

Published in final edited form as:

Free Radic Biol Med. 2009 June 1; 46(11): 1527–1533. doi:10.1016/j.freeradbiomed.2009.03.008.

Organo-Selenium (Sel-Plex) decreases amyloid burden and RNA and DNA oxidative damage in APP/PS1 mice

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Abstract

To evaluate potential antioxidant characteristics of organic selenium (Se), double knock-in transgenic mice expressing human mutations for the amyloid precursor protein (APP) and human presenilin-1 (PS-1) were provided a Se-deficient diet, a Se-enriched (Sel-Plex), or a control diet from 4 to 9 months of age followed by a control diet until 12 months of age. Levels of DNA, RNA, and protein oxidation as well as lipid peroxidation markers were determined in all mice and amyloid beta peptide (A β) plaques were quantified. APP/PS1 mice provided Sel-Plex showed significantly ($P < 0.05$) lower levels of A β plaque deposition and significantly decreased levels of DNA and RNA oxidation. Sel-Plex-treated mice showed no significant differences in levels of lipid peroxidation or protein oxidation compared to APP/PS1 mice on a control diet. To determine if diminished oxidative damage was associated with increased antioxidant enzyme activities, brain glutathione peroxidase (GSH-Px), glutathione reductase, and glutathione transferase activities were measured. Sel-Plex-treated mice showed a modest but significant increase in GSH-Px activity compared to mice on a normal diet ($P < 0.5$). Overall, these data suggest that organic Se can reduce A β burden and minimize DNA and RNA oxidation and support a role for it as a potential therapeutic agent in neurologic disorders with increased oxidative stress.

Keywords

Alzheimer's disease; DNA oxidation; RNA oxidation; organo-selenium; antioxidants

Introduction

Oxidative damage to a variety of biomolecules has been implicated in the pathogenesis of several neurologic disorders including Alzheimer's disease (AD) (reviewed [1]). Free radicals generated by amyloid beta peptide (A β), a 40 or 42 amino acid peptide generated by gamma and beta secretase cleavage of the amyloid precursor protein (APP) may play a critical role in the oxidative damage associated with AD [2-4]. Because of its high oxygen consumption rate, high lipid content, and relatively limited antioxidant capacity compared to other organs, the

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brain is particularly susceptible to oxidative damage. Several studies show significantly increased oxidative damage to lipids, protein, DNA, and RNA in AD (Reviewed [1]). More recent studies suggest that oxidative damage associated with AD may begin during earlier stages of the disease including during amnesic mild cognitive impairment, the earliest clinical manifestation of AD [5]. To protect against oxidative damage, the brain employs a variety of small molecule antioxidants (vitamins E and C); enzymatic antioxidants including glutathione peroxidase (GSH-Px), a tetrameric cytosolic selenoprotein that catalyzes the reduction of organic hydroperoxides as well as H₂O₂ [6]; and thioredoxin reductase, a multifunctional selenoprotein that functions in the reduction of protein disulfides [7] and as a scavenger of reactive oxygen species [8].

Because selenium (Se) is an essential component of GSH-Px and thioredoxin reductase, there is considerable interest in the potential ability of Se and other dietary antioxidant supplements to modulate oxidative stress. Selenium, an essential trace element at low concentrations, has been shown to be a protective anti-carcinogenic [9]. In contrast, high concentrations of Se, particularly inorganic Se, may be genotoxic or carcinogenic [9], likely mediated through reactive oxygen species generation [10-13]. In addition, recent studies suggest that speciation of Se is also critical in determining whether it is toxic or protective [14]. Both organic (selenomethionine, selenocysteine, Se-methylselenocysteine) and inorganic (sodium selenite, sodium selenate) forms of Se are utilized by humans with similar efficiencies ultimately leading to production of selenoproteins [15]. Previous studies of sodium selenite show pretreatment of HT1000 cells with low concentrations of inorganic Se for 12 h decreased H₂O₂-induced apoptosis through activation of anti-apoptotic P13/Akt pathways and inhibition of the ASK1/JNK pathways [16]. Selenomethionine (SeMet), the major component of dietary Se, represents an organic form of Se that may be protective and provide enhanced antioxidant capacities [17]. Previous studies from our laboratory showed SeMet is protective against A β and iron/hydrogen peroxide-mediated neuron death and is associated with increased GSH-Px activities [18]. In addition to numerous studies of Se in cancer prevention, a large NIH funded long term study of the efficacy of Se in the prevention of AD is currently underway [19].

To determine if the *in vitro* effects of organic Se are observed in an *in vivo* model of A β deposition, we provided APP/presenilin 1 (PS1) mice a Se-deficient, a Se-supplemented (Sel-Plex, 1.07 \pm 0.04 μ g/g Se), or a normal control diet (0.3 μ g/g) from ages 4 to 9 months of age, a period during which A β production has been shown to occur but prior to extensive amyloid plaque deposition. The mice were then switched back to a normal control diet and maintained until 12 months of age. Quantification of amyloid burden was carried out using immunohistochemistry and Image J analysis. Levels of protein oxidation and lipid peroxidation were quantified using dot-blot immunochemistry. Oxidative modification of DNA and RNA was assayed using immunohistochemistry and confocal microscopy for 8-hydroxyguanine. To determine if Se supplementation increased antioxidant capacity, GSH-Px, glutathione reductase (GSSG-R) and glutathione transferase (GST) activities were analyzed.

