SPANDIDOS PUBLICATIONS

ERK activation induced by selenium treatment significantly downregulates ß/γ-secretase activity and Tau phosphorylation in the transgenic rat overexpressing human selenoprotein M

SU Y. YIM[1,2], KAB R. CHAE[1], SUN B. SHIM[1], JIN T. HONG[3], JUNG Y. PARK[4], CHUNG Y. LEE[5], HONG J. SON[5], YHUN Y. SHEEN[2] and DAE Y. HWANG[5]

[1]Department of Laboratory Animal Resources, National Institute of Toxicological Research, Korea FDA, Seoul 122-704; [2]College of Pharmacy, Ewha Woman's University, Seoul 120-750; [3]College of Pharmacy, Chungbuk National University, Chungju 316-763; [4]National Fisheries Research and Development Institute, Pusan 619-902; [5]College of Natural Resources and Life Science, Pusan National University, Miryang 627-706, Korea

Received February 17, 2009; Accepted April 24, 2009

DOI: 10.3892/ijmm_00000211

Abstract. Selenium reportedly contribute to the modulation process of protein phosphorylation to regulate various cellular functions including growth, differentiation, proliferation and development. The aim of this study was to investigate whether selenium and Selenoprotein M (SelM) affects the mechanism of Alzheimer's disease. To achieve this, we determined the change of the MAPK pathway, secretase activity, and Tau phosphorylation in the transgenic rat overexpressing human selenoprotein M. Based on these results, we concluded that, i) CMV/GFP-hSelM Tg rats showed a high activity level of antioxidant enzyme in the brain tissues, ii) in response to selenium treatment, the ERK signaling pathway was significantly increased in Tg rats, but did not change in wild-type rats, iii) the activation of the ERK pathway by selenium treatment and SelM overexpression induced the inhibition of the α/γ-secretase activity related to the protection of Aß-42 production, iv) the activation of the ERK pathway by selenium treatment and SelM overexpression inhibited the phosphorylation in several sites of Tau protein. Therefore, these results provide strong evidence that selenium treatment and SelM activate the ERK pathway to attenuate α/γ-secretase-mediated proteolysis and Tau phosphorylation to protect brain function.

Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder represented by the progressive loss of cognitive and memory functions, neuronal and synaptic cell loss and the appearance of apoptotic cells in the brain (1,2). There are two characteristics of neuropathological lesions with AD, senile plaques and neurofibrullary tangles (3,4). The main component of senile plaques is an extracellular deposit made of Aß peptides, produced by the amyloid precursor protein by cleaving ß/γ-secretase (5,6). Neurofibrillary tangles constitute of bundles of abnormal filaments called paired helical filaments (PHF), which are highly phosphorylated forms of the microtubule associated Tau protein. Furthermore, results from previous studies suggest that the high level of phosphorylated PHF-Tau is tightly associated with microtubule disorganization and generation of neurifibrillary lesions (7-9).

Various studies suggest novel strategies for the development of medical compounds to cure and/or relieve the symptoms of AD. Selenium is a known candidate for prevention and reliever of AD. This element, long known as a ubiquitous trace compound in nature, has been proven to be essential for animal and human health (10). Recently, it was found that selenium contributes to the modulation of protein phosphorylation regulating various cellular functions. In particular, an inorganic form of this compound, sodium selenite, was shown to increase the p38 and p53 phosphorylation, activating caspase-independent cell death (11,12). Furthermore, this compound activates anti-apoptotic and the mitogen-activated protein inhibitor (MAPK) pathway to prevent damage to brain tissues and cells (13,14). However, few studies have been conducted to investigate whether selenium treatment and SelM over-expression affects the key regulator of AD pathology using a transgenic rat overexpressing the human SelM (hSelM) gene.

As demonstrated by our data, the activity of antioxidant-related enzymes involving superoxide dismutase (SOD) and glutathione peroxidase (GPX) was significantly induced by selenium treatment and SelM overexpression. In addition, selenium treatment and SelM overexpression increased only the ERK MAPK pathway, not p38 and JNK. Furthermore, the activation of this signal induced the downregulation of α/γ-secretase and Tau phosphorylation, which were related to

Correspondence to: Dr Dae Youn Hwang, Department of Biomaterials Science, College of Natural Resources and Life Science, Pusan National University, 50 Cheonghak-ri, Samnangjin-eup, Miryang-si, Gyeongsangnam-do, 627-706, Korea
E-mail: dyhwang@pusan.ac.kr

Key words: selenoprotein M, transgenic rat, secretase, Tau, ERK

the prevention of AD pathogenesis. These results suggest that selenium treatment and SelM overexpression contribute to the activation of the ERK pathway to attenuate AD pathology including Aß-42 peptide production and Tau phosphorylation.

