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Brain Research 1018 (2004) 86–96

BRAIN RESEARCH

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Research report

Antisense directed at the Aβ region of APP decreases brain oxidative markers in aged senescence accelerated mice

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Accepted 11 May 2004
Available online 24 June 2004

Abstract

Amyloid β-peptide (Aβ) is known to induce free radical-mediated oxidative stress in the brain. Free radical-mediated damage to the neuronal membrane components has been implicated in the etiology of Alzheimer's disease (AD). Aβ is produced by proteolytic processing of the amyloid precursor protein (APP). The senescence accelerated mouse prone 8 (SAMP8) strain was developed by phenotypic selection from a common genetic pool. The SAMP8 strain exhibits age-related deterioration in memory and learning as well as Aβ accumulation, and it is considered an effective model for studying brain aging in accelerated senescence. Previous research has shown that a phosphorothiolated antisense oligonucleotide directed against the Aβ region of APP decreases the expression of APP and reverses deficits in learning and memory in aged SAMP8 mice. Consistent with other reports, our previous study showed that 12-month-old SAMP8 mice have increased levels of oxidative stress markers in the brain compared with that in brains from 4-month-old SAMP8 mice. In the current study, 12-month-old SAMP8 mice were treated with antisense oligonucleotide directed against the Aβ region of APP, and the oxidative markers in brain were decreased significantly. Therefore, we conclude that Aβ may contribute to the oxidative stress found in aged SAMP8 mice that have learning and memory impairments. These results are discussed in reference to AD.

Theme: Disorders of the nervous system
Topic: Alzheimer's disease—beta amyloid

Keywords: Amyloid β-peptide; Senescence accelerated mouse; Amyloid precursor protein

1. Introduction

Amyloid β-peptide (Aβ) is the major constituent of senile plaques, a pathological hallmark of Alzheimer's disease (AD). It is generally accepted that Aβ(1–42) plays a central role in the pathophysiology of AD [95]. Our laboratory, along with others, suggested that Aβ neurotoxicity is mediated through its ability to produce free radical oxidative stress, including protein oxidation and lipid peroxidation [18,19,22,26,28,105]. The methionine residue at position 35 (Met-35) is important in this process [12,16,18,25,68,69,106,111]. Also, Aβ-induced damage can be modulated by the enzyme superoxide dismutase (SOD), suggesting $O_2^{\cdot -}$ is involved in Aβ toxicity [102]. Aβ induces $O_2^{\cdot -}$ production by stimulating NADPH oxidase [67]. Aβ is reported to produce H_2O_2 through copper or iron reduction. Aβ can also increase NO production in macrophages which are present in a microglial cell line [83]. Free radicals further oxidatively modify protein, lipid and DNA in cells resulting in cytotoxicity [19]. Other mechanisms by which Aβ can induce neurotoxicity include binding to the nicotinic acetylcholine receptor [107], forming calcium and potassium channels in cell membranes [8,44,45], decreasing glucose transport across brain endothelial cells [14], and

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doi:10.1016/j.brainres.2004.05.048

actuating the release of chemokines [48] and cytokines[2]. Aβ causes oxidative modification of glutamate synthetase [3] and the glutamate transporter [73], both of which are decreased in activity in AD brain [19]. This suggests that Aβ-mediated excitotoxic mechanisms could be important in AD [20]. Loss of enzymatic activity of glutamine synthetase results when the protein is oxidized [3], a modification that changes the conformation of this enzyme [23].

When Aβ is given exogenously, it causes deficits in learning and memory in animals [34,35,52,54,97,101,108]. Such learning and memory impairments are also present when Aβ is overly expressed in transgenic models [64] and the senescence accelerated prone (SAMP8) mouse [47,49–51,53,55,87].

It has, therefore, been proposed that reducing the levels of Aβ could slow or even prevent cognitive impairment observed in AD or age-related dementia [82]. Such reductions can be achieved by site-directed antisense oligonucleotide therapy. This type of oligonucleotide is designed to bind to a complementary sequence (referred to as the ''target sequence'') in a selected mRNA, inhibiting gene expression at the translational level. As a consequence, the protein product coded by that particular mRNA is not produced [58]. Since antisense oligonucleotides are intended to inactivate specific RNA nucleotide sequences rather than three-dimensional protein structures as antibodies do, they offer the advantage of discriminating among closely related gene products [94]. Therefore, site-directed antisense oligonucleotide therapy offers significant advantages for the specific reduction of Aβ production.

