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Effects of Selenoprotein W gene expression by selenium involves regulation of mRNA stability in chicken embryos neurons

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Abstract Selenium (Se) and Selenoprotein W (SelW) plays a pivotal role in the brain development, function, and degeneration and that SelW expression in the brain may be affected by Se. However, the mechanism which Se regulates the SelW gene expression in neurons remains to be unclear. To investigate the effects of the SelW gene expression and mRNA stability induced by Se, primary cultured chicken embryos neurons derived from 8-day-old chick embryo cerebral hemispheres were treated with 10^{-9}–10^{-5} mol/l Se as selenite for 3, 6, 12, 24 or 48 h, respectively. The morphology and viability of Neurons was detected. The SelW mRNA expression level and mRNA half-life was examined in Se-treated neurons. The relative low concentrations of Se enhanced the neurite outgrowth, increased the SelW mRNA levels and elevated the mRNA half-life of chick embryo neurons. In contrast, the high concentrations of Se presented neurotoxic to neurons, decreased the SelW mRNA levels and reduced the mRNA half-life of neuronal cells. These results suggest that the alteration of post-transcriptional stabilization of SelW mRNA is an important mechanism of Se-induced the elevation or reduction of the SelW expression level in chick embryo neurons.

Keywords Chicken embryos neurons · Selenoprotein W · Selenium · mRNA expression · mRNA stability

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Introduction

Selenium (Se) is an essential micronutrient in the diet of animals including birds, and its importance for regular development, immune functions (Hoffmann and Berry 2008), chemoprevention (Li et al. 2010), neurobiology, especially normal functioning of the brain (Chen and Berry 2003; Savaskan et al. 2003; Schweizer et al. 2004a, b; Schweizer and Schomburg 2006). When Se was deficient in the diet, the brain showed high priority to conserve this element (Chen and Berry 2003; Schweizer et al. 2004a, b; Schweizer

and Schomburg 2006). The main biological form of Se is selenocysteine (Sec) which occurs in selenoproteins. Sec, the 21st proteinogenic amino acid, is cotranslationally incorporated into the growing peptide chain in response to a UGA codon that is decoded for Sec insertion in combination with a specific stem loop structure, the selenocysteine-insertion sequence (SECIS) element (Allmang et al. 2009; Gromer et al. 2005; Lu and Holmgren 2009). Se and selenoproteins play a pivotal role in the brain development, function, and degeneration (Chen and Berry 2003). Recent findings showed that Se deficiency and reduced expression of selenoproteins in the brain impairs and brain development entails functional and behavioral defects and ultimately causes severe neurological phenotypes (Nakayama et al. 2007; Savaskan et al. 2003; Schweizer et al. 2004a, b). Furthermore, Se is a well-known toxic. The central nervous system is sensitive to Se poisoning; exposure to Se causes the nervous system disorders in animals (Himeno and Imura 2002). Therefore, depending on dosage, there appears to be a dual role for Se as a neuroprotectant and toxic agent.

Selenoproteins are expressed widely in the brain (Zhang et al. 2008). Selenoprotein W (SelW), a member of the selenoprotein family, was first purified and characterized from rat skeletal muscle (Vendeland et al. 1993), but SelW and its mRNA was later also demonstrated to be present in the brain of mammals including primates (Bellingham et al. 2003; Gu et al. 2000), pigs (Zhou et al. 2009), sheep (Sun et al. 1999) and rodents (Hoppe et al. 2008; Yeh et al. 1995). SelW is present in most tissues from rats fed a Se-adequate diet, but is highest in skeletal muscle, brain, testis, and spleen (Sun et al. 1998; Yeh et al. 1995). The expression level of SelW in the rat brain is maintained in Se deficiency (Whanger 2000, 2001, 2009). Overexpression of SelW in C6 rat glial cells resulted in a greater survival rate than control cells when incubated with 2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH) (Sun et al. 2001a). These data indicate that SelW play important roles in the brain of mammals. However, the information about SelW in birds is lacking. Very recently, we have investigated that SelW was expressed widely in the chicken neural tissues including cerebral cortex, cerebral nuclei, thalamus, cerebellum, brain stem, medulla oblongata, spinal cord and sciatic nerve (Li et al. 2011b). SelW is present in most tissues of chicken, but is highest in neural and muscle tissues (Li et al. 2011b). This raises the question as to whether SelW might be important in the functions of the nevous system.

The expression of selenoproteins in the brain depends on the supply with Se and thus, behavioural changes and neuropathological alterations following Se deficiency and excess are likely due to altered selenoprotein expression. The organ-specific modulation of SelW expression may relate to the most important sites of function in Se deficiency and excess. However, the mechanism which Se regulates the SelW gene expression in neurons remains to be incompletely understood. Therefore, the aims of the present study were to investigate the alteration of mRNA stability induced by different concentrations of Se in chicken embryos neurons and to evaluate the effects of Se on the regulation of SelW mRNA expression in the cells.