Materials and methods

Animals

Double homozygous knock-in mice expressing a Swedish familial APP mutation and a humanized A β sequence and the 246L PS-1 mutation (APP/PS1) from Cephalon Inc. were used for these experiments. These animals develop amyloid plaques by 6 months of age [20] and show impairment of hippocampal long term potentiation [21]. In addition, deposition of A β in blood vessels is observed by 12 months of age and gradually increases. Alterations in A β solubility occur in APP/PS1 mice that closely resemble what is observed in AD. Thus, this model recapitulates many of the features of A β pathology in AD, including the deposition of

diffuse amyloid plaques early and subsequent neuritic amyloid plaque formation and vascular deposits.

Animal maintenance

For treatment, 4-month-old male APP/PS1 mice were randomized and placed on a Se-deficient diet ($0.1 \pm 0.1 \mu\text{g/g Se}$) ($n = 4$), a Se-supplemented diet ($1.0 \pm 0.1 \mu\text{g/g Se}$) ($n = 5$) (kindly provided by Alltech, Nicholasville, KY), or a normal control diet ($0.3 \mu\text{g/g Se}$) ($n = 3$) and maintained until 9 months of age. Because APP/PS1 mice begin to deposit amyloid plaques at 6 months of age we hypothesized that if Se can interrupt A β processing, it should be provided before the age at which definable amyloid plaques are deposited. The diet was continued until 9 months of age, a point at which amyloid deposition becomes more prominent. The ages of treatment were chosen to mimic the period during which changes in A β solubility may begin to occur in human AD subjects. The Se supplement was incorporated into the diet as a yeast extract prepared by growing yeast in Se-supplemented medium. The mice were maintained 2 per cage under 12 h light/dark cycles and provided food and water *ad libitum*. Animal care and treatment were in accordance with University of Kentucky Institutional Animal Care and Use Committee guidelines. At 9 months of age, the animals on Se-deficient or Se-supplemented diets were switched back to a normal control diet and maintained until 12 months of age. All animals were euthanized by Halothane overdose using procedures consistent with the Panel on Euthanasia of the American Veterinarian Association followed by cervical dislocation at 12 months of age. The brains were quickly removed and dissected into the two hemispheres. One hemisphere was placed in 4% phosphate buffered paraformaldehyde for immunohistochemical studies and the other was immediately frozen in liquid nitrogen for biochemical assays.

Quantification of amyloid burden

After paraformaldehyde fixation for 7 days, the left hemisphere was dissected into 3 segments and embedded in paraffin. Sections ($10 \mu\text{m}$) were cut using a Shandon Finesse microtome, placed on Plus slides, and immunostained for A β using a monoclonal antibody raised against A β_{17-34} (Vector Laboratories, Burlingame, CA) and standard protocols. A β quantification was carried out in a blinded fashion by taking micrographs of 3 sections of the whole half hemisphere per brain using a 4X objective on a Nikon Eclipse E60 microscope. If sections were larger than the field, multiple micrographs with well defined margins were taken to cover the entire area of the section. The micrographs were converted to gray scale and percent total section area covered by A β deposits quantified using Image J (NIH) software. For analysis, threshold staining was set to the same intensity for all sections and areas calculated. Slides were analyzed without knowledge of animal treatment. Results were computed as mean \pm SEM % total area occupied by amyloid plaques.

RNA and DNA oxidation

Levels of 8-hydroxyguanine (8-OHG), the predominant oxidative base adduct in DNA and RNA, were quantified using immunohistochemistry and confocal microscopy as previously described [22]. To visualize the cellular distribution of RNA oxidation, sections ($10 \mu\text{m}$) of paraffin-embedded cortex from APP/PS1 mice provided a control, Se-deficient, or Se1-Plex diet were cut using a Shandon Finesse microtome (Thermo Fisher Scientific, Waltham, MA), placed on Plus-slides, and rehydrated through xylene, descending alcohols, and water. Following rehydration sections were digested with $10 \mu\text{g/ml}$ proteinase K (Boehringer Mannheim, Indianapolis, IN) in phosphate buffered saline (PBS, pH 7.4) for 40 min at 37°C rinsed 3 times with Tris buffered saline (TBS; 150 mM tris-HCl , 150 mM NaCl , pH 7.6) and incubated with RNase free DNase-I ($10 \text{ U}/\mu\text{l}$ in PBS, Roche, Mannheim, Germany) for 2 hr at 37°C . The sections were rinsed 3 times in TBS and blocked in 10% normal goat serum in TBS

at room temperature for 2 h and incubated overnight in a 1:100 dilution of anti-8-OHG antibody (QED Biosciences, San Diego, CA) in 1.5% goat serum/TBS. Following thorough rinsing in TBS, sections were incubated in a 1:1000 dilution of Alexa-488 conjugated IgG/1.5% goat serum/TBS for 2 h at room temperature. After 5 rinses in TBS and distilled/deionized water, sections were coverslipped using fluorescent anti-fade (Molecular Probes) and imaged using a Leica DM IRBE confocal microscope equipped with argon, krypton and HeNe lasers and a 40X oil objective. Confocal images were captured from a single z plane without optical sectioning and were the average of 3 scans. Images were captured from 10 fields/section with an average of 10 to 20 cells per field without knowledge of animal treatment. Fluorescence intensities of all cells in each field were quantified using Leica image analysis software. Mean fluorescence intensities were calculated for 8-OHG for each slide.

