluble supernatants were collected, and their pellets were resuspended in two volumes of 5% sodium dodecyl sulfate (SDS) containing the protease inhibitor cocktail and phosphatase inhibitors. The TBS-insoluble pellet mixtures were then sonicated for 1 min in an ice bath and centrifuged at 13,000 x *g* for 30 min at 4°C. The supernatants of TBS-insoluble homogenates were also collected. Protein concentration was determined using the bicinchoninic acid (BCA) assay (Sigma-Aldrich, USA). Proteins (20μg) were loaded into each lane of a 10-15% SDS-polyacrylamide gel. After electrophoresis, proteins were transferred onto 0.45 nm polyvinylidene difluoride membranes (Millipore, Massachusetts, USA) at 100 mA for 1.5 h. The membrane was then blocked with 5% fat-free milk in TBS for 2 h at 37°C, followed by incubation with primary antibodies (6E10/39320, sAPPβ/39138, Covance, USA; full-APP/ab126732, BACE1/ab108394, Selp/ab109514, SelR/ab66061, Abcam, UK) overnight at 4°C and horseradish peroxidase-conjugated secondary antibodies (anti-mouse and anti-rabbit; NeoBioscience, Shenzhen, China) for 1 h at 37°C. The bands were treated with an electroluminescence kit, scanned, and analyzed by densitometric evaluation using an imaging system (Image Station 4000 M, Kodak, Japan) and the analyzing software Quantity One (Bio-Rad, Hercules, CA, USA). α-tubulin was chosen as the control for loading protein.

Measurement of Se level

Freshly thawed hippocampi and cortices from mice brains were thoroughly rinsed with PBS, and the remaining PBS droplets were removed with tissues. An appropriate amount of hippocampus/cortex was placed in a glass beaker, digested with mixed acid of perchloric acid and nitric acid (1:4 in volume) overnight, and filled with 10% HCl to a constant volume of 5mL. The Se levels of the samples were measured by atomic fluorescence spectrometry (AFS-920; Beijing Gitan Instruments, Beijing, China). The Se standard solution (GBW(E)080215, 100 pg/mL) was obtained from the National Standard Material Research Center (Beijing, China). Five samples were randomly collected from five brains in each group.

Activity of the selenoenzyme

The activities of GPx and TrxR in brain homogenates were measured using the GPx assay kit (Beyotime Institute of Biotechnology, Nanjing, China) and TrxR assay kit (Suzhou Comin Biotechnology, Suzhou, China), respectively. The supernatant of brain homogenate was prepared as described in the immunoblot analysis.

Statistical analysis

The data were analyzed using GraphPad Prism software. All data were expressed as the mean ± SEM and considered statistically significant at a level of $P<0.05$. A two-way t-test was used to analyze the data from immunohistochemical and immunoblot analysis and from the detection of selenoenzymatic activity.

Results

Administration of Se-Met attenuated the deposition of Aβ in the hippocampus and cortex of 3×Tg-AD mice.

To determine the effects of Se-Met on the production and deposition of Aβ in AD mice, the expression level of Aβ was detected using immunohistochemistry and immunoblotting with the antibody 6E10, which can specifically react with amino acid residues 1-16 of Aβ. After 12 weeks of treatment with Se-Met, histological observation in the brain of 3×Tg-AD mice indicated that Se-Met-treated mice had fewer plaques in both the hippocampus and the cortex compared to the control mice (Fig. 1A). This was further confirmed using Western blot analysis, which showed that there was a significant decrease in the expression levels of both TBS-soluble and TBS-insoluble Aβ in the hippocampus ($P<0.01$) (Fig. 1B). Consistent with these the Western blot results, Se-Met could also reduce the formation of Aβ oligomer (20-30 kDa) (Fig. 1B), which is recognized as a more toxic form than SP in AD [20]. While in the cortex, the level of TBS-soluble Aβ showed a moderate decrease compared to controls ($P=0.057$) (Fig. 1B). With regard to TBS-insoluble Aβ, the two groups showed no difference in the cortex (Fig. 1B).