Materials and methods

Maintenance and identification of CMV/EGFP-hSelM Tg rat. All transgenic (Tg) rats used in this study were developed by microinjection of CMV/GFP-hSelM fusion gene into fertilized rat eggs and showed a high antioxidant status in various tissues (15). All the rats were kept in an accredited Korea FDA animal facility in accordance with the AAALAC International Animal Care policies (Accredited Unit-Korea Food and Drug Administration, Unit Number-000996). The rats were given a standard irradiated chow diet (Purina Mills Inc.) *ad libitum*, and were maintained in a specific pathogen free state under a strict light cycle (light on at 06:00 h and off at 18:00 h). All pedigrees were hemizygous for their transgene.

In order to identify Tg rats, PCR analysis was performed on the genomic DNA isolated from the tails of the 4-week old founder rat. This transgene DNA was synthesized using the sense and antisense primers, 5'-ATG AGC CTC CTG TTG CCT CCG CTG-3' and 5'-AGC TGG GGA AGG AAG AAA GTG G-3', respectively, with a complementary *hSelM* cDNA ranging from 90 to 113 and 675 to 696 nucleotides as the DNA template. After 25 cycles of amplification, the level of the *hSelM* product (606 bp) was quantified using a Kodak Electrophoresis Documentation and Analysis System 120 on 1% agarose gels.

Experimental design and selenium treatment. Sodium selenite (Na_2SeO_3) obtained from Sigma (S5261, USA) was dissolved in distilled water to give a final concentration of 0.2 μmol/μl. Ten-week old CMV/EGFP-hSelM Tg and wild-type rats were randomly divided into two subgroups, six rats per group. The first subgroup of the Tg and wild-type rats received a comparable volume of distilled water via intraperitoneal injections daily (vehicle-treated Tg and wild-type group), while the second subgroup received 5 μmol/kg body weight per day of sodium selenite via intraperitoneal injections for 3 weeks (selenium-treated Tg and wild-type group). After 3 weeks of injections, all animals were immediately euthanized using CO_2 gas. Blood from the abdominal vein and brain tissue samples were collected. These samples were then stored in Eppendorf tubes at -70˚C until assayed.

Western blot. The protein prepared from brain tissues of Tg and wild-type rats was separated by electrophoresis in a 4-20% SDS-PAGE gel for 3 h and transferred to nitrocellulose membranes for 2 h at 40 V. Each membrane was incubated separately with the primary, anti-Tau (H-150, Santa Cruz, sc5587), anti-p-Tau (Ser404, Santa Cruz, sc12952), anti-p-Tau (Ser396, Sigma, T 7319), anti-p-Tau (Ser202, Sigma, T 8069), anti-p-Tau (Ser231, Sigma, T7194), and antibodies for anti-p38 (Cell Signaling, 9212), anti-p-p38 Thr180/Tyr182 (Cell Signaling, 9211), anti-JNK (Cell Signaling, 9252), anti-p-JNK Thr183/Tyr185 (Cell Signaling, 9251), anti-ERK (Santa Cruz Biotechnology, SC94), anti-p-ERK (Santa Cruz Biotechnology, SC7383), and anti-actin (Sigma, A5316) antibodies for overnight at 4˚C. The membranes were washed with a washing buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, and 0.05% Tween-20) and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Zymed) at a 1:1,000 dilution at room temperature for 2 h. The membrane blots were developed using a Chemiluminescence Reagent Plus kit (ECL, Pharmacia).

Activity analysis of SOD and GPX. The level of SOD and GPX in brain cells from wild-type and Tg rat were detected using the calorimetric assay procedure and reagents in the Bioxytech SOD-525 and GPX-340 kit (OxisResearch™; Portland, USA). The neuronal cells were harvested from the blood by centrifugation at 3,000 rpm for 10 min, then 4 volume of cold deionized water to lysis cell. This lysate was stored at -70˚C until the enzyme activity assay. In order to measure the GPX activity, the spectrophotometer was set to measure the absorbance at 340 nm and 23-25˚C. Immediately prior to this assay, the erythrocytes lysate was diluted with assay buffer, typically 1:10 (for SOD assay; 200 mM Manitol, 10 mM Tris-HCl (pH 7.4), 1 mM EDTA, 50 mM sucrose, for GPX assay; 5 mM EDTA, 50 mM Tris (pH 7.5), 1 mM mercaptoethanol). Assay buffer (350 μl), NADPH reagent (350 μl) and erythrocytes lysate (70 μl) were well mixed and added to the cuvette. To this, 35 μl of working substrate, 1:10,000 dilution of tert-Butyl hydroperoxide, was also added to the cuvette and mixed by pipetting up and down twice. Finally, the absorbance was measured in A_{340} for 3 min with the GPX activity rate determined by the change in A_{340} per min. The net rate for GPX activity was calculated by subtracting the rate observed for a water blank from the rate measured for a erythrocytes lysate. Then, the net A_{340} for GPX activity was converted to NADPH consumed (nmol/min/ml) using the following relationship, 1 mU/ml = 1 nmol NADPH/min/ml = (A_{340}/min)/0.00622. For SOD activity, 900 μl of reaction buffer (2-amino-2methyl-1,3-propanediol, containing boric acid and DTPA, pH 8.8) was aliquoted to a test tube for each blank or brain sample. This mixture was added to the 40 μl of brain sample and 30 μl of R2 reagent (1-methyl-2-vinyl-pyridinium trifluoromethanesulfonate in HCl), and incubated at 37˚C for 1 min. Finally, 30 μl of R1 reagent (5,6,6a,11b-tetrahydro-3,9,10-trihydroxybenzo[c]fluorine in HCl) was added to the test tube containing mixture and vortex briefly. Immediately, the final mixture was transferred to a spectrophotometric cuvette and the absorbance was measured by a spectrophotometer at 525±2 nm. The SOD activity was calculated directly from the experimental Vs/Vc ratio using the following equation, [SOD] = 0.93 x (Vs/Vc-1)/1.073-(0.073 x (Vs/Vc)).