Materials and methods

Preparation of chick embryo neurons

Primary cultures of chick embryo neurons were prepared with the modified method according to Mangoura and Dawson (Mangoura and Dawson 1993). In brief, Cultures were prepared by dissociating 8-day-old chick embryo cerebral hemispheres. 6-well plates were used for cell culture and turning experiments. To achieve optimum growth conditions, the plate was coated overnight with 0.1 mg/ml poly-D-lysine (Sigma, USA). The cerebral hemispheres were dissected aseptically, cleaned of adhering meningeal membranes and blood vessels, and dissociated by trypsin treatment. Cell suspension was prepared by gently pushing the splenic pulp through a sterile stainless steel mesh with a pore size of 100 μM. The dispersed cells were plated initially in complete cell culture medium (DMEM containing HEPES and 2 mM glutamine, supplemented with 10% fetal bovine calf serum and antibiotics, containing 3.04×10^{-12} mol/l Se) for 24 h. To purify the chick embryo neurons, the culture medium was supplemented with 2 μg/ml cytarabine (Sigma, USA). Following culture for 24 h, medium was removed cultures and the cell monolayers were washed three times with Hanks' balanced salt solution. Then, neurons were incubated in complete cell culture medium.

Neuron cultures and treatments

Neurons were seeded into six-well plates precoated with poly-D-lysine at a density of 1.0×10^6 viable cells per well in 2 ml of complete medium. All cultures were incubated at 37°C in an atmosphere of 5% CO_2 and 95% air, saturated with water. After an attachment period of 48 h, cell monolayers were washed twice with Hanks' balanced salt solution. Then, neurons were grown in 2 ml of fresh complete medium and treated with vehicle, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} or 10^{-5} mol/l of Se as sodium selenite (Sigma, USA) for 0, 3, 6, 12, 24 or 48 h, respectively. Neurons were harvested for analysis of SelW mRNA level. Each treatment was done in six wells in three separate experiments.

Determination of the morphology and viability of neurons

The morphology of treated and untreated neurons were visualized under the light microscopy (Eclipse-*Ti*, Nikon, Japan) at ×400 magnification. The viability of cell was measured by trypan blue exclusion test.

Measurement of neurite outgrowth

Under a light microscope, digital images were taken from 10 randomly selected fields which contained more than 20 cells. Neurite lengths on the digitized images were measured using Image J, an image analysis system (NIH Image J software located at http://rsb.info.nih.gov/ij/). The clusters of cells were excluded from the measurement of neurite length. A neurite was identified as a process greater than one-cell body diameter in length and possessing a terminal growth cone. In each cell, all primary neurites and their higher order branches were measured from their points of origin to their free tips to determine the total neuritic length and the length of the longest neurite. In each set of experiments, more than 100 neurites were measured, and their mean length was calculated. This analysis was performed in a double-blinded fashion and the data was averaged and plotted for statistical analysis.

Determination of SelW mRNA level by quantitative RT-PCR

After neurons were treated with different concentrations of Se, the medium was removed. Total RNA was isolated from the neuron monolayers using the TRIZOL reagent (Invitrogen, China) according to the manufacturer's protocol. The dried RNA pellets were resuspended in 40 μl of diethyl-pyrocarbonate-treated water. The concentration and purity of the total RNA were determined spectrophotometrically at 260/280 nm. First-strand cDNA was synthesized from 2 μg of total RNA using oligo dT primers and Superscript II reverse transcriptase according to the manufacturer's instructions (Invitrogen, China). Synthesized cDNA was diluted five times with sterile water and stored at −80°C before use.

Design of the specific primers for SelW and GADPH were performed as described previously (Li et al. 2011a). Quantitative real-time PCR was performed on an ABI PRISM 7500 Detection System (Applied Biosystems, USA). Reactions were performed in a 20 μl reaction mixture containing 10 μl of 2 × SYBR Green I PCR Master Mix (TaKaRa, China), 2 μl of either diluted cDNA, 0.4 μl of each primer (10 μM), 0.4 μl of 50 × ROX reference Dye II and 6.8 μl of PCR-grade water. The PCR procedure for SelW and GADPH consisted of 95°C for 30 s followed by 40 cycles of 95°C for 15 s, 60°C for 30 s and 60°C for 30 s. The melting curve analysis showed only one peak for each PCR product. Electrophoresis was performed with the PCR products to verify primer specificity and product purity. A dissociation curve was run for each plate to confirm the production of a single product. The amplification efficiency for each gene was determined using the DART-PCR program (Peirson et al. 2003). We analysed the expression levels of the housekeeping genes (GADPH) in chick embryo neurons. The expression of GADPH was stable in Se-treated and untreated neurons ($P > 0.05$, Supplementary Fig. S1). For normalization purposes, GADPH is found to be a suitable gene for qRT-PCR analysis of gene expression in the chicken embryos neurons. Therefore, the mRNA relative abundance was calculated according to the method of Pfaffl (Pfaffl 2001), accounting for gene-specific efficiencies and was normalized to the mean expression of GADPH.

Determination of SelW mRNA half-life

The mRNA half-life determinations were performed as described previously (Blanquicett et al. 2010). Briefly, the control and Se-treated neurons were

further incubated with 5 μg/ml actinomycin D (ActD, Sigma, USA) for 0, 3, 6, 9, 12, 24 or 48 h at which time points RNAs were extracted and the level of SelW mRNA was measured by quantitative RT-PCR, as described above. Cell transcription was stopped by the addition of the RNA polymerase inhibitor ActD. The mRNA half-life was extrapolated from the SelW mRNA decay curve as the time point after ActD treatment at which there was 50% of the initial mRNA level remaining.