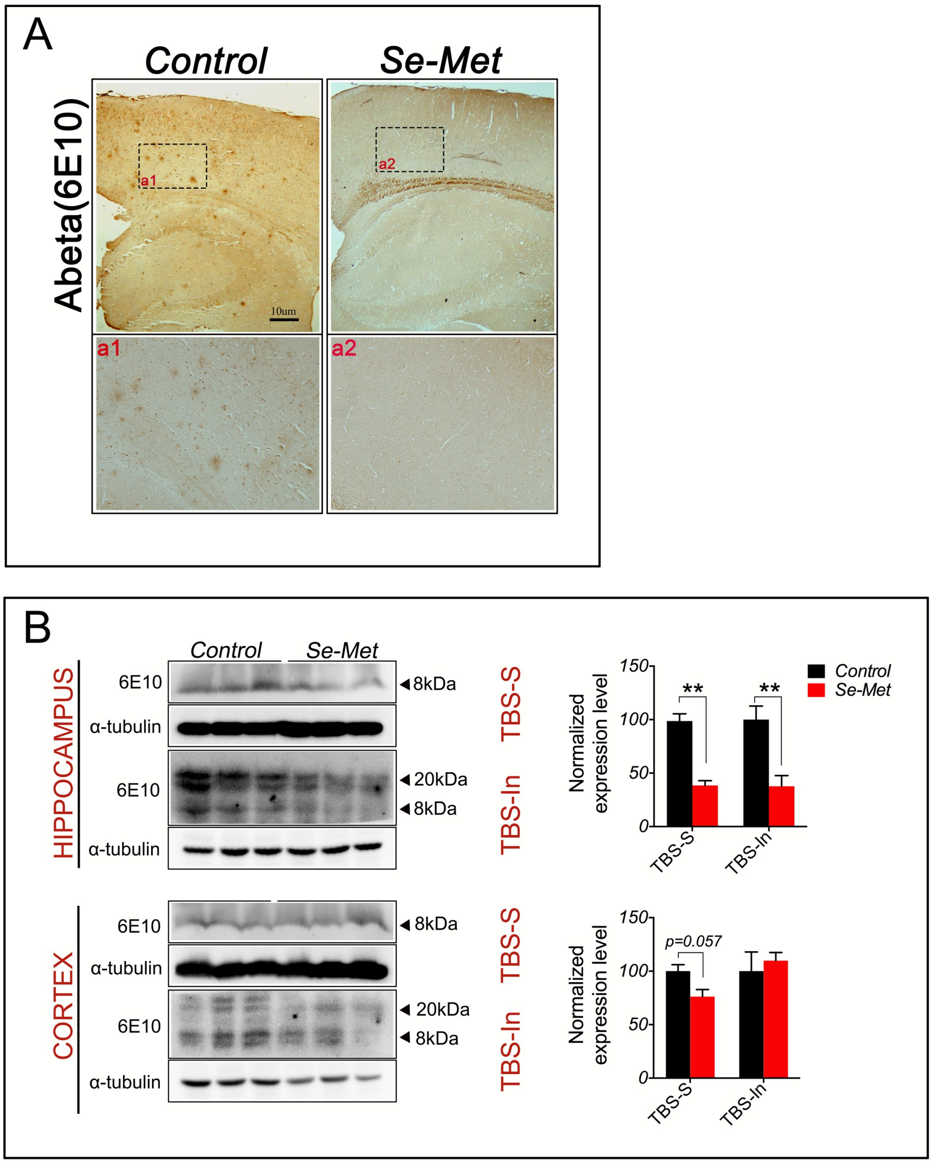

Figure 1. Treatment with selenomethionine decreased the burden of amyloid deposition and production of Aβ in the brain of 3×Tg-AD mice. (A) Immunohistochemical staining using antibody 6E10 revealed differences between vehicle-treated and Se-Met-treated 3×Tg-AD mice (The bottom panels are magnification figures of the top panels); Scale bars, 10 μm. (B) The expression of Aβ in the hippocampus and cortex was determined using immunoblot analysis (left). Representative bands and quantitative analysis (right) indicated that the level of TBS-soluble and TBS-insoluble Aβ significantly decreased in the hippocampus of Se-Met-

treated mice, but the difference in the cortex was not significant following treatment with Se-Met. Quantitative results were normalized against the expression level of α-tubulin. Values are expressed as percentages compared to the control (set to 100%) and presented as the group mean ± SEM (n=3-6), ***P*<0.01 vs the control group.