α, ß and γ-secretase activity analyses. α, ß and γ-secretase activity was detected using the detection kits for each (α-kit, FP001; ß -kit, FP002; γ-kit, FP003; R&D System Inc., MN, USA) using the recommended methods. First, brain tissue was homogenized using a glass homegenizer in ice cold 1x Cell Extraction Buffer to yeild a final protein concentration of roughly 0.5-2.0 mg. These mixtures were then incubated on ice for at least 10 min and centrifuged at 10,000 x g for 1 min to remove the unbroken fragments. Supernatants collected

rifuged mixture were used in the detection of secretase carried out using the microplate provided by the manufacturer. To perform the enzymatic reaction, 50 μl of tissue lysate was added to each well, followed by 50 μl of 2X Reaction buffer, 5 μl of substrate. The wells were gently mixed in these microplates and incubated in the dark at 37˚C for 1-2 h to induce the enzyme-substrate reaction. Final fluorescence produced from the reaction was read on a fluorescent microplate reader filtering the excitation between 335-355 nm wavelength.

Statistical analysis. Significance between selenium- and vehicle-treated rats were performed using the one-way ANOVA test of variance (SPSS for Windows, Release 10.10, Standard Version, Chicago, IL). Post-Hoc tests (SPSS for Windows, Release 10.10, Standard Version, Chicago, IL) were performed to determine the variance and significance between CMV/EGFP-hSelM Tg and wild-type rats for the levels given in the text. All the values are reported as the mean ±SD. A p-value <0.05 was considered significant.

Results

Effects of both selenium treatment and SelM overexpression on the SOD and GPX activity. It was reported that the activity of SOD and GPX were significantly higher in the CMV/GFP-hSelM Tg than the wild-type rats (15). Therefore, we first examined whether both selenium treatment and SelM overexpression induces changes in the activity levels of antioxidant enzymes. The activity level of SOD and GPX were detected in the brain tissue of CMV/GFP-hSelM Tg and wild-type rats using detection kits containing specific substrates. In the vehicle-treated group, SOD and GPX activity in CMV/GFP-hSelM Tg rats were higher than that of wild-type rats. After selenium treatment, the activity of these enzymes significantly increased in both CMV/GFP-hSelM Tg and wild-type rats (Fig. 1B). However, the increase in CMV/GFP-hSelM Tg rats was greater than wild-type rats (Fig. 1A). These results suggest that the selenium treatment and SelM overexpression contributed to the increase of GPX and SOD activity in the CMV/EGFP-hSelM Tg rats, respectively.

Effect of selenium treatment and SelM overexpression on the MAPK pathway. It was demonstrated that sodium selenite also significantly activates anti-apoptotic PI3K/AKT and ERK pathway to protect ischemia. (13,14). To investigate whether the co-stimulation with both selenium treatment and SelM overexpression affects the MAPK signaling pathway, the phosphorylation level of three major components (ERK, JNK and p38) in the MAPK signaling pathway were detected in the brain of CMV/GFP-hSelM Tg and wild-type rats using specific antibodies. In the vehicle treatment condition, there were no differences between the total and phosphate-form level of JNK and p38 protein in the brain of both CMV/GFP-hSelM Tg and wild-type rats. These results were also observed with the same patterns detected in the selenium treatment group. However, for the ERK protein, CMV/GFP-hSelM Tg rats showed high levels of p-ERK in both cortex and hippocampus regions during selenium treatment, which did not change in wild-type rats (Fig. 2). These results suggest that selenium treatment and SelM overexpression dramatically induce activation of the ERK signaling pathway.

