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Original Research Communication

Seleno-L-Methionine Protects Against β-Amyloid and Iron/Hydrogen Peroxide-Mediated Neuron Death

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ABSTRACT

Increasing evidence suggests a role for oxidative stress in several neurodegenerative diseases, including Alzheimer's disease (AD), and that selenium compounds may function as antioxidants. To evaluate the antioxidant mechanism of selenium, primary rat hippocampal neurons were pretreated with seleno-L-methionine (SeMet) for 16 h prior to treatment with iron/hydrogen peroxide (Fe^{2+}/H_2O_2) or amyloid beta peptide ($A\beta_{25-35}$); free radical generation was assessed using laser confocal microscopy and CM-H_2DCFDA and APF. Treatment with Fe^{2+}/H_2O_2 or Aβ significantly decreased cell survival and increased free radical generation compared to cultures treated with vehicle alone. In contrast, cultures pretreated with SeMet showed significantly ($p < 0.05$) increased survival and significantly lower CM-H_2DCFDA and APF fluorescence compared to Fe^{2+}/H_2O_2 or Aβ treated cultures. To determine if SeMet protection was mediated by glutathione peroxidase (GPx), levels of GPx protein and activity were measured using confocal microscopy and a selenium-dependent GPx specific antibody and an activity assay. Pretreatment with SeMet significantly ($p < 0.05$) increased GPx protein and activity in Fe^{2+}/H_2O_2- and Aβ-treated cultures compared to cultures treated with Fe^{2+}/H_2O_2 or Aβ alone. These data suggest that SeMet can decrease free radical generation induced by Fe^{2+}/H_2O_2 or Aβ through modulation of GPx and may be suitable as a potential therapeutic agent in neurodegenerative diseases where there is increased oxidative stress. *Antioxid. Redox Signal.* 9, 457–467.

INTRODUCTION

Oxidative stress has been implicated in several neurodegenerative diseases, including Alzheimer's disease (AD)(7). Amyloid β peptide (Aβ) has been identified as central to the development of AD, and iron/hydrogen peroxide (Fe^{2+}/H_2O_2) may also play a critical role in oxidative stress in AD (3, 27, 31). In aging brain, basal production of reactive oxygen species (ROS) is increased and antioxidant capacity is decreased (4, 23). Critical antioxidants in brain include glutathione and glutathione peroxidase (GPx), a cytosolic, tetrameric selenoenzyme that catalyzes the reduction of organic hydroperoxides (16).

Currently, selenium and other dietary antioxidant supplements are suggested to help protect against ROS that can cause lipid peroxidation, and protein and DNA oxidation (38). Selenium, an essential trace element, has shown benefits in human health and functions as an anticancer agent in epidemiologic and in *in vitro* studies (41). Previous studies of the effects of sodium selenite show pretreatment of HT1080 cells with low concentrations of the inorganic form of selenium for 12 h decreased H_2O_2-induced apoptosis through activation of the antiapoptotic PI3-K/Akt pathway and inhibition of the ASK1/JNK pathways (54). Selenomethionine (SeMet), the major component of dietary selenium, represents an organic form of selenium that is more bioavailable than inorganic forms (11) and may provide enhanced protection. Previous studies show SeMet exerts cancer chemopreventive properties by regulating p53 (46) and ERK (17). SeMet is also shown to increase GPx activity in cardiomy-

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ocytes (38). Although selenium is associated with antioxidant effects, the direct association between selenium and AD is not well established. In the present study, we measured ROS production in primary rat hippocampal neuron cultures treated with AD relevant insults (Aβ or Fe^{2+}/H_2O_2) using 5-(and 6-) chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester (CM-H_2DCFDA) or 3′-(p-aminophenyl)fluorescein (APF) and confocal microscopy. We then determined the response of the antioxidant defense system by pretreating cultures with SeMet prior to Aβ or Fe^{2+}/H_2O_2 treatment and measuring GPx protein levels and activity using immunohistochemistry and confocal microscopy and enzyme activity assays.

MATERIALS AND METHODS

Primary rat hippocampal and cortical neuron cultures

Primary hippocampal and cortical neurons were prepared using the methods of Xie *et al*. (29, 53) with modification. Briefly, hippocampi were obtained from E-18-rat embryos and incubated for 15–20 min in a solution of 2 mg/ml trypsin (Worthington Biochemical Corp., Lakewood, NJ) per ml of Hank's balanced salt solution (HBSS) (Gibco, Grand Island, NY) containing 2.4 g/L HEPES and 10 mg/L gentamicin, pH 7.2, at a concentration of 2 hippocampi/ml. The cortices were incubated in 0.8 mg trypsin/ml HBSS for 7 min. The hippocampi and cortices were rinsed three times in 10 ml HBSS, followed by a 5-min incubation in a solution of 1 mg trypsin inhibitor/ml of HBSS, and finally rinsed three times with 10 ml HBSS. Cells were dissociated and distributed to 35 mm (hippocampal) and 60 mm (cortical), polyethyleniminecoated plastic culture dishes containing 1 ml (35 mm dish) and 2 ml (60 mm dish) of MEM+. The dissociated neurons were plated at a density of ~80–100 neurons/mm^2 of growth substrate. After 4 h incubation at 37°C in humidified 6%CO_2/94% room air, MEM+ was replaced with 50% MEM+ and 50% neurobasal medium prepared with B-27 supplement (Gibco) and 0.5 m*M* L-glutamine. On day three, 5′-fluoro-2-deoxyuridine and uridine (Sigma Chemical, St. Louis, MO; 75 and 35 μg in 1 ml medium, respectively) were added to block glial proliferation (6). Experiments were carried out on cells 7 days in culture.

Cell viability study

Cell viability was assessed by counting the number of hippocampal neurons in premarked microscopic fields before treatment and at each time point as previously described (53) or by measuring lactate dehydrogenase (LDH) release into the medium, as described by Koh and Choi (22).