Statistical analysis

Statistical analysis of Se concentration and mRNA level was performed using SPSS statistical software for Windows (version 13; SPSS Inc., Chicago, IL, USA). When a significant value ($P < 0.05$) was obtained by one-way analysis of variance, further analysis was done. All data showed a normal distribution and passed equal variance testing. Differences between means were assessed by Tukey's honestly significant difference test for post hoc multiple comparisons. Data are expressed as mean ± standard deviation. Differences were considered to be significant at $P < 0.05$.

Results

The morphology and viability of neurons

The photomicrographs of chick embryo neurons treated with Se and without Se are shown in Fig. 1. Neurons treated with 10^{-9}–10^{-8} mol/l of Se for 48 h and 10^{-7} mol/l of Se for 24 h had more neurite branches and length than those in the control, indicating that the low concentrations of Se enhanced neurite outgrowth (Fig. 1A–D). The decreases of the neurite length and branches were found in neurons treated with 10^{-7} mol/l of Se at 48 h and 10^{-6} mol/l of Se at 24 h (Fig. 1E, F). Neurons treated with 10^{-6} mol/l of Se for 48 h and 10^{-5} mol/l of Se for 6 and 12 h demonstrated vacuolated soma, fragmented neurite and cell shrinkage, indicating that Se led cell death at higher (10^{-6} mol/l) dose (Fig. 1G–I). Therefore, the decrease of cell viability was evident when neurons were treated with 10^{-6} mol/l of Se for 24 h and 10^{-5} mol/l of Se for 3 h (data not shown). The results showed that chick embryo neurons exposed to high concentrations of Se displayed a time- and dose-dependent decrease in cell viability.

The effects of different concentration of Se and periods of treatment on neurite outgrowth in chick embryo neurons was examined (Fig. 2A–C). Following treatment with Se, the number of neurite-bearing cells was increased in the cells treated with 10^{-9}–10^{-8} mol/l of Se for 48 h and decreased in the cells treated with 10^{-7} mol/l of Se at 48 h, 10^{-6} mol/l of Se at 12 h and 10^{-6} mol/l of Se at 6 h, when compared with untreated control cells ($P < 0.001$ or 0.05, Fig. 2A). In quantitative analyses of neurons, significant increases in neurite length and branches were observed in the cells treated with 10^{-9}–10^{-8} mol/l of Se for 48 h ($P < 0.01$ or 0.05, Fig. 2B, C). The decreases of the neurite length were found in neurons treated with 10^{-7} mol/l of Se at 48 h, 10^{-6} mol/l of Se at 24 h and 10^{-5} mol/l of Se at 6 h ($P < 0.001$ or 0.01, Fig. 2B). The number of neurite branches was decreased in neurons treated with 10^{-7} mol/l of Se at 48 h, 10^{-6} mol/l of Se at 6 h and 10^{-5} mol/l of Se at 3 h ($P < 0.001$ or 0.01, Fig. 2C).

Effect of Se supplementation on SelW mRNA level

The SelW mRNA level measured by quantitative real-time RT-PCR is shown in Fig. 3. When compared with the control group, significant increases in the SelW mRNA levels were observed in neurons treated with 10^{-9}–10^{-8} mol/l of Se for 6-48 h ($P < 0.001$). Of note, the maximal increases of SelW mRNA levels were observed in the neurons incubated with 10^{-9} mol/l of Se at 48 h (about 3.6-fold vs. control), 10^{-8} mol/l of Se at 24 h (about 2.97-fold vs. control), 10^{-7} mol/l of Se at 24 h (about 2.95-fold vs. control) and 10^{-6} mol/l of Se at 6 h (about 2.1-fold vs. control). After reaching a maximal level, prolonged incubation time led to a reduction in the SelW mRNA level, especially at the dose of 10^{-6} mol/l Se. However, the SelW mRNA level was displayed a time-dependent decrease in neurons treated with 10^{-5} mol/l of Se, indicating that the high concentration of Se had the cytotoxicity effect in chick embryo neurons.

Effect of Se supplementation on SelW mRNA stability

To examine the mechanisms by which Se influenced the SelW mRNA, the SelW mRNA half-life in chick