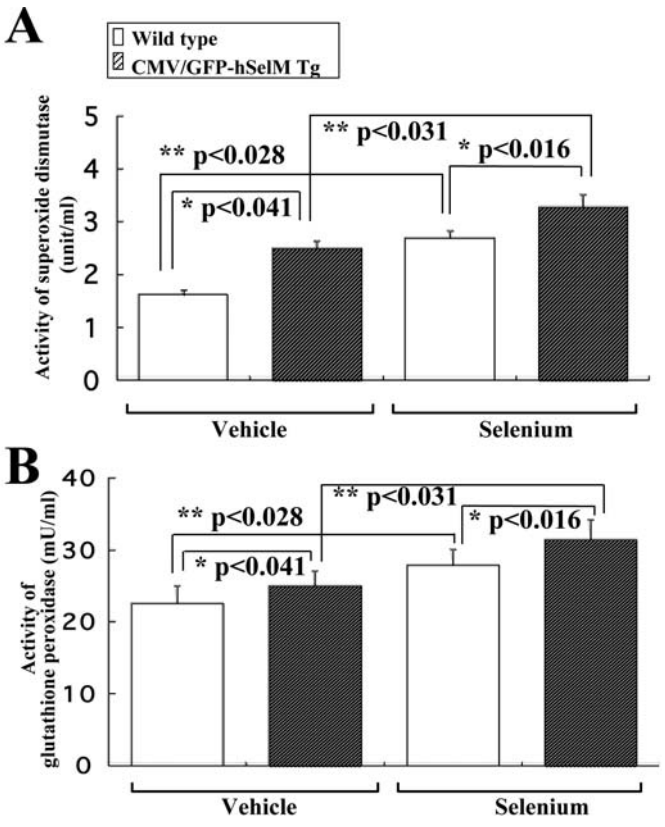

Figure 1. Effects of selenium treatment and SelM overexpression on GPX and SOD activity in brain tissue. The brains used in this assay were collected from CMV/EGFP-hSelM Tg and wild-type rats after intraperitoneal injections of sodium selenite (5 μmole/kg body weight/day) for 3 weeks. Six rats per group were assayed in the ELISA test. The data represent the mean ±SD from three replicates. *p<0.05, significant level compared with the Tg rat; **p<0.05, significant level compared with the selenium-treated group.

Effects of MAPK signaling pathway on α, ß and γ-secretase regulation. Previous studies suggest that the activation of ERK MAPK pathway by sodium selenite inhibits the activity of γ-secretase, a key enzyme regulating Aß-42 peptide production in C99-GL-T20 cells (16,17). To ascertain whether the ERK MAPK activation induced by selenium treatment and SelM overexpression regulates the activity of the three secretases, the activity levels of α, ß and γ-secretase were measured in the brain of CMV/GFP-hSelM Tg and wild-type rats. For the α and γ-secretases, the basal levels in CMV/GFP-hSelM Tg rats were slightly higher than that of wild-type rats. However, selenium treatment induced the decrease of α and γ-secretase activity in both CMV/GFP-hSelM Tg and wild-type groups, although the decrease rate in CMV/GFP-hSelM Tg rats was significantly higher than that of wild-type rats (Fig. 3A and C). On the other hand, in the case of ß-secretase, the basal activity had a similar pattern with α and γ-secretase when vehicle-treated. However, after selenium treatment, both CMV/GFP-hSelM Tg and wild-type rats exhibited significantly high activity of ß-secretase, approximately 10-20% induction after 3 weeks of treatment (Fig. 3B). These observations suggest

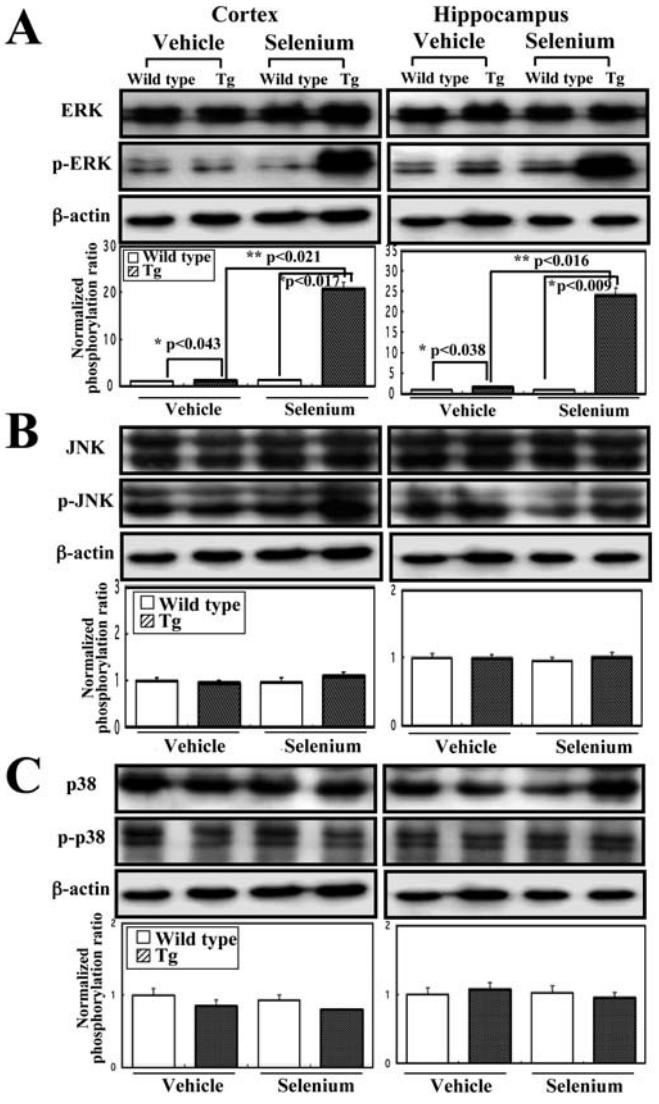

Figure 2. Effect of selenium treatment and SelM overexpression on the MAPK pathway. (A) Protein expression level of ERK. (B) Protein expression level of JNK. (C) Protein expression of p38. Protein extracts were prepared from brains of selenium treated and untreated group, as described in Materials and methods. Protein (50 μg per sample) were immunoblotted with antibodies for each protein. Three samples were assayed in triplicate via Western blot. The data represent the mean ±SD from three replicates. *p<0.05, significant level compared with the Tg rat; **p<0.05, significant level compared with the selenium-treated group.