To determine optimum concentrations of SeMet, hippocampal cultures were switched to Locke's solution (154 m*M* NaCl, 5.6 m*M* KCl, 2.3 m*M* $CaCl_2$, 1.0 m*M* $MgCl_2$, 3.6 m*M* $NaHCO_3$, 10 m*M* glucose, and 5 m*M* HEPES, pH 7.2) and incubated with 100 n*M*, 500 n*M*, 750 n*M*, 1 μ*M*, 2 μ*M*, or 5 μ*M* SeMet (Alltech, Nicholasville, KY) alone or in combination with 10 μ*M* Fe^{2+}/30 μ*M* H_2O_2 or 25 μM Aβ for 16 h. Cell survival was quantified by counting undamaged neurons in premarked microscopic fields at 0 and 16 h in a blinded fashion or by measuring LDH release. Cells were identified as damaged by the presence of dendriditic beading and vacuolization of the cell body. Concentrations of Aβ and Fe^{2+}/H_2O_2 were based on previous studies (27) from our laboratory. After identification of SeMet concentrations that provided optimal protection at 16 h, hippocampal neurons were divided into 5 groups ($n = 3$): (a) controls treated with vehicle only; (b) cells treated with 20 μ*M* Aβ (Bachem, Torrance, CA); (c) 20 μ*M* Aβ/1 μ*M* SeMet; (d) 10 μ*M* Fe^{2+}/30 μ*M* H_2O_2; or (e) 10 μ*M* Fe^{2+}/30 μ*M* H_2O_2/1 μ*M* SeMet to determine if SeMet significantly protected against ROS production by Aβ or Fe^{2+}/H_2O_2. For concomitant treatment with SeMet and insult, cultures were pretreated for 16 h with SeMet in original MEM+ medium. The culture medium was then switched to Locke's solution and Aβ or Fe^{2+}/H_2O_2 added along with 1 μ*M* SeMet. Cell counts and LDH measurements were made at 3, 6, 9, and 16 h following addition of treatments. Based on cell viability measures, a 6 h treatment time was chosen for quantification of ROS and glutathione peroxidase activity and protein.

Quantification of reactive oxygen species

Hippocampal neurons were divided into 5 groups and treated for 6 h, as described above. ROS generation in cells was assessed using the fluorescent probes CM-H_2DCFDA (Molecular Probes, Eugene, OR) that interacts with a variety of radicals and APF (Molecular Probes), a cell permeable fluorescent probe that interacts specifically with hydroxyl radical and hypochlorite anion. After 5 h, 5 μ*M* CM-H_2DCFDA or 10 μ*M* APF were added for the last hour of treatment and dishes were washed three times with PBS and immediately analyzed using a Leica DM IRBE confocal microscope equipped with argon, krypton, and HeNe lasers and a 40X oil objective. The excitation wavelength was 488 nm for both CM-H_2DCFDA and APF. Images were captured from a single z plane without optical sectioning from 10–15 fields/dish and fluorescent intensity was quantified using Leica Confocal Software. One hundred cells were quantified from each dish and the mean calculated for each dish. The data represent the mean ± SEM % control CM-H_2DCFDA or APF fluorescence intensity.

Immunohistochemistry and confocal microscopy

To examine levels of GPx-1 protein, hippocampal cultures were divided into 5 groups and treated as described above. After 6 h treatment, cells were rinsed three times with PBS and fixed 20 min with 4% paraformaldehyde in PBS. Cultures were incubated in 0.25% Triton X-100 for 30 min and blocked with 10% horse serum in PBS for another 30 min. Cultures were incubated for 2 h in anti-GPx-1 (1:500, Frontier, Seoul, Korea), followed by three washes with PBS. The cultures were then incubated with goat anti-mouse Alexa 633 labeled IgG (Molecular Probes) for 1 h. Following three washes with PBS, dishes were immediately analyzed using confocal microscopy and fluorescent intensity quantified using Leica software. One hundred cells were quantified from each dish ($n = 3$) and averaged to ob-

tain a value for each dish. Data represent the mean ± SEM % control GPx intensity.

GPx activity assay

Because of the amount of protein required for analysis of GPx activity, primary rat cortical neuron cultures were treated for 6 h with 25 μ*M* Aβ or 20 μ*M* Fe^{2+}/60 μ*M* H_2O_2 alone or in combination with SeMet, as described above. Because of differences in neuron density, slightly higher concentrations of insult were required to induce the same degree of cell death as observed for hippocampal cultures. Cortical neuron viability was assessed by LDH release and showed similar cell death as observed for hippocampal neurons. GPx activity assays were carried out as previously described by following the rate of oxidation of reduced glutathione to oxidized glutathione by H_2O_2 as catalyzed by GPx in the culture supernatant (32, 39). Cells were divided into 5 groups ($n = 6$) and treated as described above. After 6 h incubation, cells were washed three times with ice-cold PBS and scraped into 1 ml HEPES buffer (pH 7.4, containing 137 m*M* NaCl, 4.6 m*M* KCl, 1.1 m*M* KH_2PO_4, 0.6 m*M* $MgSO_4$, 0.7 μg/ml pepstatin A, 0.5 μg/ml leupeptin, 0.5 μg/ml aprotinin, and 40 μg/ml PMSF), homogenized using a micro Dounce homogenizer, and centrifuged at 100,000 *g* for 45 min at 4°C. The supernatant was freeze-dried overnight, and dissolved in 350 μl HEPES buffer. Protein concentrations were determined using the Pierce BCA method. For the assay, 100 μl aliquots of protein were added to 1.68 ml 68 m*M* KH_2PO_4 buffer (pH 7.0) containing 1 m*M* EDTA, 100 μl 2 m*M* NADPH, 10 μl 66 U/ml GSSG reductase, 10 μl 200 m*M* sodium azide. The assay was initiated with 100 μl 15 m*M* H_2O_2 and absorbance measured at 340 nm using a ThermoSpectronic UV-visable spectrometer. The decrease in absorbance due to consumption of NADPH was followed for 2 min at 340 nm. Quantification was based on a molar absorption coefficient of 6,270 M^{-1} cm^{-1} for NADPH (5). Activity of the enzyme was expressed in units/μg total protein, with 1 unit = 1 nmol NADPH oxidized per min. The data are presented as mean ± SEM % of control activity.

Statistical analysis

One-way ANOVA and Student's *t* tests were used to compare treatment-dependent differences using commercially available ABSTAT (AndersonBell, Arvada, CO) and SAS (Cary, NC) software packages. Significant differences were for $p < 0.05$.