Treatment with Se-Met regulated APP processing.

To ascertain potential mechanisms of reduced deposition of Aβ by Se-Met, the pathway by which Aβ generation takes place was investigated. First, the expression level of full-length APP (fAPP, full-APP) was assessed using immunoblot. There were no notable changes in the expression levels of fAPP in both TBS-soluble and TBS-insoluble portions of hippocampi and cortices after treatment with Se-Met (Fig. 2A), which indicated that the effect of Se-Met in reducing the production of Aβ was not due to the down-regulation of expression of fAPP. In the amyloidogenic pathway, APP is sequentially cleaved by β- and γ-secretase to generate Aβ. Therefore, the expression levels of BACE1 and sAPPβ, an APP proteolytic product processed by β-secretase, were evaluated [21]. The results showed that Se-Met could significantly reduce the expression level of BACE1 in both the hippocampus and cortex of these 3×Tg-AD mice (Fig. 2B) (P<0.05). There was also a remarkable down-regulation in the expression levels of sAPPβ in both the hippocampus and cortex compared to controls (P<0.01 and P<0.05 respectively) (Fig. 2C).

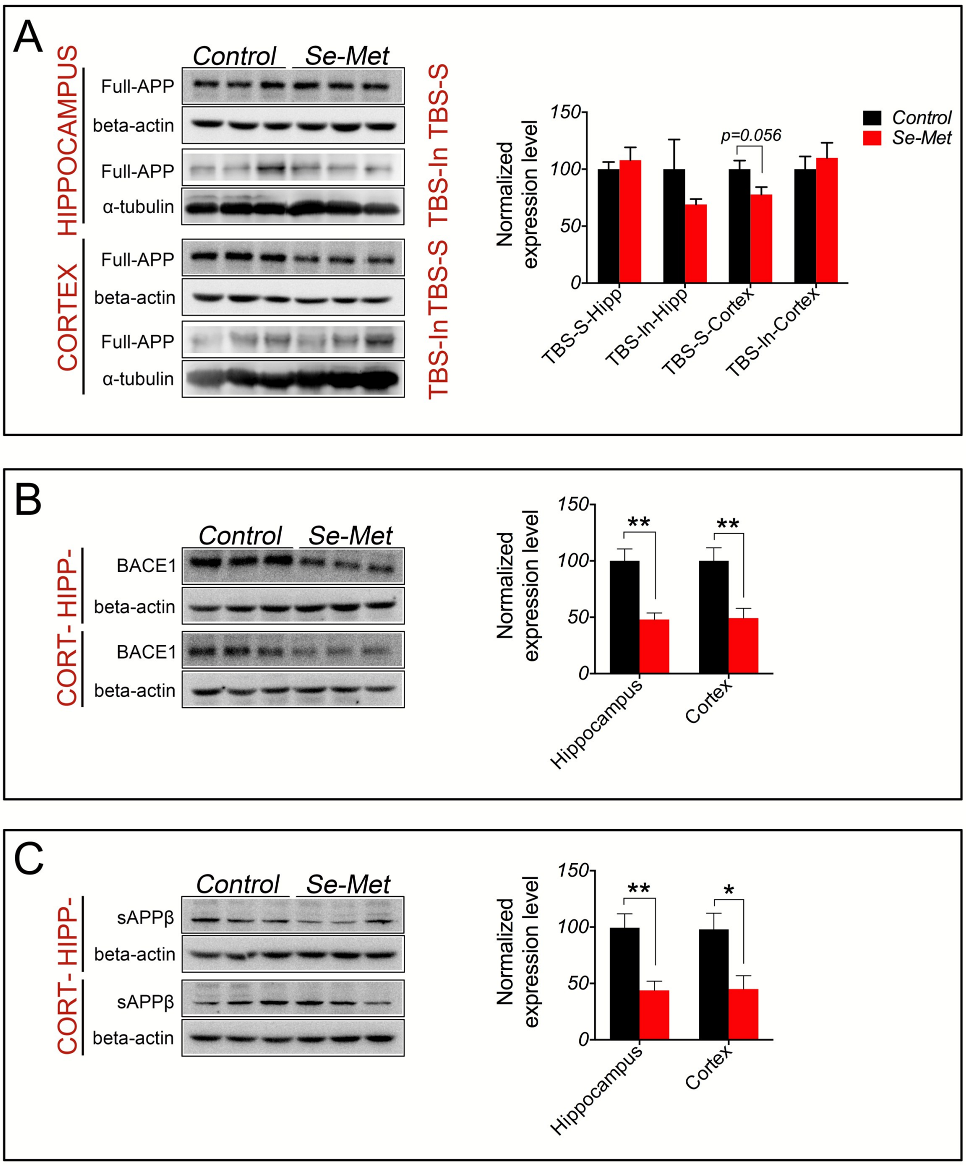

Figure 2. Treatment with Se-Met regulates APP processing by reducing the levels of BACE1 and sAPPβ. Representative western-blots of fAPP, BACE1, and sAPPβ (left of A, B, C) in both TBS-soluble and TBS-insoluble cortical and hippocampal brain homogenates. Quantitative analysis showed that (1) there were no obvious changes in the