The SAMP8 mouse strain has undergone a natural mutation which has resulted in age-dependent defects of learning and memory beginning at about 8 months of age [50]. At the same time, SAMP8 mice produce increased amounts of amyloid precursor protein (APP) and Aβ in brain similar to those moieties observed in AD [86]. The nucleotide sequence of APP in SAMP8 mice is 89.2% homologous with that in the human. Unlike transgenic mice that have 5–14 times the normal amount of Aβ increased in their brains as function of age, the Aβ level of SAMP8 mice increases only 100% from 4 to 12 months [71]. Such an increase is much closer to the estimated 50% increase in Aβ seen in AD [91] than is observed in transgenic mouse models. The APP and APP mRNA of SAMP8 mice also increase significantly as the mice age from 4 to 12 months [71,86]. Hence, SAMP8 mice serve as a useful model in the study of age-related cognitive impairment.

It is possible to decrease the APP production in the brain by giving an intracerebroventricular injection of a 42-mer phosphorothiolated antisense oligonucleotide (AO) directed at the Aβ region of the APP gene. Injection of AO twice or more has been shown to improve acquisition and retention in a footshock avoidance paradigm [70]. Although many in vitro studies show the benefits of reducing APP level by antisense RNA [74], antisense ribozymes [40–42], or AOs [7,37,77], little is known about the relation between the reduced Aβ level by AO and oxidative stress. Therefore, in this current study, we hypothesized that the cognitive improvement resulting from AO treatment of SAMP8 mice is caused by a decrease in Aβ-induced free radical insults. To test this hypothesis, we compared the protein oxidation and lipid peroxidation markers in brain from 4-month-old SAMP8 mice treated with saline (4m), 12-month-old SAMP8 mice treated with random AO (12mR), and 12-month-old SAMP8 mice treated with the 42-mer AO directed at the Aβ region of the APP gene (12mA).

2. Materials and methods

All chemicals are purchased from Sigma Aldrich unless specified.

2.1. Subjects

The SAMP8 mice were from an in-house colony, inbred for 10 years from stock obtained from Dr. Takeda of Kyoto University, Japan. Mice were on a 12:12-h light/dark cycle with lights on at 0600 h. Food and water was available ad libitum. Sentinels from the colony have remained free of pathogens including mycoplasma, salmonella/shigella, ectoparasites, pneumonia virus, Sendai virus, mouse hepatitis, Reo 3, ectromelia, GBVII, and lymphcytic choriomeningitis.

2.2. Antisense oligonucleotides (AO) and its permeability to cell membrane

We designed phosphorothiolated antisense oligonucleotides (AO) directed at positions 17–30 of the Aβ region of the APP gene (Midland Certified Reagent, Midland, TX). The sequences of the designated AO and of a random AO used as a control are given in Table 1. These AOs were previously used to reverse the learning and memory deficit observed in aged SAMP8 mouse [70].

Hippocampal neuronal cultures were prepared from 18-day-old Sprague–Dawley rat fetuses as described previously [43]. Incorporation of AO into neurons was observed using fluorescein-conjugated AO. Briefly, neuronal cells were incubated with 10 ng of 5′-FITC-AO for 2 h at 37 °C followed by washing with PBS (three times) to remove the excess of 5′-FITC-AO and the cells were examined under a fluorescence microscope equipped with an argon laser (λ_{ex} 485 nm, λ_{em} 530 nm).