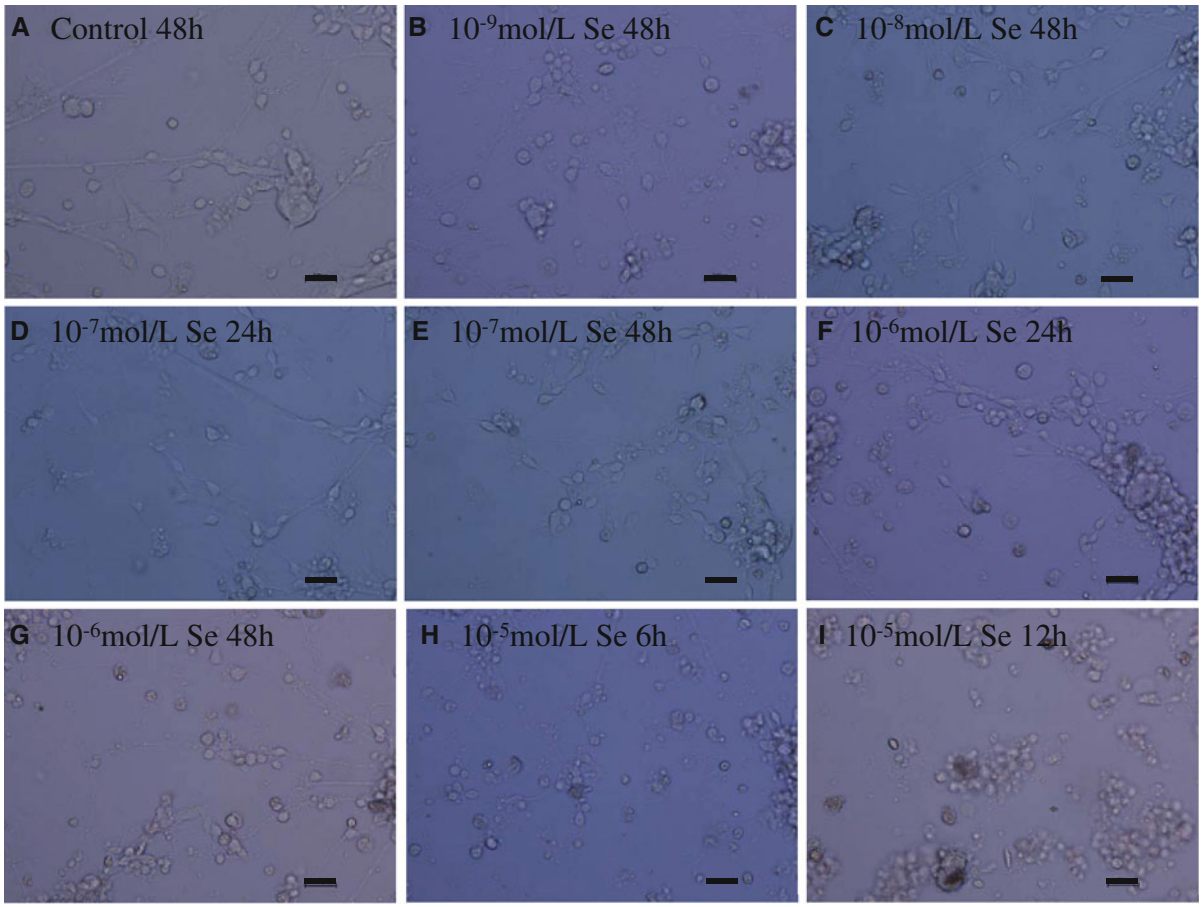

Fig. 1 Effects of Se on the morphology of chicken embryo neurons. The chicken embryo neuron monolayers were treated with 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} or 10^{-5} mol/l of Se as sodium selenite for 0, 3, 6, 12, 24 or 48 h, respectively. The morphology of treated and untreated neurons was visualized under the light microscopy (magnification: ×400, *bar* 50 μm). Note the neurite length and branches and the morphological alterations of neurons

embryo neurons was measured in the presence or absence of ActD. Cells were incubated with the RNA polymerase inhibitor ActD to block the novo mRNA synthesis and harvested at the indicated time points. The SelW mRNA half-life was observed in chick embryo neurons incubated with ActD (about 12.85 h), ActD + 10^{-8} mol/l of Se (about 16.40 h), ActD + 10^{-7} mol/l of Se (about 24.08 h), ActD + 10^{-6} mol/l of Se (about 13.23 h) and ActD + 10^{-5} mol/l of Se (about 3.24 h) (Supplementary Fig. S2). Of note, in the presence of 10^{-8}–10^{-7} mol/l Se, the SelW mRNA level was higher than in the presence of ActD (Fig. 4). However, the SelW mRNA level of neurons treated 10^{-5} mol/l Se was significantly reduced (Fig. 4). The results showed that the SelW mRNA stability in neurons treated with 10^{-7} mol/l of Se was considerably increased and the mRNA stability in cells treated with 10^{-5} mol/l of Se was significantly reduced.

Discussion

Se compounds play important roles in the central nervous system, including elevation of selenoenzyme activity, reduction of lipid peroxidation and protection from cell death (Brauer and Savaskan 2004; Savaskan et al. 2003). However, the effects of Se could be blocked with inhibitors of protein biosynthesis (Savaskan et al. 2003). It suggested that Se acts mainly after incorporation into selenoproteins. The