RESULTS

To select the optimally protective dose of SeMet for neuron treatment, primary hippocampal neurons were treated with 100 n*M*, 500 n*M*, 750 n*M*, 1 μ*M*, 2 μ*M,* and 5 μ*M* SeMet alone or in combination with 10 μ*M* Fe^{2+}/30 μ*M* H_2O_2 or 20 μ*M* Aβ for 16 h and cell viability measured by blinded cell counts or LDH release. Figures 1 and 2 show there were no significant differences in survival between cultures treated with vehicle and those treated with any concentration of SeMet. Figure 1 shows 100, 500*,* and 750 n*M* SeMet did not significantly increase cell survival compared to cultures treated with Fe^{2+}/H_2O_2 alone. In contrast, treatment with SeMet at 1, 2, or 5 μ*M* in combination with Fe^{2+}/H_2O_2 led to significant ($p < 0.05$) increases in neuron survival compared to Fe^{2+}/H_2O_2 treatment alone, although 5 μ*M* SeMet protec-

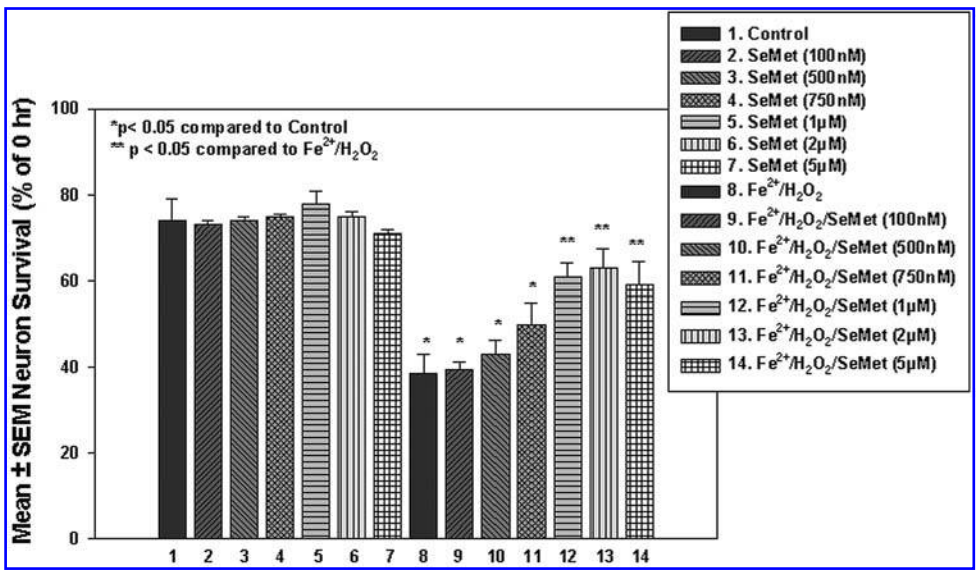


FIG. 1. Neuron viability (cell counts) for primary rat hippocampal cultures treated for 16 h with 10 μ*M* Fe^{2+}/30 μ*M* H_2O_2 alone or pretreated with SeMet. Data represent the mean ± SEM % initial number from three separate experiments and three replicates for each experiment, with a total of 400–600 cells counted for each treatment. *$p < 0.05$ compared to control, **$p < 0.05$ compared to Fe^{2+}/ H_2O_2 treatment. Correlation analysis of cell counts and cell viability measured by LDH release showed statistically significant positive relationships between the two independent measures of cell viability.

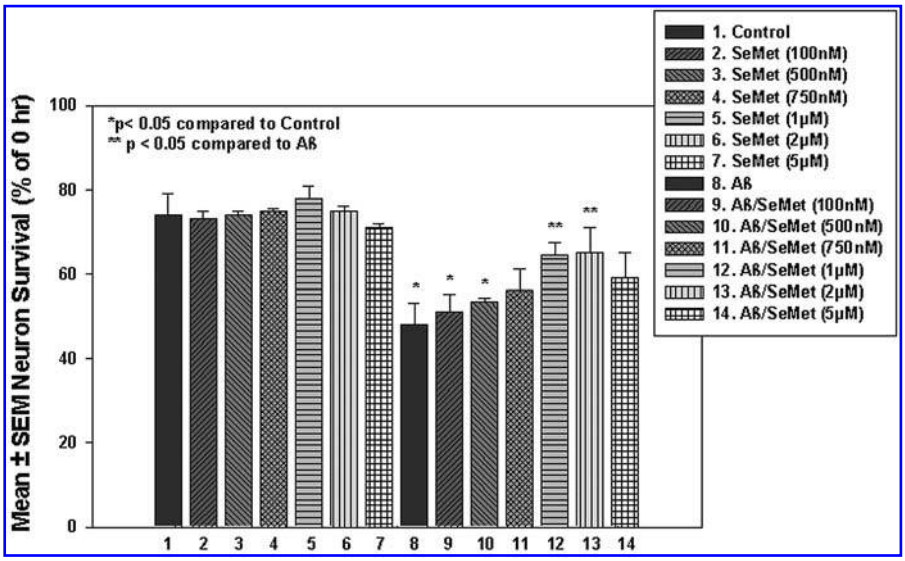
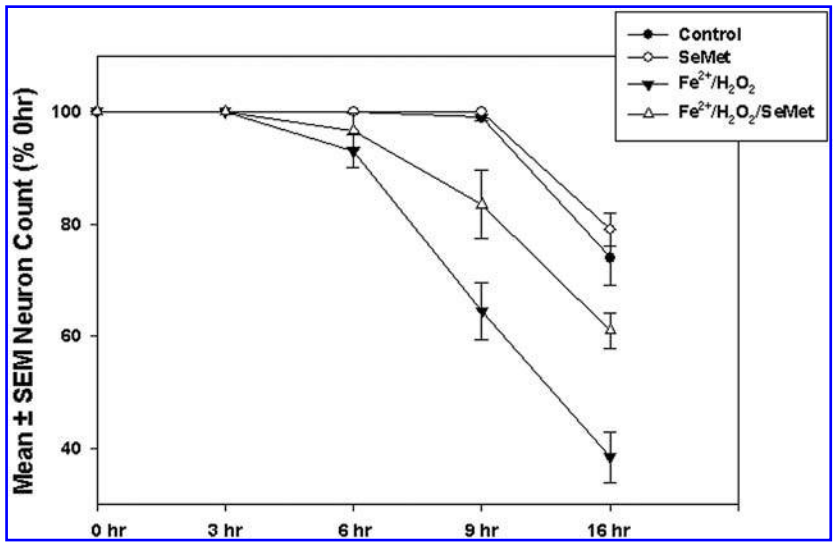


FIG. 2. Hippocampal neuron viability for cultures treated for 16 h with 20 µ*M* Aβ alone or pretreated with SeMet. Data represent mean ± SEM % initial number from three separate experiments and three replicates for each experiment, with a total of 400–600 cells counted for each treatment. $^{*}p < 0.05$ compared to control, $^{**}p < 0.05$ compared to Aβ treatment. Correlation analysis of cell counts and cell viability measured by LDH release showed statistically significant positive relationships between the two independent measures of cell viability.