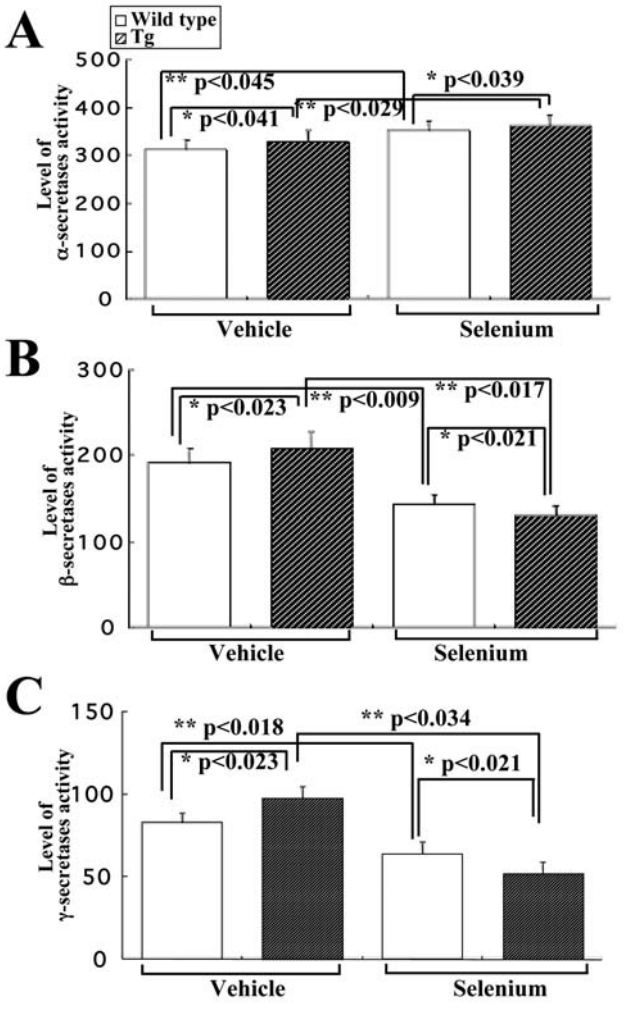

Figure 3. Effects of the activated ERK pathway induced by selenium treatment and SelM overexpression on the activity of the three secretases. The microsomal proteins isolated from the three groups of mice were used to determine the secretase activity, as described in Materials and methods. The data represent the mean ±SD from three replicates. *p<0.05, significant level compared with the Tg rat; **p<0.05, significant level compared with the selenium-treated group.

that the activation of ERK MAKP pathway by selenium treatment and SelM overexpression contribute to the decrease of α and γ-secretase activity in the brain.

Effects of ERK MAPK signal pathway on Tau phosphorylation. Finally, to determine whether Tau phosphorylation, which causes the formation of neurofibril tangles, is directly affected by the ERK MAPK pathway, the levels of Tau and p-Tau were detected in the brain of both CMV/GFP-hSelM Tg and wild-type rat after selenium injections for a 3-week period. In the cortex region, Tau phosphorylation at Ser404 and Thre231 was significantly decreased in the selenium-treated group compared the to vehicle-treated group in CMV/EGFP-hSelM Tg rats, but was not affected in wild-type rats. However, in the Ser396 and -202 sites, the levels of p-Tau were decreased in both CMV/EGFP-hSelM Tg and wild-type rat after selenium treatment. For the hippocampus region, the levels of p-Tau were lower at the Ser404, Ser202 and Thre231 site after selenium treatment, but increased at the Ser396 site (Fig. 4A and B). Therefore, these results suggest that the activation of the ERK MAPK signaling pathway by selenium treatment and hSelM overexpression induces the decrease of Tau phosphorylation.