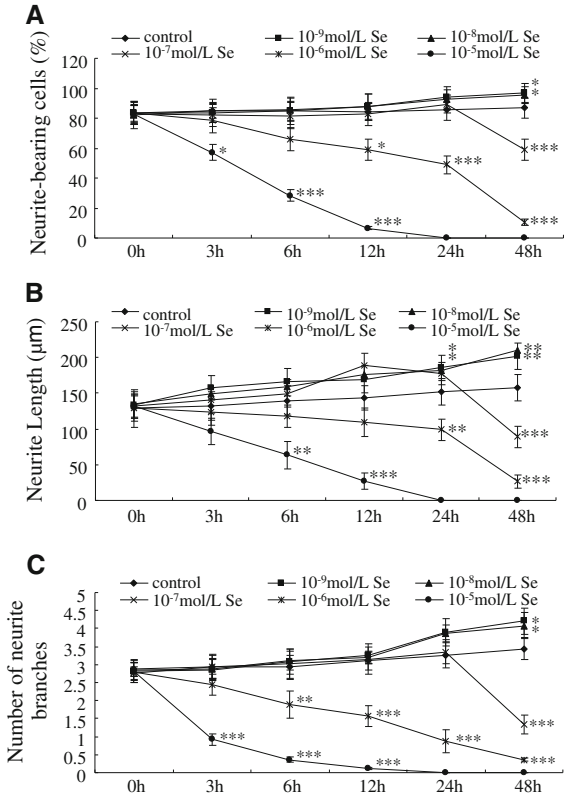

Fig. 2 Quantitative measurement of neurite outgrowth in chicken embryo neurons. (**A**) Population analysis of neurite bearing cells. (**B**) Average neurite lengths. (**C**) Population analysis of neurite branches. *Bars* represent mean ± standard deviation of triplicate cultures. *Bars* with * are statistically significantly different from control by one-way analysis of variance followed by Tukey's multiple comparison test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$)

expression of individual selenoproteins is differentially affected by cellular Se content. For example, SelW is expressed in the rat brain and its expression level is maintained in Se deficiency (Whanger 2000, 2001, 2009). However, the information of SelW in the avian brain was unknown. In our previous study, we demonstrated that SelW is expressed widely in chicken tissues, with predominant expression in the neural tissue and muscle tissue (Li et al. 2011b). SelW gene expression in the avian neural tissues is sensitive to dietary Se content (unpublished data), consistent with these studies about the avian gastrointestinal tract (Li et al. 2011a). In this study, we observed the mRNA expression of SelW in chicken embryos neurons and the effects of different concentrations Se on the morphology and viability of cells. The results of the present work confirmed that Se not only effected the SelW mRNA expression in chicken embryos neurons but also altered mRNA stability in the cells.

Se is a potent modulator of eukaryotic cell growth with strictly concentration-dependant effects. Lower concentrations are necessary for cell survival and growth, whereas higher concentrations inhibit growth and induce cell death (Selenius et al. 2010; Zeng and Combs 2008). It has been determined that Se protects neurons against insults relevant to the pathogenesis of neurodegenerative disorders (Gwon et al. 2010). Selenite sodium induces neurodegeneration at μM level in primary cultured cortical neurons through an apoptotic pathway, and the apoptotic cells increase proportionally to the concentration and the exposure time of sodium selenite (Xiao et al. 2006). The present study indicates that in cultures of chick embryo neurons, relative low concentration of Se (10^{-9}–10^{-8} mol/l) had no effect on neuronal survival. In contrast, the high concentrations of Se (10^{-6}–10^{-5} mol/l) were found neurotoxic to chick embryo neurons and significantly decreased the cell viability. These results suggest that the effect of Se on the survival of neurons in cultures is at least associated with its treatment concentration and time.

One of the critical steps in neuronal differentiation is the outgrowth of neuronal processes, axons and dendrites, because these processes establish a neuron's structural and functional polarity (Cline 2001; Poirazi and Mel 2001). Our further study showed that in cultures of chick embryo neurons, relative low concentrations of Se (10^{-9}–10^{-8} mol/l) enhanced neurite outgrowth (Fig. 1). In contrast, the high concentration of Se (10^{-6} mol/l) was found neurotoxic to chick embryo neurons and significantly decreased the neurite length and branches. The fact suggested that outgrowth of both axons and dendrites of chick embryo neurons was affected by Se. An increase in the number of neurites would appear to reflect an effect of Se treatment on growth of dendrites since they are the more numerous type of neurite. Correspondingly, an increase in the length of the longest neurite probably reflected a change in the axonal growth since the longest neurite with a prominent growth is usually the growing axon. These results suggested that Se may affect the growth of dendrites and axons in different ways.

Selenoproteins are expressed widely in the brain and most selenoproteins are expressed in neurons of

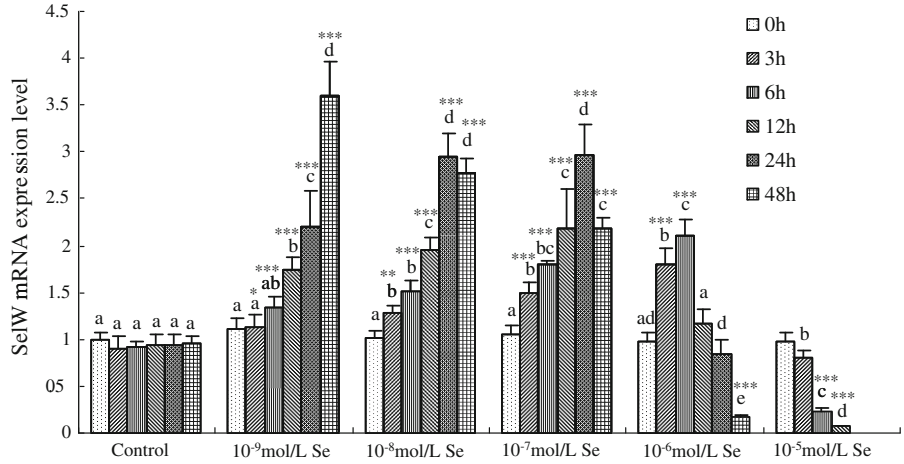

Fig. 3 Effects of Se on the SelW mRNA expression level in chicken embryo neurons. The chicken embryo neuron monolayers were treated with 0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6} or 10^{-5} mol/l of Se as sodium selenite for 0, 3, 6, 12, 24 or 48 h, respectively. SelW mRNA expression level in chicken embryo neurons was measured by quantitative real-time RT-PCR, and the ratio of the level of SelW mRNA to that of the GADPH internal control was used for statistical comparison. *Bar*s represent mean ± standard deviation of triplicate cultures. *Bars* with * are statistically significantly different from control by one-way analysis of variance followed by Tukey's multiple comparison test (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). Within the groups treated with various concentrations of Se, *bars* sharing a common *letter* are not significantly different ($P > 0.05$)