A



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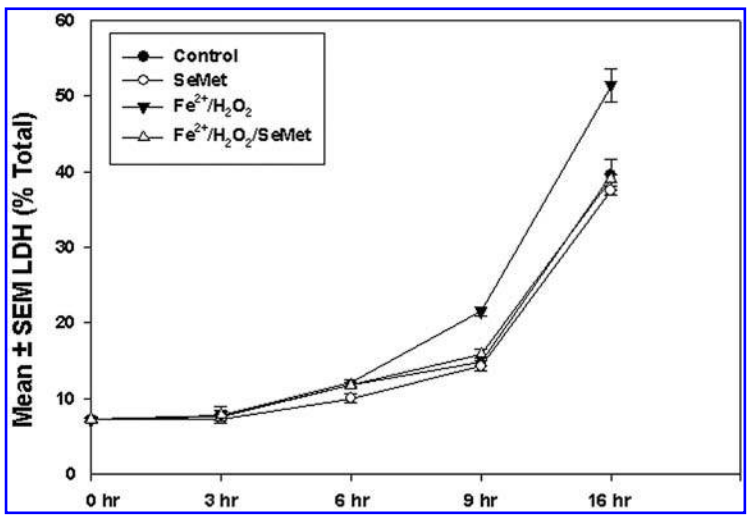


FIG. 3. Time dependent decrease in cell count of primary hippocampal neurons treated with 10 µ*M* Fe^{2+}/30 µ*M* H_2O_2. (**A**) Note concomitant treatment with 1 µ*M* SeMet led to statistically significant protection beginning at 9 h. Results represent the mean ± SEM % initial number for three separate experiments and three replicates for each experiment. (**B**) Time-dependent decrease in cell viability measured by quantifying increased LDH release into the medium. Note concomitant treatment with 1 µ*M* SeMet led to statistically significant protection against 10 µ*M* Fe^{2+}/30 µ*M* H_2O_2 beginning at 9 h. Results are expressed as mean ± SEM % total LDH release.

tion was not as robust as observed for 1 and 2 μ*M*. Similarly, Fig. 2 shows concomitant treatment with 1 and 2 μ*M* SeMet led to significant ($p < 0.05$) dose-dependent protection against 20 μ*M* Aβ. In contrast to results observed for Fe^{2+}/H_2O_2 , 5 μ*M* SeMet did not provide protection against Aβ. To minimize any potentially detrimental effects of SeMet, the lowest concentration that provided significant protective effects (1 μ*M*) was chosen for further analyses. Correlation analyses showed statistically significant positive correlations between cell counts and LDH release ($r = 0.7$ to 0.97; $p < 0.05$) for suggesting the two independent measures of cell viability showed similar results.

After optimum SeMet concentrations were chosen, cell survival was measured at 3, 6, 9, and 16 h using cell counts and LDH release. Because of the limited viability of control cultures after 24 h in Locke's medium, treatment times longer than 16 h were not studied. Figures 3A and 4A show a time dependent decrease in cell count and that SeMet significantly protected against Fe^{2+}/H_2O_2 and Aβ beginning at 9 h. To verify cell counts, LDH release into the medium was also measured at the same time points. Figures 3B and 4B show a similar time dependent decrease in cell viability as evidenced by increased LDH release. Measures of LDH showed that SeMet decreased Fe/H_2O_2 (Fig. 3B) and Aβ (Fig. 4B) mediated LDH release beginning at 9 h. Because of increased cell death beginning at 9 h, a treatment time of 6 h was chosen to evaluate ROS generation and GPx protein and activity.

Figures 5A and B show treatment with Fe^{2+}/H_2O_2 or Aβ alone resulted in significantly increased ($p < 0.05$) CM-H_2DCFDA fluorescence. In contrast, cells pretreated with 1 μ*M* SeMet showed a significant decrease in fluorescence intensity compared to Fe^{2+}/H_2O_2 or Aβ alone. The fluorescence intensity in cultures treated with SeMet alone was decreased but was not significantly different from control levels. Figure 5A shows confocal micrographs of CM-H_2DCFDA fluorescence and the corresponding phase contrast (Nomarski) images as well as a merged image. The Nomarski image shows that the cell morphology is intact for 6 h treatment times.