2.3. Antisense administration

Administration of AO was performed according to Kumar et al. [70]. Mice were anesthetized in a stereotactic instrument with methoxyflurane. Three injections of vehicle or AO were given to the mice by intracerebroventricular (ICV) injection on 2 weeks intervals (from 11 to 12 months). All substances (65 ng) were injected in a 2 μl volume by drilling a hole through the skull over the third

Table 1
The antisense and control random phosphorothiolated oligonucleotides used for inhibition of APP translation

AO	Sequence
AO inhibiting APP translation	GGCGCCTTTGTTCGAACCCACATCTTCAAAAGAACACCAG
Random AO	GATCACGTACACATCGACACCAGTCGCCATGACTGAGCTT

ventricle (− 0.5 relative to Bregma; 0.5 mm right of central suture). The scalp wound was closed and the mice were returned to their cages.

2.4. Sample preparation and methods employed

Mice were sacrificed 2 weeks after the last injection. SAMP8 mouse whole brains were flash frozen in liquid nitrogen. The samples were homogenized in PBS with protease inhibitors (2 mM EDTA, 2 mM EGTA, 20 mM HEPES, 20 μg/ml trypsin inhibitor, 4 μg/ml leupeptin (ICN Biomedicals, Ohio), 4 μg/ml pepstatin (ICN Biomedicals), 5 μg/ml aprotinin (ICN Biomedicals)) by sonication. Protein concentration was determined by the bicinchoninic acid (BCA) assay method (Pierce, Rockford, IL).

2.5. Immunochemistry

2.5.1. Slot blot and Western blot of Aβ levels

About 12.5 μl of the samples was treated with an equal volume Laemmli buffer (0.125 M Trizma base, 4% SDS, 20% glycerol) for 20 min. Levels of Aβ were measured by slot blot with 1 μg per slot. For Western blot, 12.5% linear Gradient Precast criterion Tris–HCl gels (Bio-Rad, California) were used to perform the separation of the proteins. Precision Protein™ Standards (Bio-Rad) were run along with the sample at 200 V for 45 min. The proteins from gels were transferred to a nitrocellulose paper (Bio-Rad) using the Transblot-Blot® SD Semi-Dry Transfer Cell (Bio-Rad) at 8 V for 45 min. Aβ levels from both Western blot and slot blot were detected on the nitrocellulose paper using a primary mouse antibody (a generous gift from Dr. Ralph N. Martins) specific for Aβ (1:50) and then a secondary anti-mouse IgG (AnaSpec, California) antibody. The resultant stain was developed by application of Sigma-Fast 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium (BCIP/NBT) tablets followed by density measurement by Scion-Image software package (Scion, Maryland).

2.5.2. Protein carbonyl, 3-nitrotyrosine (3-NT) and 4-hydroxynonenal (HNE) detection

Detection of protein carbonyl, 3-NT and HNE level by slot blots were performed as described previously [47]. Levels of 3-nitrotyrosine (3-NT), 4-hydroxynonenal (HNE) and protein carbonyls were determined immunochemically. Protein carbonyl levels were determined as adducts of 2,4-dinitrophenylhydrazine (DNPH) [21,98]. Five microliters of the samples was treated with an equal volume of 12% SDS. Samples were then derivatized with 10 μl of 20 mM 2,4-DNPH (Chemicon, California) for 20 min. The reaction was stopped by addition of neutralizing reagent (7.5 μl of 2 M Tris/30% glycerol buffer, pH = 8.0). Levels of protein carbonyls were measured by using the slot blot technique with 250 ng of protein loaded per slot. The 2,4-dinitrophenyl hydrazone (DNP) adduct of the carbonyls is detected on nitrocellulose paper using a primary rabbit antibody (Chemicon) specific for DNP-protein adducts (1:100) followed by a secondary goat anti-rabbit IgG (Sigma) antibody. The resultant stain was developed by application of Sigma-Fast (BCIP/NBT) tablets; the line densities were quantified by Scion-Image software package. HNE and 3-NT levels were determined in the same manner. Five microliters of the samples was treated with an equal volume of 12% SDS. Samples were then further denatured with 10 μl of Laemmli buffer (0.125 M Trizma base, 4% SDS, 20% glycerol) for 20 min. Levels of 3-NT and HNE were measured by using the slot blot technique: 500 ng of protein per slot for protein 3-NT levels and 250 ng of protein per slot for HNE levels. The HNE levels were detected on the nitrocellulose paper using a primary rabbit antibody (Alpha Diagnostics, Texas) specific for HNE-modified protein (1:8000). The 3-NT levels were detected by primary rabbit antibody (Chemicon) specific for 3-NT (1:100). The same secondary goat anti-rabbit IgG (Sigma) antibody was then used. The resultant stain was developed by application of Sigma-Fast (BCIP/NBT) tablets; the line densities were also quantified by Scion-Image software package.