levels of full-APP in both the cortex and hippocampus between the control and the Se-Met-treated groups (right A). (2) However, Se-Met significantly reduced the levels of BACE1 and sAPPβ in both the hippocampus and cortex of 3×Tg-AD mice. Quantitative results were normalized against the levels of α-tubulin. Values were

expressed as percentages in comparison to the control (set to 100%) and presented as the group mean ± SEM (n=3-6). *P<0.05, **P<0.01 vs. the control group.

Se-Met increases brain Se levels but does not significantly alter selenoprotein P and selenoprotein R levels.

To examine the status of Se in the 3×Tg-AD mice after the 12-week treatment with Se-Met, the level of Se in the brain was determined using atomic fluorescence spectrometry. Se concentrations of the hippocampus and cortex in Se-Met-treated mice were all significantly increased in comparison with control mice ($P<0.01$) (Fig. 3A). Selenoproteins are a distinct class of proteins characterized by the specific incorporation of Se, which are highly expressed in the brain and may have an important role in protecting against neurological pathologies such as AD [22]. Here, the expression levels of Sel-P and Sel-R in the hippocampus and cortex were investigated, and the results showed that there were no significant differences in Sel-P and Sel-R between the Se-Met-treated and the control groups, although there was a trend (p=0.06) of increasing expression of Sel-R in the hippocampus and cortex after treatment with Se-Met (Fig. 3B).