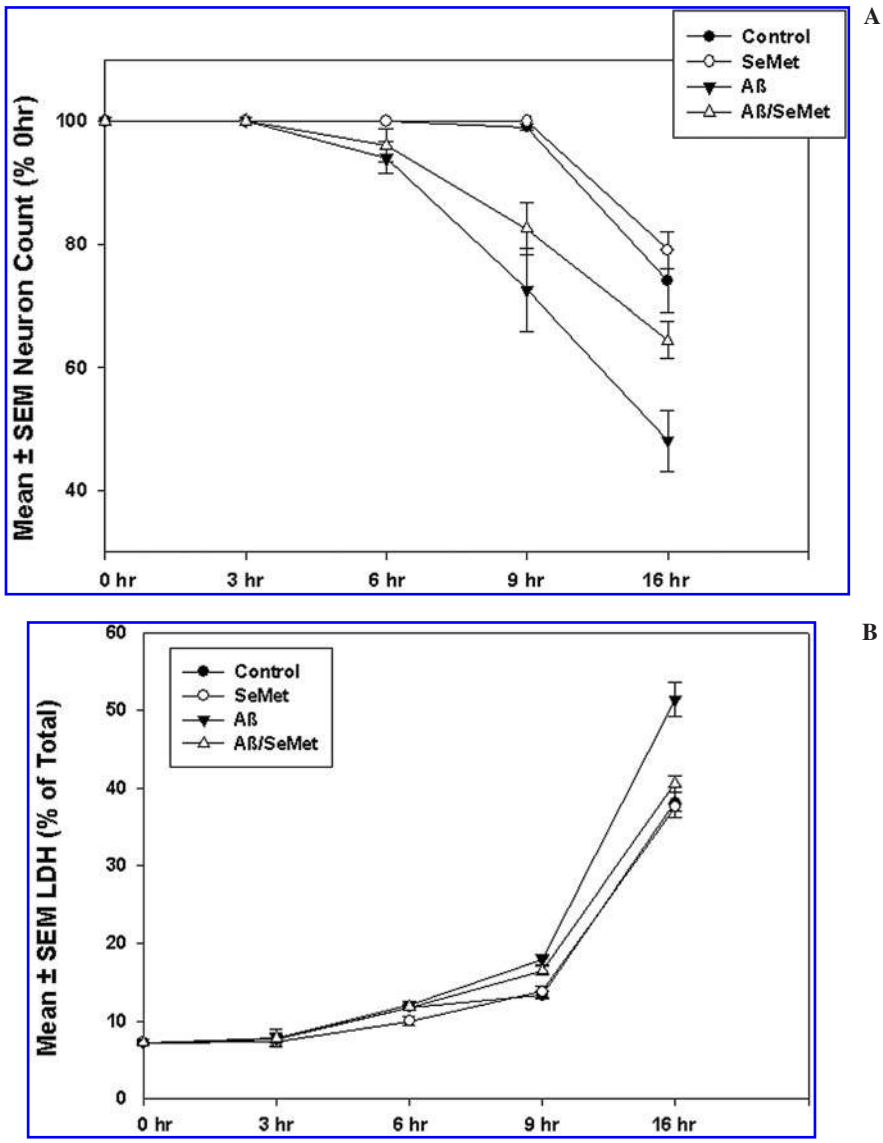


FIG. 4. (A) Time-dependent decrease in cell viability of primary hippocampal neurons treated with 20 μ*M* Aβ measured by counting the number of viable cells in premarked microscopic fields. Note concomitant treatment with 1 μ*M* SeMet led to statistically significant protection at all time points studied ($p < 0.05$). Results represent the mean ± SEM % initial number for three separate experiments and three replicates for each experiment. (**B**) Time dependent decrease in cell viability measured by quantifying increased LDH release into the medium at indicated time points. Note concomitant treatment with 1 μ*M* SeMet led to statistically significant protection against Aβ at all time points studied. Results represent the mean ± SEM % total LDH release for three separate experiments and three replicates for each experiment.

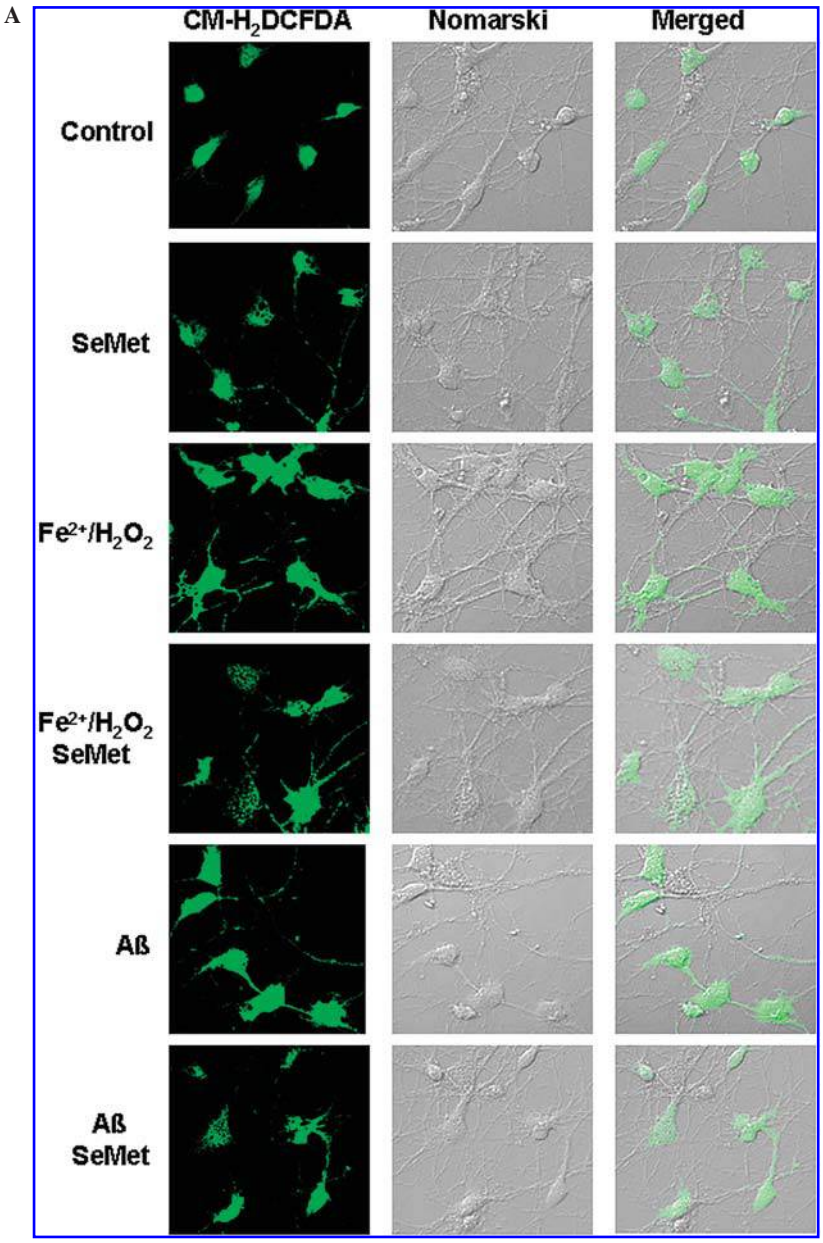


FIG. 5. ROS generation measured by CM-H2DCFDA fluorescence. (**A**) representative fluorescence, phase contrast (Nomarski), and merged images of hippocampal neurons. (**B**) mean ± SEM % control fluorescence. Data in (**B**) represent the mean ± SEM % control fluorescence intensity for three experiments and three replicates for each treatment, with a total of 900 cells counted for each treatment. $*p < 0.05$ compared to control, $**p < 0.05$ compared to Fe^{2+}/ H_2O_2 treatment, $***p < 0.05$ compared to Aβ treatment.