Discussion

Selenium compounds have been demonstrated to be required for normal growth and reproduction during spermatogenesis (18). Its deficiency was demonstrated to induce multiple diseases associated with oxidative damage an endemic fatal cardiomyopathy in Keshan, China (19) as well as muscular dystrophy in patients subjected to long-term, unsupplemented and parenteral nutrition (20). It is also well known that selenium deficiency was associated with a variety of serious diseases including infectious diseases, cardiovascular disease, cancer and neurodegenerative disorders (21). Savaskan *et al* (22) provided direct evidence that selenium was a

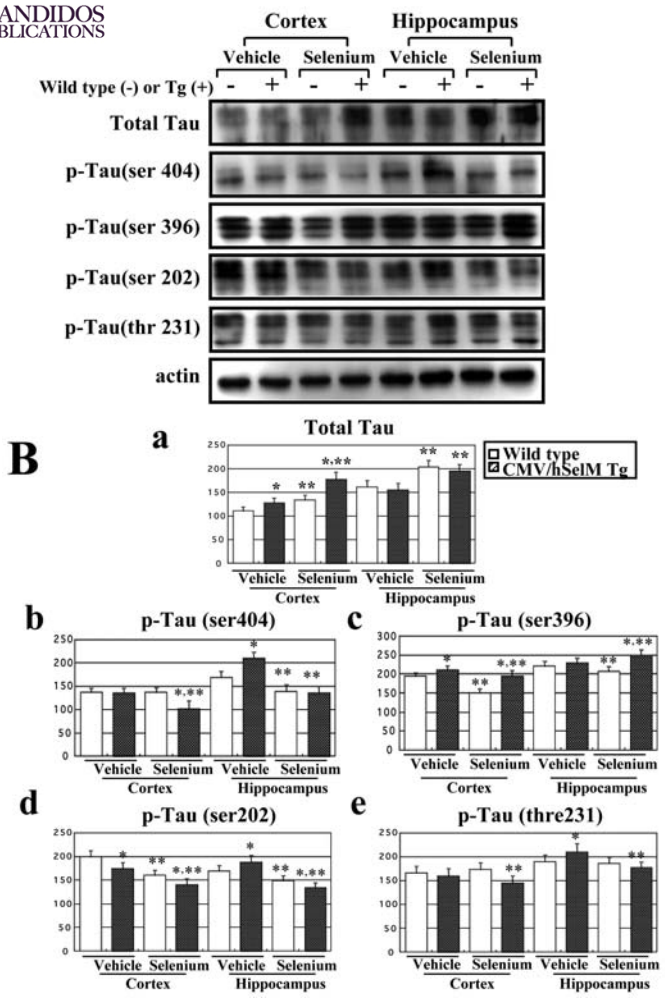

Figure 4. Effects of the activated ERK pathway induced by selenium treatment and SelM overexpression on the Tau phosphorylation. Nitrocellulose membranes trasferring 50 μg of protein from Tg and wild-type rat brains were incubated with the specific antibody to total Tau, p-Tau (Ser404), p-Tau (Ser396), p-Tau (Ser202), p-Tau (Ther231) and actin, and followed by horseradish peroxidase-conjugated goat anti-rabbit IgG. The data represent the mean ±SD from three replicates. $^{*}p<0.05$, significant level compared with the Tg rat; $^{**}p<0.05$, significant level compared with the selenium-treated group.

nutritionally essential trace element, having a pivotal role in neuronal susceptibility to excitotoxic lesions. Also, this study suggests that the neuroprotective effect of selenium is not directly mediated via antioxidative effects of selenite, but requires *de novo* protein synthesis. Therefore, the selenium deficiency in the brain tissue leads to increased oxidative stress with subsequent NF-κB activation and neuronal cell death.

Several studies reported that intracellular ETKs were activated either with sodium selenite, an inorganic salt known to activate ERK (13), or transfected with a constitutively active mutant of MEK1, an immediate upstream activator of ERKR (23,24). Until now, the results of some studies suggest that selenium compounds and selenoprotein were regarded as novel candidates to protect brain damage and improve neuronal dysfunction. In this study, CMV/EGFP-hSelM Tg rats have shown high activity of antioxidant enzymes, such as SOD and GPX after selenium treatment. Furthermore, selenium treatment and SelM overexpression significantly induced the ERK MAPK pathway, but not other MAPK pathways involving p38 and JNK. Therefore, these results suggest that SelM overexpression as well as selenium treatment contribute to the activation of the ERK signal pathway.

In the pathogenesis of AD, secretase-mediated generation of the Aß-42 peptide is closely correlated with deposition at neuritic plaques (17,25). Specifically, γ-secretase plays an important role in the production of the Aß-42 peptide. This enzyme is composed of one obligatory component, presenilin, and three additional cofactors, including nicastrin, Aph-1 and Pen-2, to make a multimeric protease complex (26). In this study, the activity levels of α, ß and γ-secretase were detected in the brain of CMV/EGFP-hSelM Tg and wild-type rats to examine whether the ERK MAPK signaling pathway induced by selenium treatment and SelM overexpression affects secretase activity levels. As seen in the studies of the Tung group (16), our studies also show that γ-secretase activity was significantly decreased with selenium treatment, respectively. Furthermore, our results suggest that selenium treatment also induced the decrease of α-secretase activity as well as γ-secretase, while the ß-secretase enzyme activity was significantly increased. In this study, SelM is regarded as a novel regulator factor affecting the secretase activity and Tau phosphorylation. Therefore, all these results were considered important data in AD pathology study, because it was suggested that selenium treatment and SelM overexpression contribute to the decrease of Aß-42 peptide production through downregulation of α/γ-secretase and upregulation of ß-secretase.