2.5.3. Thiobarbituric acid reactive substances (TBARS)

The concentration of TBARS in brain tissue was determined according the method of Ohkawa et al. [88]. Four hundred microliters of 10% w/v of ice cold TCA was added into 0.25 ml of 4 mg/ml tissue homogenate. The samples were spun in an Eppendorf centrifuge tube for 5 min at $3000 \times g$. The supernatant was collected (0.5 ml) and treated with TBA reagent (20 mM TBA in 50% v/v glacial acetic acid). The samples were then heated at 100 °C for 1 h. After the cooling period, 500 μl of water-saturated butanol was added, and the organic layer was removed and redistributed to a black microtiter plate (Corning). End point fluorescence was measured at $\lambda_{ex} = 515$ nm and $\lambda_{em} = 585$ nm by SpectMX UltraXS® (Molecular Devices).

2.5.4. Glutamine synthetase (GS) assay

Fresh, non-frozen brains, shipped on ice overnight from St. Louis to Lexington, were homogenized in a 0.32 M sucrose isolation buffer (2 mM EDTA, 2 mM EGTA, 20 mM HEPES, 20 μg/ml trypsin inhibitor, 4 μg/ml leupeptin,

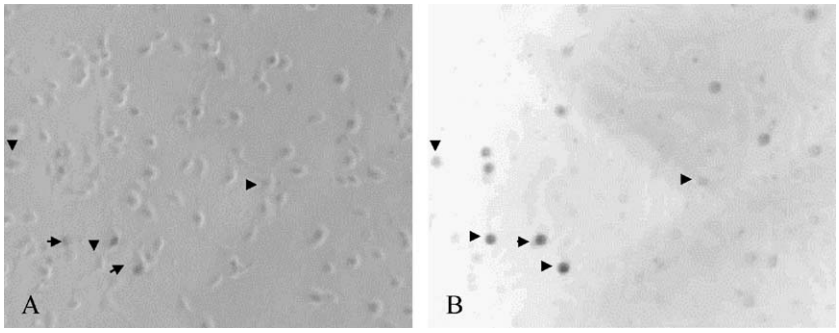

Fig. 1. (A) Bright filed micrograph showing live neurons (arrowed). (B) Fluorescence micrograph showing dark spots which represent the fluorescence from the 5′-FITC-AO remaining in the cells after washes.

4 μg/ml pepstatin, 5 μg/ml aprotinin). The samples were then centrifuged at 20,000 × *g* at 4 °C for 10 min. Supernatants that contain GS and other cytosolic proteins were extracted. The protein concentration was determined by the BCA method and normalized to 0.4 mg/ml by addition of the 0.32 M sucrose solution. GS activity was determined by the