To verify our ROS measurements using CM-H_2DCFDA, ROS production was also quantified using APF, a cell permeable fluorescent probe that specifically interacts with hydroxyl radicals and hypochlorite anions. Figures 6A and B show results of confocal analyses of primary hippocampal neurons treated with SeMet alone or in combination with Aβ or Fe^{2+}/H_2O_2. As was observed using CM-H_2DCFD levels of APF fluorescence were significantly decreased in cultures treated with SeMet compared to those treated with Aβ or Fe^{2+}/H_2O_2 alone. The phase contrast images (Fig. 6A) show that cell morphology was maintained after 6 h treatment and that the ROS generation being measured is not simply due to extensive cell death.

Comparison of GPx-1 protein expression after 6 h treatment showed pretreatment with SeMet led to a significant increase of GPx-1 in cultures receiving SeMet compared to control cultures or cultures treated with Aβ and Fe^{2+}/H_2O_2 alone (Fig. 7A and B). To determine if increased GPx-1 expression affected total GPx activity, GPx activity assays of protein extracts were carried out using cortical neuron cultures exposed to Aβ or Fe^{2+}/H_2O_2 alone for 6 h or pretreated with SeMet prior to addition of insults. Figure 8 shows total GPx activity was significantly decreased in cultures treated with Fe^{2+}/H_2O_2 or Aβ alone compared to controls. In contrast, GPx activity was significantly increased 29 ± 5.1% and 21 ± 4%, respectively, in cultures pretreated with SeMet compared to Fe^{2+}/H_2O_2 or Aβ alone. Results of cortical neuron viability assays using LDH release showed cultures treated with Fe/H_2O_2 alone showed no significant increase in LDH release (12.9 ± 0.7% total LDH) compared to control cultures (11.8 ± 0.4% total LDH) at 6 h. Cultures treated with Fe/H_2O_2/SeMet showed similar levels of LDH release (11.6 ± 0.4% total LDH). Similar results were observed for cultures treated with Aβ (12.1 ± 0.4% total LDH) compared to controls (11.8 ± 0.4% total LDH). Cultures treated with Aβ/SeMet showed similar levels of LDH release (11.8 ± 0.3% total LDH). Because both cell counts and measures of LDH release showed there was minimal cell death at 6 h, results of GPx activity assays were calculated as units of activity/mg protein and are expressed as mean ± SEM % control activity.