To visualize DNA oxidation subsequent sections were digested with proteinase K as described above followed by digestion of RNA with DNase free RNase (10U/ml in PBS, Roche, Mannheim, Germany) for 2 h at 37° C. The sections were then labeled for 8-OHG and imaged as described above.

Tissue processing for enzyme analysis

Tissue samples for enzyme assays, lipid peroxidation, and protein carbonyl assays were homogenized using a chilled Dounce homogenizer on ice in 1 mL HEPES buffer (pH 7.4) containing 137 mM NaCl, 4.6 mM KCl, 1.1 mM KH₂PO₄, 0.6 mM MgSO₄, pepstatin A (1 μM), leupeptin (1.2 μM), aprotinin (0.1 μM) and PMSF (0.23 mM). Samples were centrifuged at 100,000 × g for 1 h at 4°C and the supernatant used for analyses. Total protein concentrations were determined using the Pierce BCA method (Sigma, St. Louis, MO).

4-Hydroxynonenal and acrolein assay

Dot-blot analyses of 4-hydroxynonenal (HNE) and acrolein modified proteins were carried out using a Schleicher & Schuell dot-blot apparatus and the method of Saiki et al [23] with modification. Briefly, 20 μg aliquots of 100,000 × g supernatant protein were loaded in triplicate onto nitrocellulose in a 40-50 μL volume. Vacuum was applied until the solution was evacuated from the wells. After air drying, blots were incubated overnight in 5% dry milk in 0.05% Tween-20/tris buffered saline (TTBS) to block nonspecific binding of the primary antibody. Blots were probed with a 1:2000 dilution of anti-HNE (Alpha Diagnostic, San Antonio, TX) or anti-acrolein (United States Biological, Swampscott, MA) polyclonal antibodies for 1 h at room temperature, washed in TTBS 3 × 10 minutes and then incubated with a 1:3000 dilution of horseradish peroxidase-conjugated goat anti-rabbit antibody (Jackson ImmunoResearch, West Grove, PA) for 1 h [24]. Detection of bound antibodies was by enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) and exposure of the membrane to Hyperfilm (Amersham). Dots were quantified using Scion Image (NIH). The average of triplicate dots indicates protein bound HNE or acrolein content in an individual sample. Our previous study showed a linear response of immunostaining for protein samples incubated with increasing HNE or acrolein concentrations [24]. Protein carbonyls were determined using an oxy-blot kit (Chemicon, Temecula, CA) per manufacturer's instructions. Briefly, 5 μg protein samples were derivatized with 10 mM 2,4-dinitrophenylhydrazine (DNPH) in the presence of 5 μL of 12% sodium dodecyl sulfate for 20 min at room temperature. The samples were then neutralized with 7.5 μL of the neutralization solution (2 M Tris in 30% glycerol) and blotted onto a nitrocellulose membrane using a Schleicher and Schull dot blot assembly. The membrane was washed with 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20 (wash buffer) and blocked by incubation with 5% bovine serum albumin/wash buffer, followed by incubation with rabbit polyclonal anti-DNPH antibody (1:100 dilution) for 1 h. The membranes were washed 3 to 5 times with wash buffer and incubated with horse radish peroxidase-conjugated goat anti-rabbit antibody/TTBS for 1 h. Blots were developed using

enhanced chemiluminescence per manufacturer's instruction and staining intensity quantified using Scion Image.

Enzyme activity assays

The method for assay of total superoxide dismutase (SOD) (Cu/Zn- and MnSOD) activity is that of Misra and Fridovich [25] as modified by Mizuno [26]. Briefly, 70- μ L aliquots of the 100,000 \times g supernatant were placed in UV-grade cuvettes with 1.46 mL 68mM NaH₂PO₄ containing 1.35 mM EDTA (pH 7.8), 100 μ L 4 mM xanthine and 170 μ L 3.53 mM epinephrine (pH 11.5). Following incubation at 30°C for 5 min, 10 μ L xanthine oxidase (Calbiochem, San Diego, CA) was added to the solution and the absorbance followed at 320 nm (Genesys 10 UV, ThermoSpectronic, Waltham, MA) for 3 minutes. The blank for the assay contained 70 μ L tissue supernatant boiled in water for 5 minutes. For MnSOD, the same protocol was followed with the addition of 200 μ L 20 mM KCN. Enzyme activities are expressed as units per microgram protein, where 1 unit reduces the absorbance change by 50%. Activities are expressed as mean \pm SEM percent of control activity. Cu/Zn activity was obtained by subtracting MnSOD activity from total activity.

For GSH-Px activity, the method of Paglia and Valentine [27] as modified by Mizuno [26] was followed, which measures the rate of oxidation of reduced glutathione to oxidized glutathione by H₂O₂ as catalyzed by GSH-Px present in the tissue supernatant. For the assay, 100 μ L aliquots of the 100,000 \times g supernatant were added to 1.68 mL 68 mM KH₂PO₄ buffer (pH 7.0) containing 1mM EDTA, 100 μ L 2 mM NADPH, 10 μ L 66 U/mL GSSG reductase and 10 μ L 200 mM sodium azide. The assay was initiated with 100 μ L 15 mM H₂O₂. The decrease in absorbance was followed for 2 minutes at 340 nm. Quantification was based on a molar absorption coefficient of 6,270 M⁻¹ cm⁻¹ for NADPH. The blank for the enzyme assay consisted of 100 μ L supernatant heated at 100 °C for 5 min, and its value was subtracted from the sample value to correct for nonenzymatic oxidation of glutathione. Activity of the enzyme is expressed in units/ μ g total protein, with 1 unit = 1 nmol NADPH oxidized per minute, and final data for activity is expressed as mean \pm SD percentage of control.