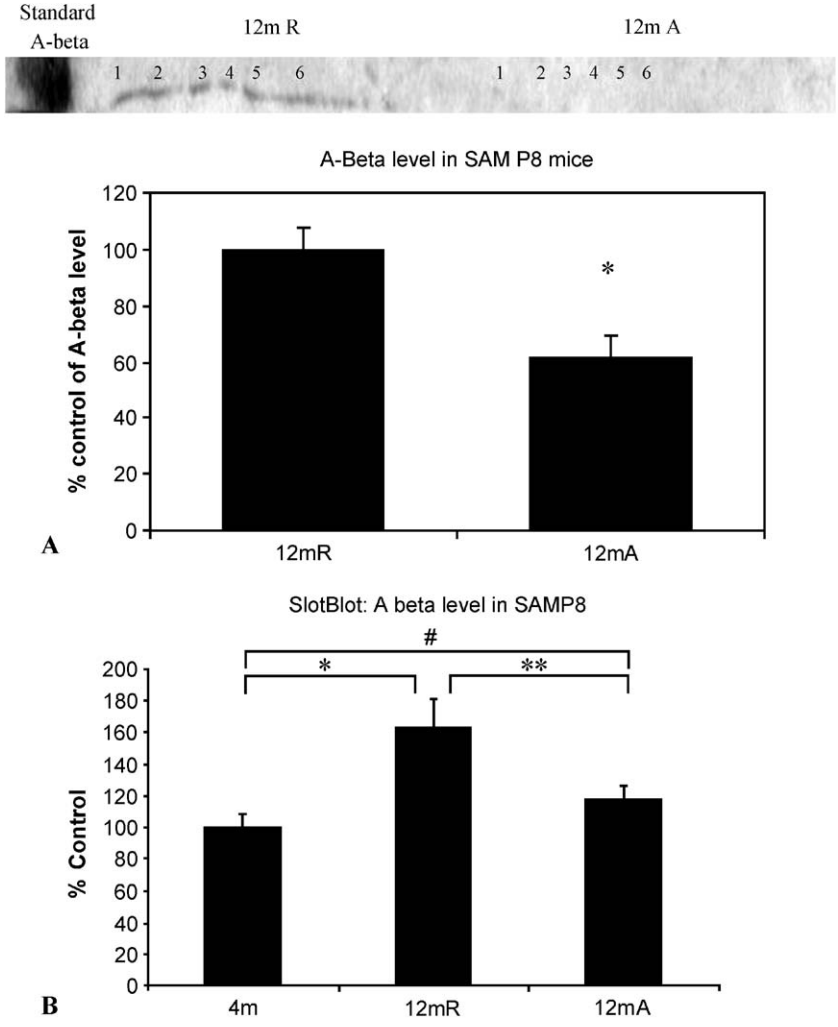

Fig. 2. (A) Western blot of Aβ levels in brains of 12mR and 12mA mice. Data represents the average of the level of Aβ of 12-month-old SAMP8 mice treated with random AO (12mR) and 12-month-old SAMP8 mice treated with AO directed to the Aβ region of APP (12mA). Error bars indicate SEM for six animals in each group. Measured values are normalized to the 12mR values. $*p < 0.05$. (B) Slot blot of Aβ levels in brains of 4m, 12mA and 12mA. Data represents the average of the level of Aβ of 4-month-old SAMP8 mice treated with vehicle (4m), 12-month-old SAMP8 mice treated with random AO (12mR) and 12-month-old SAMP8 mice treated with AO directed to the Aβ region of APP (12mA). Error bars indicate SEM for eight animals in each group. Measured values are normalized to the 4m values. $*p < 0.05$; $**p < 0.05$; $^{\#}p > 0.2$.

method of Meister [84,92] as modified by Miller et al. [85]. The absorbance was recorded at 505 nm in a microtiter plate reader (PowerWaveX®, Bio-Tek Instruments).

2.5.5. Statistics

The data were analyzed by Student's *t*-tests. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. Accessibility to cells and effects on Aβ levels of AO

3.1.1. Accessibility of AO in cell cultures

The AO approach previously was used to reverse the learning and memory deficits of aged SAMP8 mice [70], consistent with the notion that the AO penetrated nucleuses in vivo. To model this result, we used neuronal cultures. Fig. 1A shows bright-field microscopy of living neurons (arrows) and Fig. 1B shows the fluorescence intensity of AO inside the neurons, indicating that the AOs successfully cross the cell membrane and remain in the neurons after washes. Approximately 8% of the neurons successfully took up the AO.

3.1.2. Effect of AO on Aβ levels in SAMP8 mice brain

Fig. 2A shows the Western blot of Aβ levels in 12mR and 12mA brain homogenate. The Aβ level in the 12mA SAMP8 mice brain is decreased by 40% due to the effect of AO ($p < 0.05$), while the Aβ level in brain from SAMP8 mice treated with 12mR was equal to that of untreated 12-month-old SAMP8 mice. Slot blot analysis of levels of Aβ in brains of 4-month-old SAMP8 mice reveals that Aβ levels are equivalent to those of 12-month-old treated with AO, i.e., AO treatment of aged SAMP8 mice lower Aβ levels in brain to those of young mice (Fig. 2B).