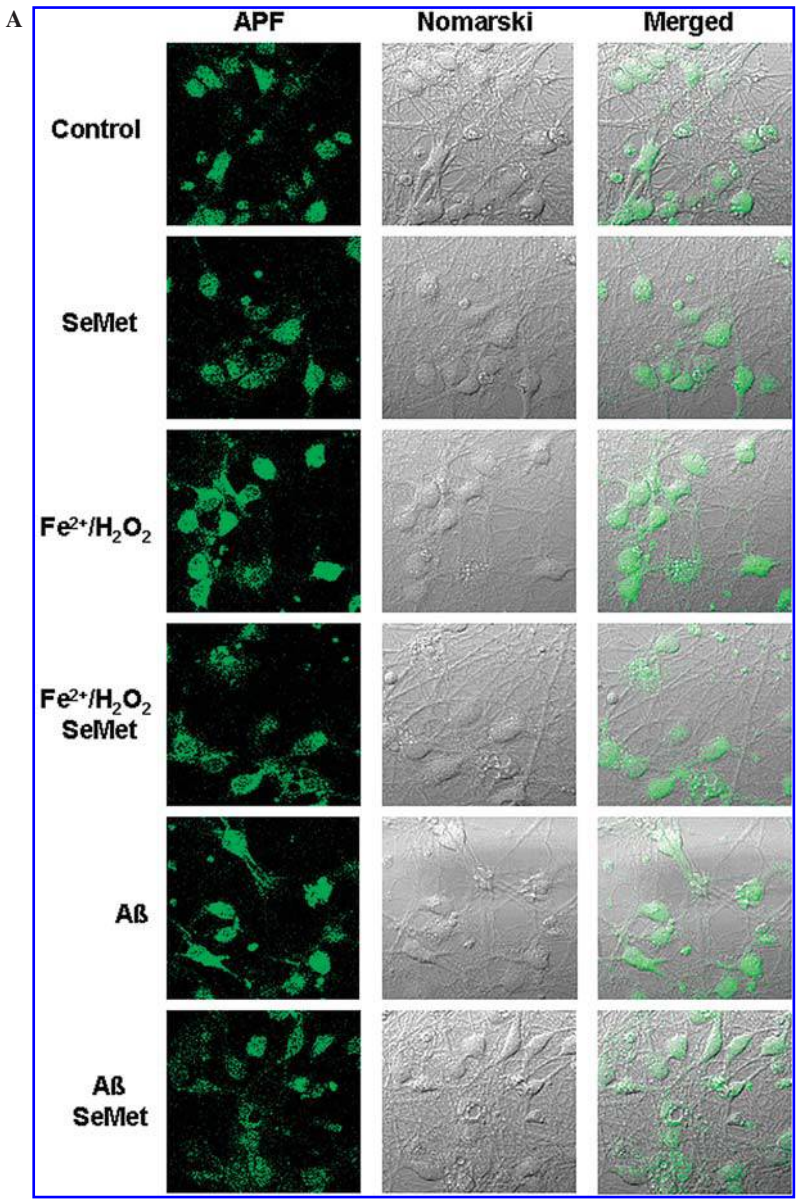
DISCUSSION

Previous studies show the AD brain is under oxidative stress as evidenced by increased levels of protein oxidation, lipid peroxidation, and other oxidative damage markers (reviewed in Ref. 8). Aβ has been identified as central to the pathogenesis of AD (7, 27), and Fe^{2+}/H_2O_2 may also play a critical role in oxidative stress (27). In the present study, we show Aβ and Fe^{2+}/H_2O_2 mediated ROS measured using CM-H_2DCFDA, a general oxidant sensing fluorescent probe or APF, a fluorescent probe for hydroxyl radicals and hypochlorite anions, are decreased by pretreatment with SeMet. Concurrent with increased ROS production, we show decreased neuron viability. Our data also show that protective effects of SeMet begin to diminish at 5 μ*M*, suggesting that SeMet concentrations must be carefully titrated when used as a therapeutic agent. Additionally, we show seleno-dependent GPx-1 expression and activity were significantly decreased in the presence of Aβ or Fe^{2+}/H_2O_2. By analyzing ROS generation and GPx protein and activity prior to pronounced cell death, our data show that SeMet inhibits ROS production and limits cell death induced by Aβ or Fe^{2+}/H_2O_2. Previous studies show antioxidant defense enzymes in general are altered in AD (18, 20, 26, 45). GPx is primarily located in the cytosol and functions in the detoxification of lipid hydroperoxides and H_2O_2. Studies of GPx in AD have been contradictory with reports of significant increases in AD (26, 45) no differences (20), or significant decreases in AD (18). These contradictory results may be related to different APOE genotype in AD patients (8), or a compensatory response to increased free radical formation (26). Similar results were reported for *in vitro* studies in rats (4).

Selenium, an essential trace element, is a critical component of ~12 selenoproteins, including iodothyronine deiodinases (1, 2, 10, 12), thioredoxin reductases (24, 25, 37, 49, 51, 52), selenophosphate synthetases (37), selenoprotein P (33), and glutathione peroxidases (9, 15, 16, 30, 42, 47, 50). Several epidemiological studies show selenium is protective against the development of cancer, particularly prostate cancer (reviewed in Ref. 21). In experimental models of tumorgenesis, selenium is postulated to inhibit proliferation and induce apoptosis (14, 55, 56). Additionally, selenium is thought to be protective in diseases where oxidative stress levels are high including rheumatoid arthritis and HIV/AIDS (reviewed in Ref. 43). Previous studies of the effects of selenium show that treatment of HT1080 cells with 5 μ*M* sodium selenite led to decreased apoptosis induced by H_2O_2 through activation of an antiapoptotic (PI3-K/Akt) pathway and inhibition of the apoptotic apoptosis signaling regulatory kinase 1 (ASK) and c-jun N-terminal protein kinase (JNK) pathways (54). Additionally, it was shown that sodium selenite increases glucose uptake and ATP production in the same model (54).

Studies of ebselen [2,4,4-trimethyl-2H-1,2-benzoselenazin-3 (4H)-one], a selenium containing heterocyclic compound, show it prevents NO-induced apoptosis of differentiated PC-12 cells by blocking activation of ASK1 and inhibition of p38 mitogen activated protein kinase (MAPK) and JNK (44). In addition, ebselen demonstrates potent antioxidant effects acting as a GPx mimic (28, 35) and is neuroprotective in experimental models of cerebral ischemia (13, 48). In studies using a rodent model of focal cerebral ischemia, Imai *et al*. (19) showed ebselen significantly decreased levels of 4-hydroxynonenal, a marker of lipid peroxidation and 8-hydroxy-deoxyguanosine (8-OHdG), a marker of DNA oxidation. Based on these observations, it was postulated that the effects of ebselen were through its antioxidant function and modulation of oxidative stress (19).

Although SeMet, the organic form of selenium, is more bioavailable than inorganic forms (11), there have been few studies of its effects on oxidative stress. Rafferty *et al.* (40) showed treatment of primary human keratinocytes with SeMet or sodium selenite protected against UV-induced oxidative DNA damage. Our data show that pretreatment of primary cortical neurons with SeMet significantly decreased free radical production by Aβ or Fe/H_2O_2 and increased GPx activity. This increased GPx may detoxify H_2O_2 and resultant lipid hydroperoxides to nontoxic alcohols as well as protect the biological membranes from lipid peroxidation (38). The



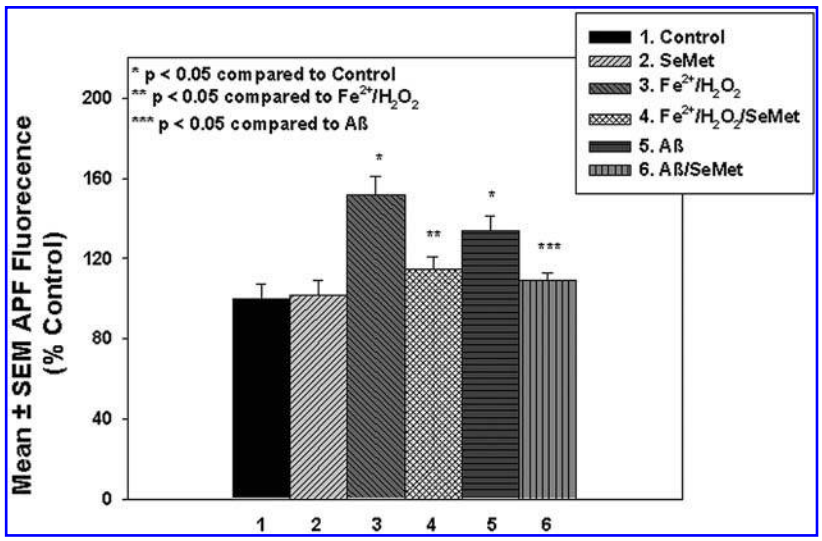


FIG. 6. ROS generation measured by APF fluorescence. (**A**) representative fluorescence, phase contrast (Nomarski), and merged images of hippocampal neurons. (**B**) mean ± SEM % control fluorescence. Data in (**B**) represent the mean ± SEM % control fluorescence intensity for three experiments and three replicates for each treatment, with a total of 900 cells counted for each treatment. *$p < 0.05$ compared to control, **$p < 0.05$ compared to Fe^{2+}/ H_2O_2 treatment, ***$p < 0.05$ compared to Aβ treatment.