GST activity was assayed using the method of Ricci et al [28], and measures the rate of conjugation of 7-chloro-4-nitrobenzo-2-oxa-1, 3-diazole (NBD-Cl) with glutathione catalyzed by glutathione transferase. The NBD-Cl complex is a stable, yellow compound that strongly absorbs at 419 nm with a molar absorption coefficient of 14.5 mM⁻¹cm⁻¹. For the assay, 1.6 mL of 0.1 M sodium acetate buffer (pH 5.0) was mixed with 200 μ L 0.2 mM NBD-Cl and 100 μ L 0.5 mM reduced glutathione. The reaction was initiated by addition of 100 μ L aliquots of the 100,000 \times g tissue supernatant. The absorbance was followed for 3 min. Enzyme activity was expressed in units/ μ g total protein, with 1 unit = 1 nmol NBD-Cl complex formed per minute. Results are expressed as mean \pm SD percentage of control.

Statistical analysis

Two-way ANOVA and Dunnett's *post hoc* test were used for differences in enzyme activity and oxidative stress markers using the commercially available ABSTAT (AndersonBell Corp, Arvada, CO) software. Significant differences were set at $P < 0.05$.

Results

Amyloid burden

Consistent with previous studies [21] immunostaining of sections of cortex for amyloid beta peptide showed the presence of numerous amyloid plaques throughout the cortex of animals maintained on a normal diet (Figure 1A, 2). In contrast, quantification of A β burden in the cortex of animals maintained on the Sel-Plex diet showed a significant ($P < 0.05$) decrease in

the volume occupied by A β -positive amyloid plaques (Figures 1B, 2) compared to animals maintained on normal control or Se-deficient diets. Comparison of Se-deficient animals to those maintained on a normal control diet showed no significant differences in amyloid burden.

Lipid peroxidation and protein oxidation

Quantification of levels of lipid peroxidation as demonstrated by protein bound HNE and acrolein showed no significant differences between any of the three treatment groups, although mice on a Se-deficient diet demonstrated a modest but statistically insignificant increase in HNE (Figure 3). Quantification of levels of oxidized protein showed a modest decrease in mice maintained on the Sel-Plex diet, although the difference was not statistically significant. In contrast, levels of oxidized protein were significantly ($P < 0.05$) elevated (~40%) in APP/PS1 mice maintained on a Se-deficient diet (Fig. 3).

DNA and RNA oxidation

To quantify levels of RNA and DNA oxidation, sections of cortex were subjected to DNase free RNase treatment for DNA analysis or RNase free DNase for RNA analysis and immunostained for 8-OHG, the predominant oxidative adduct resulting from hydroxyl radical attack of DNA and RNA. Figure 4 shows representative confocal micrographs of 8-OHG immunostaining in DNA (A) or RNA (B). Quantification of immunostaining intensity shows animals maintained on the Sel-Plex diet had significantly less DNA oxidation ($66.9 \pm 6.2\%$ APP/PS1 mice on a control diet) compared to APP/PS1 mice maintained on a control diet or Se-deficient diet (Fig. 5). Similarly, levels of 8-OHG in RNA were significantly lower ($45.6 \pm 10.6\%$) in animals provided the Sel-Plex enriched diet compared to animals on a normal control diet. There were no significant differences in 8-OHG levels in RNA of animals of a Se-deficient diet compared to animals provided a normal control diet.

Antioxidant enzymes

To determine if Se supplementation alters antioxidant enzyme capacity, activities of GSH-Px and activities of GSSG-R, were determined. Figure 6 shows Se supplementation did not significantly alter GSSG-R or GST activities. However, animals provided the Sel-Plex diet showed a modest but statistically significant increase in GSH-Px activity. In a surprising finding, GSH-Px activities were also significantly increased in animals provided a Se-deficient diet.

Discussion

Multiple studies show significant alterations of markers of oxidative stress including HNE and acrolein (markers of lipid peroxidation), protein oxidation, RNA and DNA oxidation in the brain in AD including subjects early in disease progression (MCI) (reviewed reviewed [1]). Oxidative stress, possibly mediated by A β , has been suggested to be central to the pathogenesis of AD [3,29]. One of the goals of preventive or early treatment of AD is to decrease oxidative stress.

The present study used the APP-PS1 knock-in mouse as a model of amyloid plaque formation that has some similarities to those in AD. Our study shows that Sel-Plex-treated animals have significantly decreased RNA and DNA oxidative damage compared to mice on a normal diet or a Se-deficient diet. An increase in GSH-Px activity also was found in animals treated with Sel-Plex compared to mice on a normal diet. In addition, these oxidative and antioxidant changes were accompanied by a significant decrease in amyloid burden in the Sel-Plex- treated animals.

In addition to the effect on nucleic acid oxidation, animals fed Sel-Plex showed a 20% decrease in HNE and a 15% decrease in protein oxidation, although the differences were not statistically significant for either compared to animals on a normal diet. This suggests that Sel-Plex causes a generalized decrease in oxidative damage, which is most pronounced in DNA and RNA.