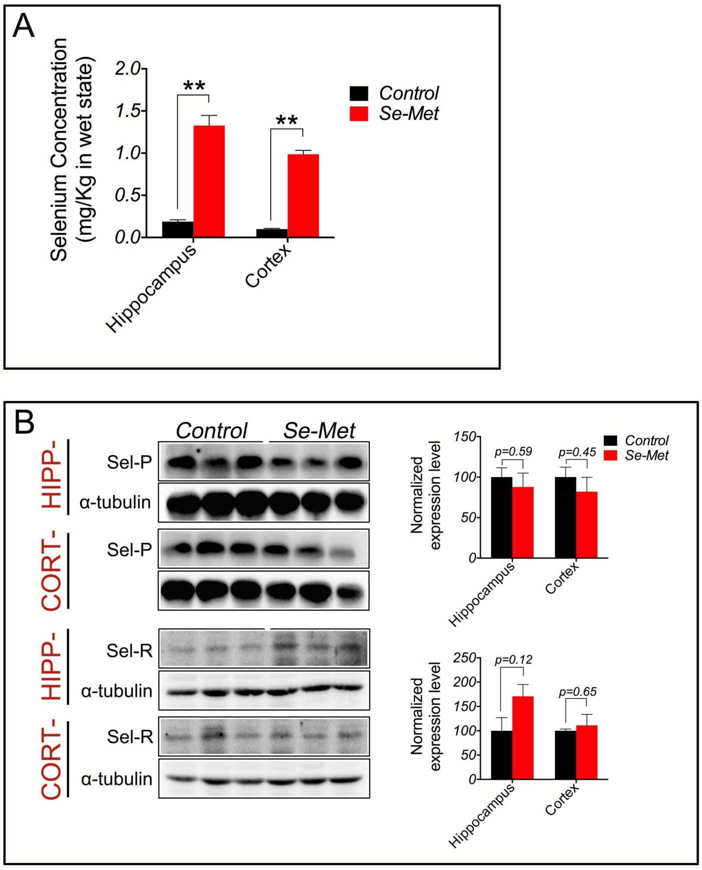

Figure 3. The effects of Se-Met treatment on Se levels and the expression of selenoprotein P and selenoprotein R in the hippocampus and cortex of 3×Tg-AD mice. (A) Levels of Se were significantly increased in both the hippocampus and cortex in Se-Met-treated group in comparison with the control (values are expressed as the mean ± SEM (n=5), $**P<0.01$ vs the control group). (B) Western blot analysis showed that treatment with Se-Met had no significant effect on the expression level of both selenoprotein P (Sel-P) and selenoprotein R (Sel-R) in the hippocampus and cortex.

Se-Met increased selenoenzyme activity and antioxidation ability in the hippocampi of 3×Tg-AD mice.

GPx and TrxR are the two key selenoenzymes that regulate redox tone in vivo. The effect of Se-Met treatment on the expression levels and activity of GPx and TrxR was measured in the samples from the hippocampi and cortices of 3×Tg-AD mice. As shown in Figure 4A, the expression level of TrxR1 in the hippocampi of Se-Met-treated mice were significantly increased compared to the controls ($P<0.05$), and there was no significant change in the level of GPx4 between the Se-Met and control groups. Interestingly, the activities of GPx and TrxR in the hippocampi of Se-Met-treated mice were significantly increased compared to the controls ($P<0.05$). GSH is a marker of antioxidative capacity in the brain and serves as the substrate of GPx to reduce hydrogen peroxide. And there was increased GSH levels in hippocampi of Se-Met mice compared to the controls ($P<0.01$). However, in the cortex, the expression levels and activity of these two enzymes in Se-Met treated mice was not notably changed in comparison with the control group, and there was also no significant change in GSH levels (Fig. 4B).