Tau proteins belong to the microtubule-associated protein (MAP) family and are mainly distributed in the neurons of the brain (27,28). These proteins were changed in various physiological processes by destructing the balance of the MAPK signal pathway, which is involved in apoptoic signaling as well as in control of growth and differentiation (29-31). The previous studies provide strong evidence that phosphorylation of Tau proteins cause the formation of neurofibril tangles in the cytoplasm of neurodegenerative disease cells (8). Most of the kinases, including glycogen synthase kinase-3ß (GSK-3ß), cyclin-dependent kinase-5 (Cdk-5), extracellular signal-regulated kinase (ERK), microtubule-affinity regulating kinase, and fyn kinase, contributed to Tau phosphorylation *in vitro* and *in vivo* (32-34). Therefore, we investigated the activation effect of the ERK pathway on Tau phosphorylation using CMV/EGFP-hSelM Tg and wild-type rats. Tau phosphorylation at three sites, Ser404, Ser202 and Thre231, was significantly decerased by ERK activation in CMV/EGFP-hSelM Tg rats but, Tau phosphorylation at the Ser396 site was slightly increased in the hippocampus region of CMV/ EGFP-hSelM Tg rat brains. These results support that selenium treatment and SelM overexpression induce the decrease of Tau phosphorylation in CMV/EGFP-hSelM Tg rats.

Taken together, our results show that selenium and SelM are tightly associated with neurodegenerative diseases, in the formation of amyloid plaques and neurofibril tangles during AD pathogenesis, indicating that selenium and SelM relieve or prevent the incidence of AD. It is postulated that selenium and SelM have crucial roles in the regulation of neurodegenerative diseases via modulation of secretase activity and Tau phosphorylation through the activation of the ERK

MAPK pathway. Therefore, it appears that selenium and SelM serve as potential new drug candidates for the relief of AD by modulating ERK signaling. However, intensive work is still needed to define the role of SelM and selenium in preventing AD in the human population.

Acknowledgements

This research was supported by a grant from Pusan National University Research.