Our data showing pronounced levels of RNA and DNA oxidation in APP/PS1 mice are consistent with multiple studies of RNA and DNA oxidation in subjects with a variety of neurological disorders including late AD (LAD) (reviewed [30]). In studies of RNA oxidation, Nunomura et al. [31] showed significantly increased cytoplasmic staining of 8-OHG in early AD brain that decreased as amyloid beta peptide and neurofibrillary tangle burden increased. Increased RNA oxidation has also been observed in a presymptomatic subject who expressed a familial AD mutation [32] and Down syndrome subjects with early AD pathology [33] leading to the suggestion that RNA oxidation is an early event in the pathogenesis of AD. Studies of DNA oxidation in the progression of AD show similar findings with significantly increased levels of 8-OHG in nuclear and mitochondrial DNA from subjects with MCI and LAD (reviewed [1]).

Selenium, an essential trace element is a critical component in 25 selenoproteins [34] including iodothyronine deiodinases [35-38] selenoprotein P [39], seleno synthetases [40] and the antioxidant enzymes thioredoxin reductase [40-45] and glutathione peroxidases [6,43,46-50]. Dietary selenium is taken up from soil by plants and enters the human food chain as inorganic Se (sodium selenate or sodium selenite) that is converted to organoselenium (selenomethionine, selenocysteine and selenium methylselenocysteine) by animals [51]. Recommended daily allowances for Se are 50 – 70 µg/day for healthy adults [52,53] with deficiencies occurring with intakes less than 11 µg/day and chemical selenosis occurring at intakes between 3200 and 5000 µg/day [54]. Selenomethionine and methionine are equally recognized during protein synthesis leading to incorporation of Se into proteins [15]. Selenomethionine may also be converted to selenocysteine by trans esterification ultimately leading to generation of H₂Se, the key intermediate between reductive metabolism of Se and its methylation pathway and serves as a precursor for synthesis of selenocysteine [14,55]. Selenomethionine may also be processed by methionine α,β lyase to generate methyl selenium [55].

Selenocysteine from dietary sources or converted from selenomethionine is converted to H₂Se that may be reconverted to selenocysteine following conversion of H₂Se to selenophosphate by selenophosphate synthetase [51]. Insertion of selenocysteine into proteins is initiated by the UGA codon in mRNA and forms the catalytic site of many selenoproteins [56].

Our previous study using cultured fetal rodent neurons showed that SeMet significantly decreased ROS generation against Aβ and iron/hydrogen peroxide and significantly increased GSH-Px activity. Others have shown that ebselen (2-phenyl-1, 2-benzisoxanazol-3(2H)-one), a Se-containing heterocyclic compound, prevents nitric oxide-induced apoptosis of differentiated PC-12 cells by blocking activation of ASK1 and inhibition of p38 mitogen-activated protein kinase and JNK. Ebselen also appears to act as a GSH-Px mimetic. Studies of focal cerebral ischemia in rodents showed ebselen significantly decreases levels of HNE and levels of 8-OHG [57]. Another study showed that treatment of primary human keratinocytes with SeMet or sodium selenite protected against UV-induced oxidative damage [58]. These studies, our recent study, and the present study suggest that Se modulates oxidative stress *in vivo* and *in vitro*.

The most pronounced effect of Sel-Plex in our study was on DNA and RNA. Levels of 8-OHG were significantly decreased in both DNA and RNA in Sel-Plex fed animals compared to those

on normal diets or Se-deficient diets. Although the reason for the decrease in DNA oxidation is not clear, it could be due to an increase in base excision repair as previously observed by Seo et al. in *in-vitro* studies [59]. We can only speculate that the decrease in RNA oxidation could relate to the general decrease of oxidative stress in the model.

Our finding of significantly decreased amyloid plaque in Sel-Plex treated animals compared to controls is of considerable interest. The role of Se in decreasing A β plaque formation is not clear. Previous studies show treatment of Chinese hamster ovary cells with Se decreased gamma secretase activity by ~50% [60]. In addition, Se alters levels of AP-1, a transcription factor associated with APP transcription, and induces prolonged stimulation of ERK leading to reduced A β secretion [61]. Although insufficient tissue was available in the current study to measure AP-1 or ERK levels, it is quite possible that a combination of these effects led to the decrease of A β plaque deposition that was observed.

Overall our data indicate that supplementation of low levels of organic Se leads to decreased oxidative damage to RNA and DNA in a transgenic mouse model of A β . This suggests that Se could be a neuroprotective agent possibly useful in prevent.

Acknowledgements

Supported by NIH grants 5-P01-AG05119 and 5-P30-AG028383 and by grants from the Abercrombie Foundation and the Healey Family Foundation. The authors thank Ms. Paula Thomason for technical and editorial assistance and Alltech Biotechnology for providing the Sel-Plex diet.

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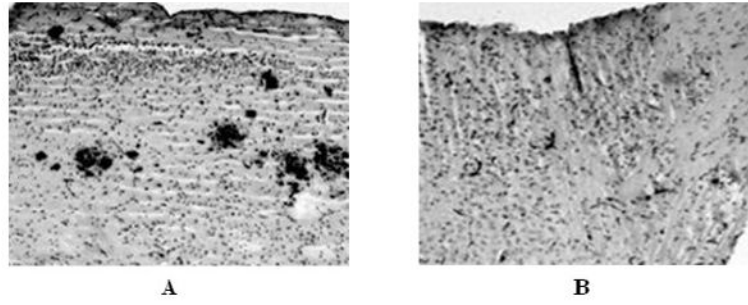


Fig. 1. Representative micrographs of APP/PS1 mice provided a normal diet (A) or a Se-supplemented diet (Sel-Plex) (B) immunostained for amyloid beta peptide. Note a significant decrease in A β burden in APP/PS1 mice provided the Sel-Plex diet.