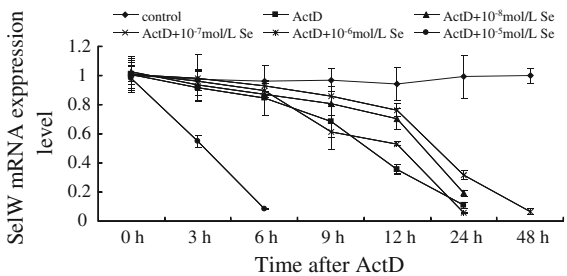

Fig. 4 Effects of Se on SelW mRNA stability in chicken embryo neurons. The chicken embryo neuron monolayers were treated with 0 mol/l, ActD, ActD + 10^{-8} mol/l Se, ActD + 10^{-7} mol/l Se, ActD + 10^{-6} mol/l Se or ActD + 10^{-5} mol/l Se for 0, 3, 6, 9, 12, 24 or 48 h, respectively. SelW mRNA expression level in chicken embryo neurons was measured by quantitative real-time RT-PCR. The mRNA stability was denoted with the SelW mRNA decay curve after 5 μg/ml ActD treatment mRNA expression level in chicken embryo neurons was measured by quantitative real-time RT-PCR. The RNA half-life was extrapolated from the SelW mRNA decay curve as the time point after 5 μg/ml ActD treatment

animals (Zhang et al. 2008). Although SelW exists in many tissues, its expression is preserved in certain regions of the rat brain in response to different stages of Se deficiency (Sun et al. 2001b; Yeh et al. 1997). It has also been reported that SelW mRNA is highly expressed in the developing central nerve system of rats, and its expression is maintained until the early postnatal stage (Jeong et al. 2004). These facts have indicated an important role of SelW in brain. Overexpression of SelW protects rat glial cells against oxidative stress induced by heavy metals or AAPH (Amantana et al. 2002; Sun et al. 2001a). Treatment of SH-SY5Y neuronal blastoma cells with methylmercury, a neurotoxicant, results in decreased SelW mRNA expression in a glutathione dependent manner (Kim et al. 2005). Recently, it was found that SelW may play a crucial neuroprotective role in oxidative stress-induced primary neuronal cell damage (Chung et al. 2009). Collectively, these results suggest SelW can safeguard developing neuronal cells from oxidative attack of endogenous and exogenous origins. In this study, relative low concentration of Se increased in the SelW mRNA levels of chick embryo neurons and enhanced the neurite length and branches. In contrast, the high concentrations of Se were found to decrease the SelW mRNA levels and neurotoxic to neurons, including vacuolated soma, fragmented neurites, cell shrinkage and a significantly decrease of the cell viability. These results suggest that Se may affect the development of neuronal cells through the alteration of the SelW expression level in chick embryo neurons. The effects of Se on neurons may be attributed to the biochemical activity of the SelW

including a potential role as an antioxidant and redox regulation in neurons (Chung et al. 2009; Ralston et al. 2008). However, further studies using genetically modified chick embryo neurons models such as the SelW knockout and overexpression cells would be required to test the biological function of the avian SelW.

Determination of mRNA half-life is important to our understanding of gene expression and mechanisms involved in the regulation of the level of transcripts in response to environmental changes or developmental cues. Dietary Se exerts its effect on pretranslational Se-GPX gene expression at the level of cytosolic mRNA stabilization (Christensen and Burgener. 1992). Se depletion of H4 rat hepatoma cells reduced cytosolic glutathione peroxidase (cGSH-Px) mRNA abundance and decreased the stability of cGSH-Px mRNA. Se supply extended the half-life of cGSH-Px mRNA in these cells (Bermano et al. 1996). We also investigated the underlying mechanisms of the effect in the SelW expression of neurons in response to Se. In the present study, the SelW mRNA half-life in chick embryo neurons was measured in the presence or absence of ActD. Relative low concentration of Se enhanced the SelW mRNA half-life in chick embryo neurons. In contrast, the high concentration of Se significantly reduced the SelW mRNA half-life of neuronal cells. These data suggested that Se may alter the SelW mRNA stability in cells. Numerous studies demonstrated that Se could regulate the SelW transcription and effect the SelW expression level in vivo and vitro (Amantana et al. 2002; Chung et al. 2009; Gu et al. 2000, 2002; Jeong et al. 2004; Loflin et al. 2006; Sun et al. 2001a, b; Yeh et al. 1995; Zhang et al. 2008). In addition to transcription regulation, it is apparent that post-transcriptional control of gene expression through mRNA transcript stability is important in the regulation of genes. The mRNA stability is a highly regulated post-transcriptional step tightly coordinated with mRNA translation (De Rubeis and Bagni 2010). The results of this paper strongly suggest that the alteration of post-transcriptional stabilization of SelW mRNA is an important mechanism of Se-induced the elevation and reduction of the SelW expression level in chick embryo neurons.