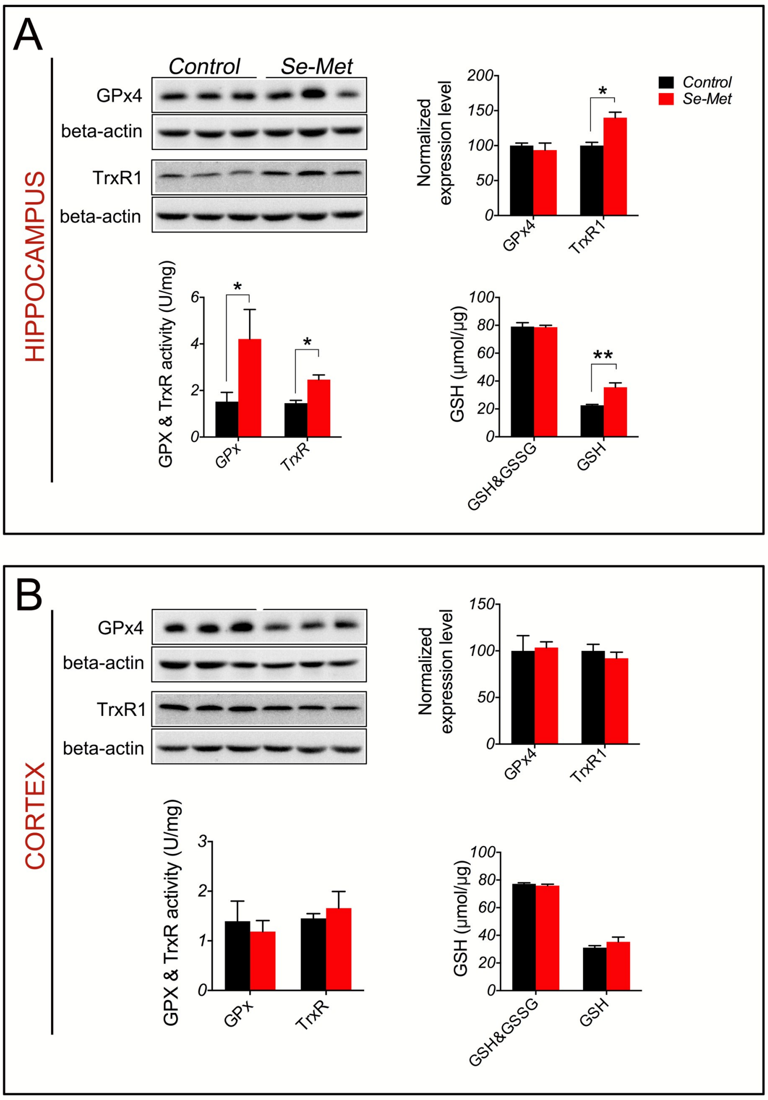

Figure 4. Se-Met enhances the activity of two selenoenzymes and increases the level of GSH in the hippocampus of 3×Tg-AD mice. The expression levels of GPx4 and TrxR1 were measured using western blot. The activities of GPx and TrxR and the level of GSH were measured using specific assay kits. Se-Met caused significant increases in TrxR1 expression level, GPx and TrxR activities and GSH level in the hippocampus in comparison with that of the control group (values are expressed as the mean ± SEM (n=4), *P<0.05 vs the control group). GPx, glutathione peroxidase; Se-Met, selenomethionine; SEM, standard error of the mean; TrxR, thioredoxin reductase.

Discussion

As a vital trace element with numerous health benefits, it has been demonstrated that Se is one of the essential dietary nutrients in humans, and it has been regarded as an anticarcinogen for a long time [23, 24]. Recently, Se has been shown to decrease with age and this is correlated with cognitive competence. There is evidence that a time-dependent decrease in Se is associated with a decline in cognitive function in patients with AD [25, 26]. Supplementation of mice or cell lines with sodium selenite, sodium selenate, and organic Se has been found to protect primary neurons from apoptosis, enhance mitochondrial functional performance in the hippocampal neuronal cells, mitigate tau pathology, and ameliorate cognitive deficits and oxidative damage in AD mice [14, 15, 27, 28]. In our previous study, it was shown that the predominant form of Se in food sources, Se-Met could also ameliorate the decline in cognitive function by reducing tau hyperphosphorylation, increasing oxidation resistance, and reversing synaptic deficits in the 3×Tg-AD mice[16]. In comparison with other inorganic forms of Se, Se-Met has a higher bioavailability and lower toxicity, which makes it a more promising agent in the treatment of AD. It was reported that selenium status is associated with the production and/or the clearance of the Aβ peptide [29], a two-fold increase in the total area of Aβ plaques was observed in AD mice fed with selenium-deficient diet in comparison to that of the selenium-adequate diet. However, to the best of our knowledge, there has been no study on the effect of Se-Met on the generation of Aβ in AD mice, which is still regarded as an important biomarker and/or contributing factor in the pathogenesis of AD.