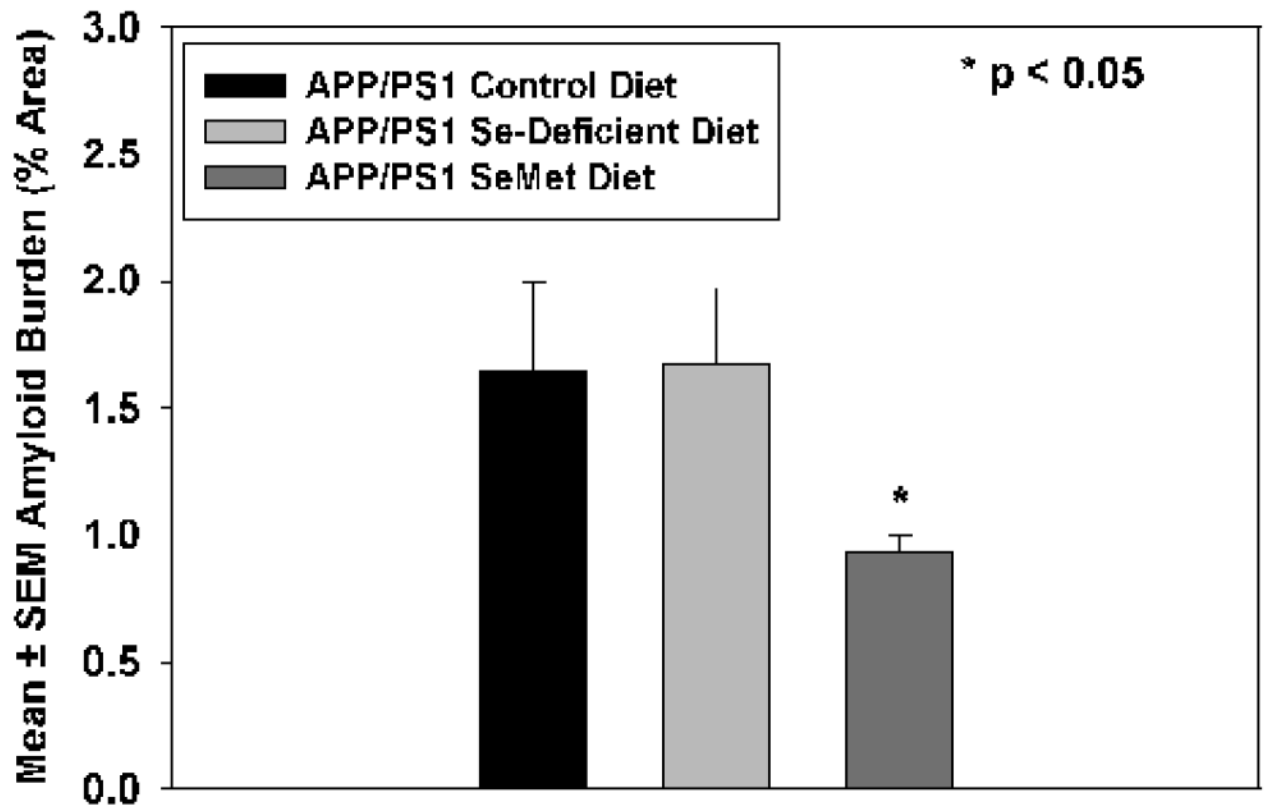


Fig. 2. Mean \pm SEM amyloid plaque area (% total area) in APP/PS1 mice provided a normal control diet, a Se-deficient diet, or the Sel-Plex diet. The A β plaque area was significantly decreased ($P < 0.05$) in mice provided Sel-Plex.