In summary, our data demonstrated that the relative low concentration of Se enhanced the neurite outgrowth and increased in the SelW mRNA levels of chick embryo neurons. However, the high concentrations of Se presented neurotoxic to neurons and decreased the SelW mRNA levels of neuronal cells. We further identify altered mRNA stability as the underlying mechanism for the observed effect of Se on the SelW gene expression in neurons. These data provide novel insights into the regulation of SelW transcription and post-transcription in Se-treated neurons.

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References

Allmang C, Wurth L, Krol A (2009) The selenium to selenoprotein pathway in eukaryotes: more molecular partners than anticipated. Biochim Biophys Acta 1790(11):1415–1423

Amantana A, Vorachek WR, Butler JA, Costa ND, Whanger PD (2002) Effect of copper, zinc and cadmium on the promoter of selenoprotein W in glial and myoblast cells. J Inorg Biochem 91(2):356–362

Bellingham J, Gregory-Evans K, Fox MF, Gregory-Evans CY (2003) Gene structure and tissue expression of human selenoprotein W, SEPW1, and identification of a retroprocessed pseudogene, SEPW1P. Biochim Biophys Acta 1627(2–3):140–146

Bermano G, Arthur JR, Hesketh JE (1996) Selective control of cytosolic glutathione peroxidase and pholipid hydroperoxide glutathione peroxidase mRNA stability by selenium supply. FEBS Lett 387(2–3):157–160

Blanquicett C, Kang BY, Ritzenthaler JD, Jones DP, Hart CM (2010) Oxidative stress modulates PPAR gamma in vascular endothelial cells. Free Radic Biol Med 48(12): 1618–1625

Brauer AU, Savaskan NE (2004) Molecular actions of selenium in the brain: neuroprotective mechanisms of an essential trace element. Rev Neurosci 15(1):19–32

Chen J, Berry MJ (2003) Selenium and selenoproteins in the brain and brain diseases. J Neurochem 86(1):1–12

Christensen MJ, Burgener KW (1992) Dietary selenium stabilizes glutathione peroxidase mRNA in rat liver. J Nutr 122(8):1620–1626

Chung YW, Jeong D, Noh OJ, Park YH, Kang SI, Lee MG et al (2009) Antioxidative role of selenoprotein W in oxidant-induced mouse embryonic neuronal cell death. Mol Cells 27(5):609–613

Cline HT (2001) Dendritic arbor development and synaptogenesis. Curr Opin Neurobiol 11(1):118–126

De Rubeis S, Bagni C (2010) Fragile × mental retardation protein control of neuronal mRNA metabolism: Insights into mRNA stability. Mol Cell Neurosci 43(1):43–50

Gromer S, Eubel JK, Lee BL, Jacob J (2005) Human selenoproteins at a glance. Cell Mol Life Sci 62(21):2414–2437

Gu QP, Sun Y, Ream LW, Whanger PD (2000) Selenoprotein W accumulates primarily in primate skeletal muscle, heart, brain and tongue. Mol Cell Biochem 204(1–2):49–56

Gu QP, Ream W, Whanger PD (2002) Selenoprotein W gene regulation by selenium in L8 cells. Biometals 15(4):411–420

Gwon AR, Park JS, Park JH, Baik SH, Jeong HY, Hyun DH et al (2010) Selenium attenuates A beta production and A beta-induced neuronal death. Neurosci Lett 469(3):391–395

Himeno S, Imura N (2002) Selenium in nutrition and toxicology. In: Sarkar B (ed) Heavy metals in the environment, 1st edn. CRC Press, New York, pp 614–618

Hoffmann PR, Berry MJ (2008) The influence of selenium on immune responses. Mol Nutr Food Res 52(11):1273–1280

Hoppe B, Brauer AU, Kuhbacher M, Savaskan NE, Behne D, Kyriakopoulos A (2008) Biochemical analysis of selenoprotein expression in brain cell lines and in distinct brain regions. Cell Tissue Res 332(3):403–414

Jeong DW, Kim EH, Kim TS, Chung YW, Kim H, Kim IY (2004) Different distributions of selenoprotein W and thioredoxin during postnatal brain development and embryogenesis. Mol Cells 17(1):156–159

Kim YJ, Chai YG, Ryu JC (2005) Selenoprotein W as molecular target of methylmercury in human neuronal cells is down-regulated by GSH depletion. Biochem Biophys Res Commun 330(4):1095–1102

Li JL, Gao R, Li S, Wang JT, Tang ZX, Xu SW (2010) Testicular toxicity induced by dietary cadmium in cocks and ameliorative effect by selenium. Biometals 23(4):695–705

Li JL, Li HX, Li S, Jiang ZH, Xu SW, Tang ZX (2011a) Selenoprotein W gene expression in the gastrointestinal tract of chicken is affected by dietary selenium. Biometals 24(2):291–299. doi:10.1007/s10534-010-9395-0