According to the amyloid hypothesis, in the amyloidogenic pathway, β-secretase cleaves at the N-terminal side Asp1 of the Aβ sequence, thereby producing a sAPPβ peptide fragment. The C-terminal portions of APP are subsequently cleaved by γ-secretase to generate Aβ. Generation and accumulation of Aβ are the critical pathogenic events in AD, in addition to direct toxic effects on neurons, which further induces a deleterious cascade, including the hyperphosphorylation of tau and activation of microglia in the brain, and ultimately results in cognitive impairment [30-32]. In this study, after treatment with Se-Met for three months, the deposition of Aβ in the brain of 3×Tg-AD mice was significantly decreased. Initially we considered the possibility that decreased expression level of APP might result in the decreased generation of Aβ, but also effects on β-secretase were investigated due to its role as the key rate-limiting enzyme that initiates the formation of Aβ. As indicated in the Western blot analysis, Se-Met does not have a notable effect on the expression level of APP protein, but there is a significant down-regulation in the expression level of BACE1. At the same time, the expression of sAPPβ also significantly decreased in Se-Met-treated AD neurons. These results suggest that Se-Met can alleviate pathological Aβ by reducing the expression and activity of BACE1, which results in a reduction in the cleavage of APP by BACE1.

Se-Met is the primary form of Se in yeasts and it represents the main nutritional form of Se for humans. Supplementation of Se-Met contributes to the functions of a number of selenoproteins, including some redox-regulating selenoenzymes [33, 34]. The level of Se in Se-Met-treated mice was strikingly increased by approximately five to eight times in comparison with that of the control mice. This confirms that Se is abundantly bioavailable from the Se-Met diet in the AD mice. Because Se mainly exerts its effect through selenoproteins, the level and activity of selenoproteins in the brain of 3×Tg-AD mice were explored. Among the 25 known selenoproteins, Sel-P and Sel-R are regarded as functionally related to AD. In neuronal cells, the histidine-rich domain of Sel-P is capable of binding metal ions, such as Zn^{2+}, Cu^{+} and Cu^{2+}, with high affinity and of modulating metal ions, which are related to the aggregation of Aβ and might have an important role in the pathogenesis of AD [35]. Mice lacking Sel-P exhibit severe neurological dysfunction, neurodegeneration, and audiogenic seizures in vivo [36]. In addition to its role as a metal chelator, Sel-P is a major Se transport protein in vivo [37-39]. In mouse models with a Sel-P gene knock-out, the Se concentration of the brain decreased to 40% of controls [40]. However, the capacity of the brain to retain selenium is higher than most other tissues, resulting in the expression level of brain

Sel-P exhibiting relatively high stability even under changing of selenium status of the organism [41]. Our previous study was consistant with this notion in that the concentration of Se did not decrease obviously in the hippocampus and cortex of 3×Tg-AD mice at 12 months of age in comparison with WT mice, but it showed a significant decline in the plasma and liver of AD mice (Supplementary Fig. 1). This supports the concept that the brain has priority in obtaining Se over other organs of the body. In the current study there were no changes in the expression levels of Sel-P after treatment of Se-Met, which might be the result of saturated selenium concentration in the brain of 3×Tg-AD mice.

Sel-R belongs to the methionine sulfoxide reductase B (MsrB) family, and represents one of the selenoenzymes along with GPx(s), TrxR(s), and deiodinase(s). Sel-R has an important role in maintaining proper redox status of methionines in proteins within the cells of the brain and other tissues in organisms. It has been reported that Sel-R was highly expressed in the brain and resisted the aggregation of Aβ in AD model cells [42]. Because of the inability to attain specific substrate of MsrB in vivo, the expression level of Sel-R was used instead of the activity of MsrB. After 3×Tg-AD mice were treated with Se-Met, the expression level of Sel-R in the brain of the treated group showed an increase without reaching a statistically significant higher level. As SelR is a selenoenzyme, the ability to measure differences in Sel-R between the control and Se-Met group might only be possible by detecting enzymatic instead of expression level.