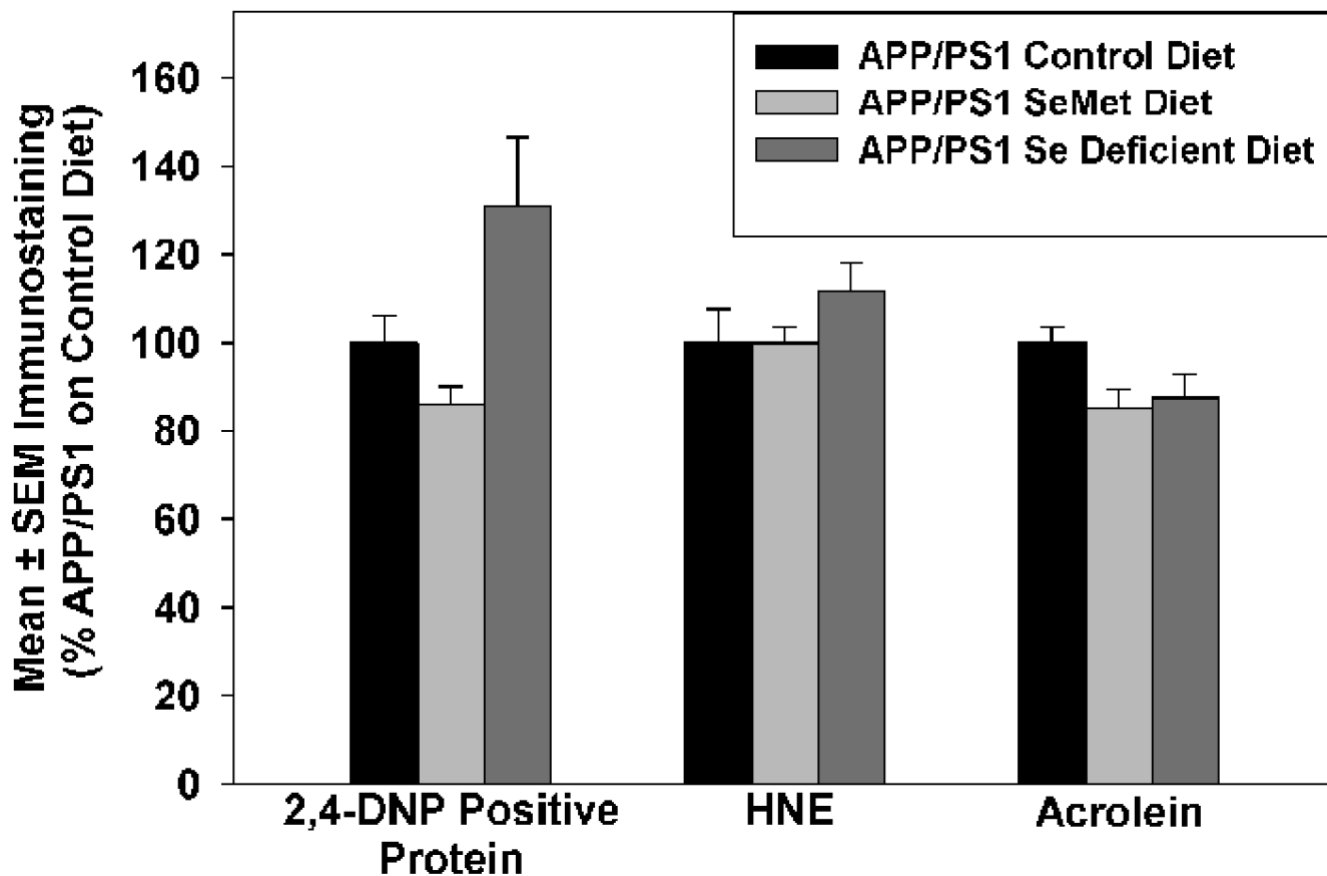


Fig. 3. Mean \pm SEM protein-bound HNE and acrolein and oxidized protein in mice provided a normal control, Se-deficient, or Sel-Plex diet. Levels of protein-bound HNE or acrolein were not significantly different between any of the three groups.