Li JL, Ruan HF, Li HX, Li S, Xu SW, Tang ZX (2011b) Molecular cloning, characterization and mRNA expression analysis of a novel selenoprotein: avian Selenoprotein W from chicken. Mol Biol Rep 38(6):4015–4022. doi:10.1007/s11033-010-0520-5

Loflin J, Lopez N, Whanger PD, Kioussi C (2006) Selenoprotein W during development and oxidative stress. J Inorg Biochem 100(10):1679–1684

Lu J, Holmgren A (2009) Selenoproteins. J Biol Chem 284(2):723–727

Mangoura D, Dawson G (1993) Opioid peptides activate phospholipase D and protein kinase C-epsilon in chicken embryo neuron cultures. Proc Natl Acad Sci USA 90(7):2915–2919

Nakayama A, Hill KE, Austin LM, Motley AK, Burk RF (2007) All regions of mouse brain are dependent on selenoprotein P for maintenance of selenium. J Nutr 137(3):690–693

Peirson SN, Butler JN, Foster RG (2003) Experimental validation of novel and conventional approaches to quantitative real-time PCR data analysis. Nucleic Acids Res 31(14):e73

Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29(9):e45

Poirazi P, Mel BW (2001) Impact of active dendrites and structural plasticity on the memory capacity of neural tissue. Neuron 29(3):779–796

Ralston NV, Ralston CR, Blackwell JL 3rd, Raymond LJ (2008) Dietary and tissue selenium in relation to methylmercury toxicity. Neurotoxicology 29(5):802–811

Savaskan NE, Brauer AU, Kuhbacher M, Eyupoglu IY, Kyriakopoulos A, Ninnemann O et al (2003) Selenium deficiency increases susceptibility to glutamate-induced excitotoxicity. FASEB J 17(1):112–114

Schweizer U, Schomburg L (2006) Selenium, selenoproteins and brain function. In: Hatfield DL, Berry MJ, Gladyshev VN (eds) Selenium: its molecular biology and role in human health. Springer, New York, pp 233–248

Schweizer U, Schomburg L, Savaskan NE (2004a) The neurobiology of selenium: lessons from transgenic mice. J Nutr 134(4):707–710

Schweizer U, Brauer AU, Kohrle J, Nitsch R, Savaskan NE (2004b) Selenium and brain function: a poorly recognized liaison. Brain Res Brain Res Rev 45(3):164–178

Selenius M, Rundlof AK, Olm E, Fernandes AP, Bjornstedt M (2010) Selenium and the selenoprotein thioredoxin reductase in the prevention, treatment and diagnostics of cancer. Antioxid Redox Signal 12(7):867–880

Sun Y, Ha PC, Butler JA, Ou BR, Yeh JY, Whanger PD (1998) Effect of dietary selenium on selenoprotein W and glutathione peroxidase in 28 tissues of rat. J Nutr Biochem 9:23–27

Sun Y, Forsberg N, Whanger PD (1999) Selenoprotein W, selenium and glutathione peroxidase in rat and sheep brains and in brain cell cultures. Nutr Neurosci 2:227–237

Sun Y, Gu QP, Whanger PD (2001a) Selenoprotein W in overexpressed and underexpressed rat glial cells in culture. J Inorg Biochem 84(1–2):151–156

Sun Y, Butler JA, Whanger PD (2001b) Glutathione peroxidase activity and selenoprotein W levels in different brain regions of selenium-depleted rats. J Nutr Biochem 12(2):88–94

Vendeland SC, Beilstein MA, Chen CL, Jensen ON, Barofsky E, Whanger PD (1993) Purification and properties of selenoprotein W from rat muscle. J Biol Chem 268(23):17103–17107

Whanger PD (2000) Selenoprotein W: a review. Cell Mol Life Sci 57(13–14):1846–1852

Whanger PD (2001) Selenium and the brain: a review. Nutr Neurosci 4(2):81–97

Whanger PD (2009) Selenoprotein expression and function—selenoprotein W. Biochim Biophys Acta 1790(11):1448–1452

Xiao R, Qiao JT, Zhao HF, Liang J, Yu HL, Liu J et al (2006) Sodium selenite induces apoptosis in cultured cortical neurons with special concomitant changes in expression of the apoptosis-related genes. Neurotoxicology 27(4):478–484

Yeh JY, Beilstein MA, Andrews JS, Whanger PD (1995) Tissue distribution and influence of selenium status on levels of selenoprotein W. FASEB J 9(5):392–396

Yeh JY, Vendeland SC, Gu Q, Butler JA, Ou BR, Whanger PD (1997) Dietary selenium increases selenoprotein W levels in rat tissues. J Nutr 127(11):2165–2172

Zeng H, Combs GF Jr (2008) Selenium as an anticancer nutrient: roles in cell proliferation and tumor cell invasion. J Nutr Biochem 19(1):1–7

Zhang Y, Zhou Y, Schweizer U, Savaskan NE, Hua D, Kipnis J et al (2008) Comparative analysis of selenocysteine machinery and selenoproteome gene expression in mouse brain identifies neurons as key functional sites of selenium in mammals. J Biol Chem 283(4):2427–2438

Zhou JC, Zhao H, Li JG, Xia XJ, Wang KN, Zhang YJ et al (2009) Selenoprotein gene expression in thyroid and pituitary of young pigs is not affected by dietary selenium deficiency or excess. J Nutr 139(6):1061–1066