References

1. Ray WJ, Ashall F and Goate AM: Molecular pathogenesis of sporadic and familial forms of Alzheimer's disease. Mol Med Today 4: 151-157, 1998.
2. Cotman CW and Anderson AJ: A potential role for apoptosis in neurodegeneration and Alzheimer's disease. Mol Neurobiol 10: 19-45, 1995.
3. Boutajangout A, Leroy K, Touchet N, Authelet M, Blanchard V, Tremp G, Pradier L and Brion JP: Increased tau phosphorylation but absence of formation of neurofibrillary tangles in mice double transgenic for human tau and Alzheimer mutant (M146L) presenilin-1. Neurosci Lett 318: 29-33, 2002.
4. Hwang DY, Chae KR, Kang TS, Hwang JH, Lim CH, Kang HK, Goo JS, Lee MR, Lim HJ, Min SH, Cho JY, Hong JT, Song CW, Paik SG, Cho JS and Kim YK: Alterations in behavior, amyloid beta-42, caspase-3, and Cox-2 in mutant PS2 transgenic mouse model of Alzheimer's disease. FASEB J 16: 805-813, 2002.
5. Haass C, Koo EH, Mellon A, Hung AY and Selkoe DJ: Targeting of cell-surface beta-amyloid precursor protein to lysosomes: alternative processing into amyloid-bearing fragments. Nature 357: 500-503, 1992.
6. Murphy MP, Hickman LJ, Eckman CB, Uljon SN, Wang R and Golde TE: gamma-Secretase, evidence for multiple proteolytic activities and influence of membrane positioning of substrate on generation of amyloid beta peptides of varying length. J Biol Chem 274: 11914-11923, 1999.
7. Johnson GV and Stoothoff WH: Tau phosphorylation in neuronal cell function and dysfunction. J Cell Sci 117: 5721-5729, 2004.
8. Shim SB, Lim HJ, Chae KR, Kim CK, Hwang DY, Jee SW, Lee SH, Sin JS, Leem YH, Lee SH, Cho JS, Lee HH, Choi SY and Kim YK: Tau overexpression in transgenic mice induces glycogen synthase kinase 3beta and beta-catenin phosphorylation. Neuroscience 146: 730-740, 2007.
9. Robert M and Mathuranath PS: Tau and tauopathies. Neurol India 55: 11-16, 2007.
10. Wilber CG: Toxicology of selenium: a review. Clin Toxicol 17: 171-230, 1980.
11. Li GX, Hu H, Jiang C, Schuster T and Lü J: Differential involvement of reactive oxygen species in apoptosis induced by two classes of selenium compounds in human prostate cancer cells. Int J Cancer 120: 2034-2043, 2007.
12. Rudolf E, Rudolf K and Cervinka M: Selenium activates p53 and p38 pathways and induces caspase-independent cell death in cervical cancer cells. Cell Biol Toxicol 24: 123-141, 2008.
13. Hu H, Jiang C, Li G and Lü J: PKB/AKT and ERK regulation of caspase-mediated apoptosis by methylseleninic acid in LNCaP prostate cancer cells. Carcinogenesis 26: 1374-1381, 2005.
14. Wang Q, Zhang QG, Wu DN, Yin XH and Zhang GY: Neuroprotection of selenite against ischemic brain injury through negatively regulating early activation of ASK1/JNK cascade via activation of PI3K/AKT pathway. Acta Pharmacol Sin 28: 19-27, 2007.
15. Hwang DY, Sin JS, Kim MS, Yim SY, Kim YK, Kim CK, Kim BG, Shim SB, Jee SW, Lee SH, Bae CJ, Lee BC, Jang MK, Cho JS and Chae KR: Overexpression of human selenoprotein M differentially regulates the concentrations of antioxidants and H_2O_2, the activity of antioxidant enzymes, and the composition of white blood cells in a transgenic rat. Int J Mol Med 21: 169-179, 2008.
16. Tung YT, Hsu WM, Wang BJ, Wu SY, Yen CT, Hu MK and Liao YF: Sodium selenite inhibits gamma-secretase activity through activation of ERK. Neurosci Lett 440: 38-43, 2008.
17. Kim SK, Park HJ, Hong HS, Baik EJ, Jung MW and Mook-Jung I: ERK1/2 is an endogenous negative regulator of the gamma-secretase activity. FASEB J 20: 157-159, 2006.
18. Smith AM and Picciano MF: Evidence for increased selenium requirement for the rat during pregnancy and lactation. J Nutr 11: 1068-1079, 1986.
19. Yang G, Chen J, Wen Z and Ge K: The role of selenium in Keshan disease. In: Advances in Nutritional Research. Drapper HH (ed). Plenum Press, New York, pp203-231, 1984.
20. Van Rij AM, Thomson CD, McKenzie JM and Robinson MF: Selenium deficiency in total parenteral nutrition. Am J Clin Nutr 32: 2085-2086, 1979.
21. Birringer M, Pilawa S and Flohe L: Trends in selenium biochemistry. Nat Prod Rep 19: 693-718, 2002.
22. Savaskan NE, Brauer AU, Kuhbacher M, Eyupoglu IY, Kyriakopoulos A, Ninnemann O, Behne D and Nitsch R: Selenium deficiency increases susceptibility to glutamate-induced excitotoxicity. FASEB J 17: 112-114, 2003.
23. Jaaro H, Rubinfeld H, Hanoch T and Seger R: Nuclear translocation of mitogen-activated protein kinase kinase (MEK1) in response to mitogenic stimulation. Proc Natl Acad Sci USA 94: 3742-3747, 1997.
24. Seger R, Seger D, Reszka AA, Munar ES, Eldar-Finkelman H, Dobrowolska G, Jensen AM, Campbell JS, Fischer EH and Krebs EG: Overexpression of mitogen-activated protein kinase kinase (MAPKK) and its mutants in NIH 3T3 cells. Evidence that MAPKK involvement in cellular proliferation is regulated by phosphorylation of serine residues in its kinase subdomains VII and VIII. J Biol Chem 269: 25699-25709, 1994.
25. Selkoe DJ: Alzheimer's disease: genes, proteins, and therapy. Physiol Rev 81: 741-766, 2001.
26. Iwatsubo T: The gamma-secretase complex: machinery for intramembrane proteolysis. Curr Opin Neurobiol 14: 379-383, 2004.
27. Goedert M, Hasegawa M, Jakes R, Lawler S, Cuenda A and Cohen P: Phosphorylation of microtubule-associated protein tau by stress-activated protein kinases. FEBS Lett 409: 57-62, 1997.
28. Sergeant N, Delacourte A and Buée L: Tau protein as a differential biomarker of tauopathies. Biochim Biophys Acta 1739: 179-197, 2005.
29. Marshall CJ: MAP kinase kinase kinase, MAP kinase kinase and MAP kinase. Curr Opin Genet Dev 4: 82-89, 1994.
30. Waskiewicz AJ and Cooper JA: Mitogen and stress response pathways: MAP kinase cascades and phosphatase regulation in mammals and yeast. Curr Opin Cell Biol 7: 798-805, 1995.
31. Fanger GR, Gerwins P, Widmann C, Jarpe MB and Johnson GL: MEKKs, GCKs, MLKs, PAKs, TAKs, and tpls: upstream regulators of the c-Jun amino-terminal kinases? Curr Opin Genet Dev 7: 67-74, 1997.
32. Lee VM, Goedert M and Trojanowski JQ: Neurodegenerative tauopathies. Annu Rev Neurosci 24: 1121-1159, 2001.
33. Lucas JJ, Hernández F, Gómez-Ramos P, Morán MA, Hen R and Avila J: Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. EMBO J 20: 27-39, 2001.
34. Lee G, Thangavel R, Sharma VM, Litersky JM, Bhaskar K, Fang SM, Do LH, Andreadis A, Van Hoesen G and Ksiezak-Reding H: Phosphorylation of tau by fyn: implications for Alzheimer's disease. J Neurosci 24: 2304-2312, 2004.