Many studies have shown that oxidative injury, as a disturbance in the balance between the production of ROS (free radicals) and antioxidant defenses, has an important role in normal aging and neurodegenerative diseases, such as Parkinson's disease and AD [43]. It has been reported that oxidative stress induced an increased production of Aβ in vitro [44, 45] and also be implicated in the neuronal injury induced by Aβ. GSH is critical for protecting the brain from oxidative stress, acting as a free radical scavenger and inhibitor of lipid peroxidation. The overexpression of SOD-2 and elevation of GSH concentration can reduce BACE1-mediated APP processing in AD mice [46-48]. . Our previous study found that Se-Met significantly improved the level of GSH in the brain of AD mice at four months of age. In this study, the level of GSH also significantly increased after the treatment of Se-Met. Evidence has indicated that the effect of Se-Met treatment may be directly linked to the activity of selenium-dependent enzymes (selenoenzymes) in the brain. Selenoenzymes are important in modulating the antioxidant metabolism and maintaining intercellular reducing conditions, particularly in the brain [49]. The activity of GPx and TrxR, the two main selenoenzymes that are directly involved in cellular protection against damage from redox reaction in vivo, were all notably increased in the hippocampus of Se-Met-treated mice. Many neurodegenerative diseases like AD are associated with low

GSH status, which results in the inhibition of GPx4 activity [50]. Increased GPx activity could eliminate harmful peroxide metabolites, thus blocking the lipid peroxidation chain reaction and prevent the production of MDA in cells. TrxR facilitates the reduction of oxidized proteins by regenerating reduced thioredoxin and is involved in the prevention and repair of damage that is caused by H_2O_2-based oxidative stress. Thus, Se-Met might improve the level of GSH and mitigate oxidative damage in the brain by enhancing the activity of selenoenzymes.

The mechanistic link between oxidative stress and APP processing remains unclear. Related research has provided evidence that oxidative stress contributes to Aβ accumulation because of JNK/c-Jun activation, which could increase levels of β- and γ-secretases and then induce mild oxidative stress, which could also alter BACE1 subcellular compartmentalization to favor the amyloidogenic processing of APP [51, 52]. Thus, Se-Met might inhibit the production and accumulation of Aβ by modulating oxidative stress through selenoenzymes. In addition, the indirect relationship between the activity of selenoenzymes and Aβ pathway should be considered. In Aβ (1-42)-treated hippocampal neurons, the activation of GSK3β is a crucial pathological feature that can further increase the level of BACE1 and Aβ aggregation [53]. Hyperactivation of GSK-3β has also been reported to induce neuronal cell death [54] and oxidative stress [55]. Oxidative stress could activate GSK-3β by inhibiting the Ser9 phosphorylation of GSK-3β (the inactivated GSK-3β) and promotes mitochondrial dysfunction mediated by GSK3β in AD mice, thus reinforce a vicious cycle and enhance the production of Aβ [56, 57]. Se-Met treatment could significantly decrease the level and activity of GSK-3β in both the hippocampus and cortex of 3×Tg-AD mice [16], which suggests that Se-Met may reduce the production of Aβ by promoting antioxidation through increase of selenoenzymatic activity and decrease GSK3β activity. Although several details in these pathways require further clarification, a direct link between the effect of Se-Met on the activity of selenoenzymes and the anti-oxidation processes in the Aβ pathway has been shown.

In conclusion, this study used Se-Met to treat 3×Tg-AD mice and demonstrated that Se-Met effectively reduced the production of Aβ by inhibiting the expression and activity of BACE1 in 3×Tg-AD mice. Moreover, it was demonstrated that Se-Met significantly enhanced the activity of selenoenzymes GPx and TrxR in the hippocampus, resulting in enhanced antioxidation and decreased Aβ production, which were probably generated through suppressing the activation of GSK3β. These data provide new insights into the therapeutic potential of Se-Met in AD.

Acknowledgements

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Abbreviations:

Alzheimer's disease	AD
Senile plaques	SPs
Beta-amyloid	Aβ
Neurofibrillary tangles	NFTs
Selenomethionine	Se-Met
Selenoprotein P	SelP
Selenoprotein R	SelR
Glutathione peroxidase	GPx
Thioredoxin reductase	TrxR
Glutathione	GSH

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