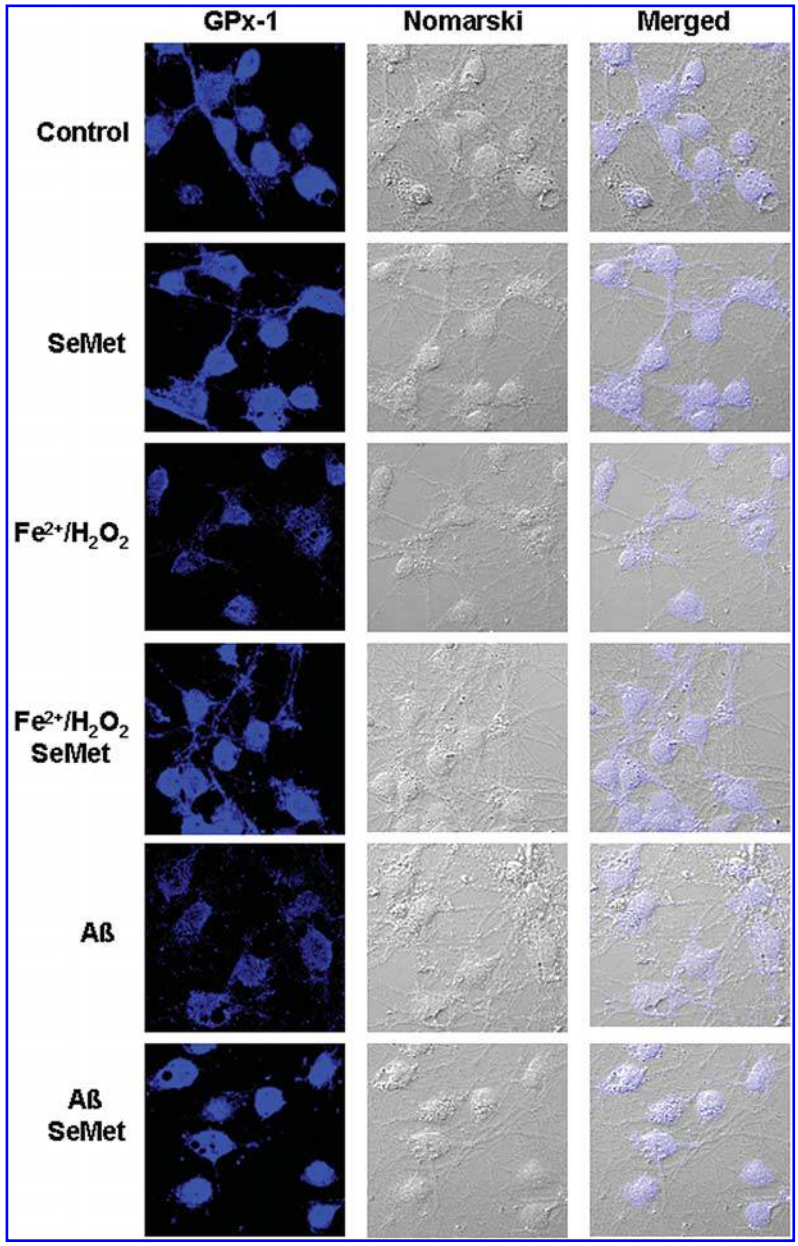


FIG. 7. Representative fluorescence, Nomarski, and merged images of hippocampal cultures immunostained for GPx-1. (**A**) Mean ± SEM % control fluorescence intensities for a total of 900 cells counted for each treatment. (**B**) $*p < 0.05$ compared to control, $**p < 0.05$ compared to Fe^{2+}/ H_2O_2 treatment, $***p < 0.05$ compared to Aβ treatment.

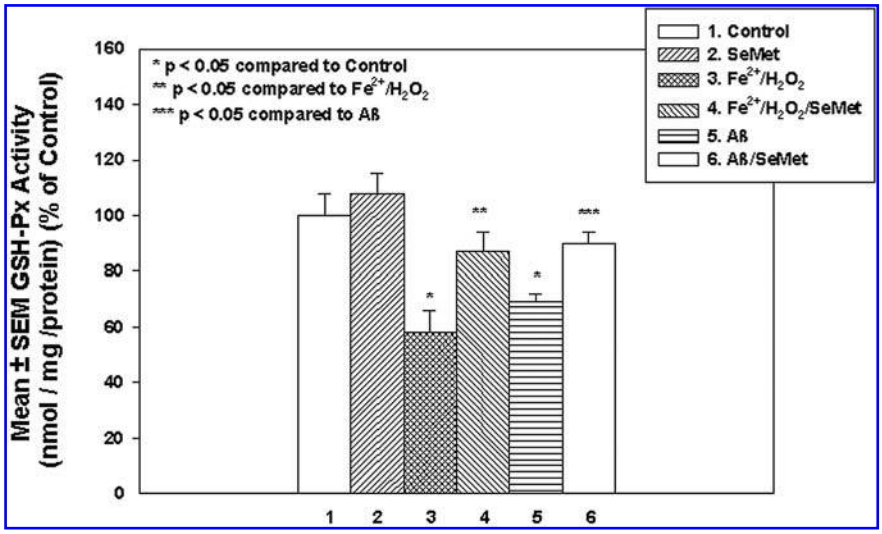


FIG. 8. GPx activity in primary cortical neurons treated as controls, with 20 μ*M* Fe^{2+}/60 μ*M* H_2O_2 or 25 μ*M* Aβ alone, or pretreated with SeMet prior to Fe^{2+}/H_2O_2 or Aβ. Data in the figure represent the mean ± SEM % control activity for six experiments and three replicates for each experiment. $*p < 0.05$ compared to control, $**p < 0.05$ compared to Fe^{2+}/H_2O_2 treatment, $***p < 0.05$ compared to Aβ treatment. Cortical neuron survival measured using LDH release showed no significant differences between control and Fe/H_2O_2 or Aβ treated cultures.

diminished oxidative damage significantly increased cell viability compared to cultures treated with Fe^{2+}/H_2O_2 or Aβ alone. These results are supported by other studies that demonstrate increased GPx activity and GPx-1 mRNA levels positively associated with SeMet supplements in *in vivo* and *in vitro* studies (4, 34, 36, 38). Although our experiments were directed toward the evaluation of the effects of SeMet on free radical generation, it is also possible that SeMet pretreatment may impact pro- and antiapoptotic pathways as has been described for ebselen.

Overall, our data demonstrate that SeMet increases GPx protein and activity that diminishes neuron oxidative damage induced by Fe^{2+}/H_2O_2 and Aβ. These data suggest SeMet may be suitable as a potential antioxidant therapeutic agent in AD.

ACKNOWLEDGMENTS

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ABBREVIATIONS

Aβ, amyloid beta peptide; APF, 3′-(p-aminophnyl) fluorescein; CM-H_2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate acetyl ester; GPx, glutathione peroxidase; SeMet, seleno-L-methionine.

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