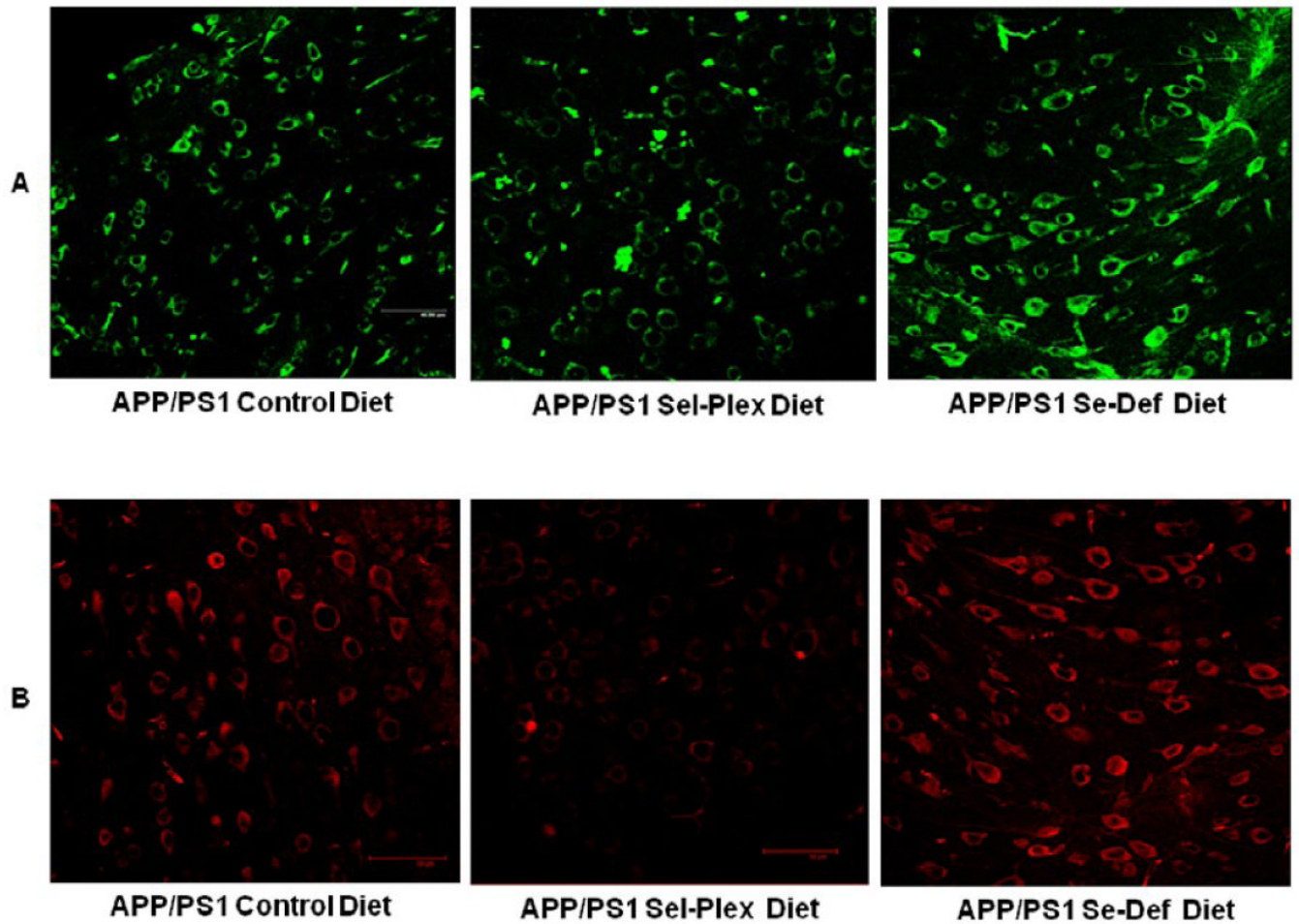


Fig. 4. Representative confocal micrographs of APP/PS1 mice provided control, Sel-Plex supplemented, or Se-deficient diets immunostained for 8-hydroxyguanine in DNA (A) or RNA (B). Scale bar = 50 μ m. Note the pronounced decrease of 8-OHG immunostaining in both RNA and DNA of mice provided Sel-Plex.

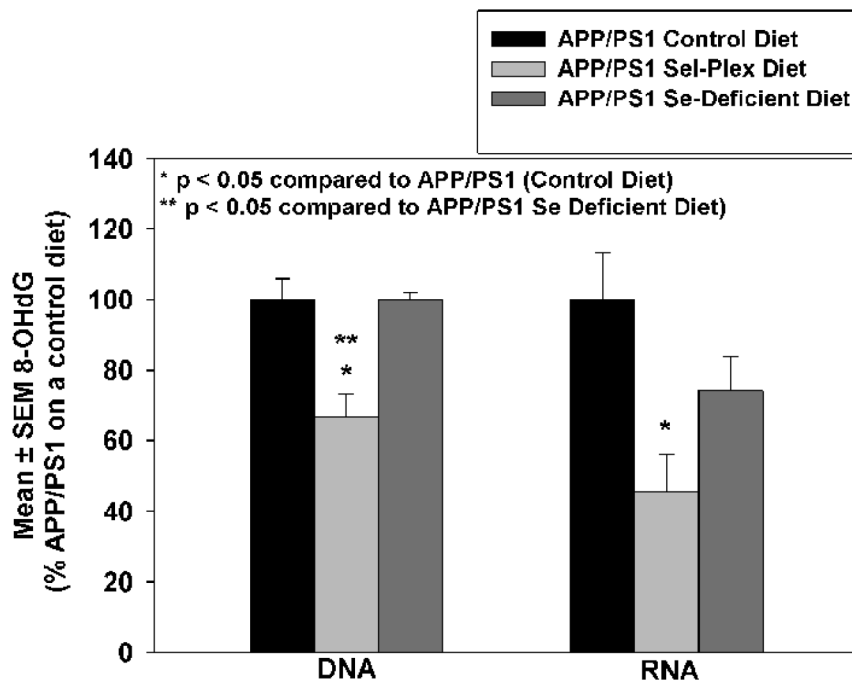


Fig. 5. Mean ± SEM 8-OHG immunostaining (% APP/PS1 mice on control diet) in DNA and RNA. Note a significant decrease of 8-OHG immunostaining in DNA and RNA of mice provided Sel-Plex ($P < 0.05$).

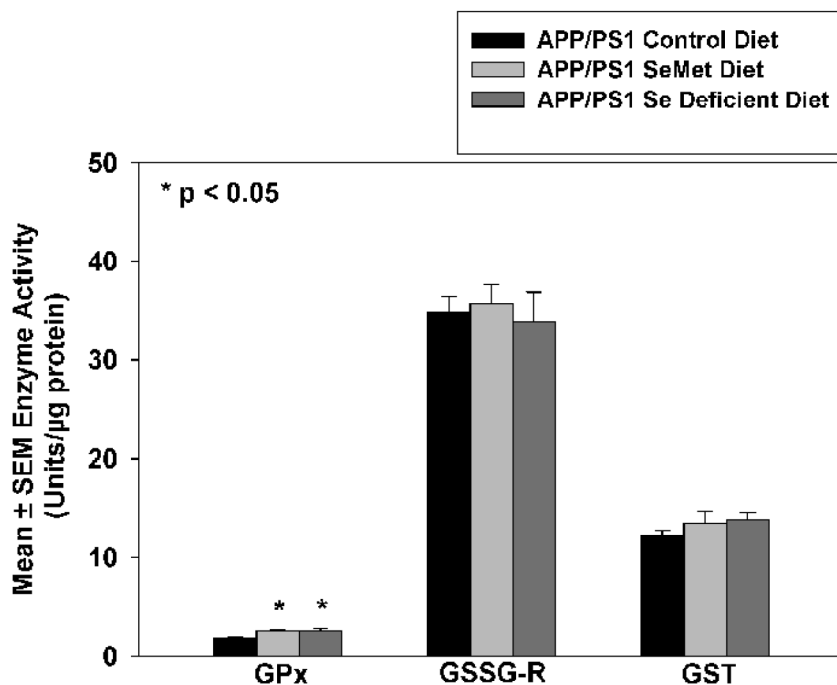


Fig. 6. Mean ± SEM activities of glutathione peroxidase (GSH-Px), glutathione reductase (GSSG-R) and glutathione transferase (GST) of mice provided a control, Sel-Plex, or Se-deficient diet. GSH-Px activity in Sel-Plex treated mice was increased modestly yet significantly ($P < 0.05$)