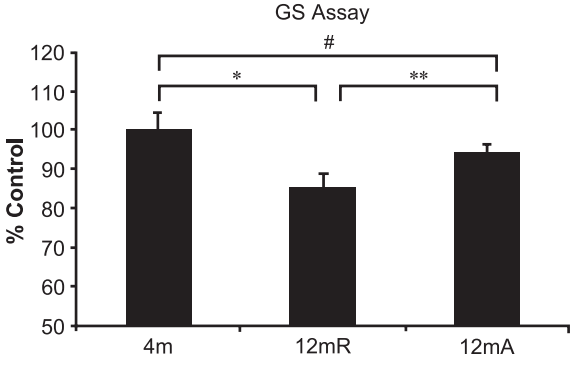

Fig. 3. Glutamine synthase activity. Data represents the average GS activity of 4-month-old SAMP8 mice treated with vehicle (4m), 12-month-old SAMP8 mice treated with random AO (12mR) and 12-month-old SAMP8 mice treated with AO directed to the Aβ region of APP (12mA). Error bars indicate SEM for six animals in each group. Measured values are normalized to the 4m values. *$p < 0.01$; **$p < 0.05$; $^{\#}p > 0.2$.

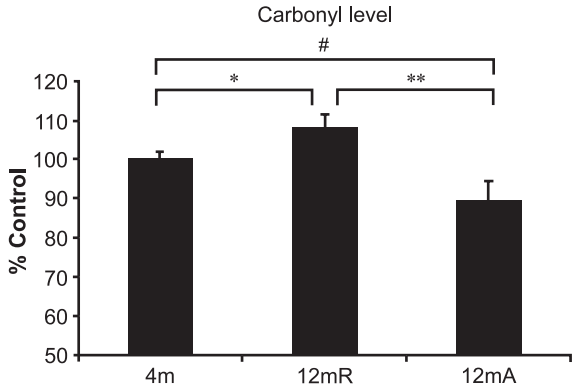

Fig. 4. Protein carbonyl level. Results show the average protein carbonyl level of 4-month-old SAMP8 mice treated with vehicle (4m), 12-month-old SAMP8 mice treated with random AO (12mR), and 12-month-old SAMP8 mice treated with AO directed to the Aβ region of APP (12mA). Error bars indicate the SEM for six animals in each group. Measured values are normalized to the 4m values. *$p < 0.05$; **$p < 0.01$; $^{\#}p > 0.05$.

3.2. Protein oxidation

3.2.1. Glutamine synthetase (GS) assay

GS is an oxidatively sensitive enzyme [24], whose activity is decreased in AD brain [61], probably a result of specific oxidation [20,31]. Also, it was shown that Aβ(1–40) and Aβ(25–35) can inactivate GS in cell-free incubates as well as in cell culture, indicating that Aβ-mediated oxidative stress maybe responsible for inactivation of GS [5].

Fig. 3 shows that the activity of GS in 12mR mice brains was decreased to 85.7 ± 3.1% compared to the 4m mice brains ($p < 0.01$). Antisense directed against the Aβ region of APP partially reversed this decrease ($p < 0.05$), with the activity of GS restored to 94.3 ± 1.9% of the mean GS activity from 4m brains. This value was statistically identical to that between the 4-month-old brain ($p > 0.2$).

3.2.2. Protein carbonyl level

Protein carbonyls are an index of protein oxidation [21] and are increased in AD brain [19,26,31,32,61]. Consistent with the GS assay results, Fig. 4 shows that the brain protein carbonyl levels were increased in 12mR mouse brains compared to those in brains from 4m mice ($p < 0.05$). However, by decreasing the production of Aβ by antisense against APP, the brain protein carbonyl levels of 12-month-old SAMP8 mice were significantly decreased to 89.6 ± 4.5% ($p < 0.01$), and found to be insignificantly different to those in brain from 4-month-old mice ($p > 0.05$).

3.2.3. 3-Nitrotyrosine (3-NT)

Fig. 5 shows that the 3-NT levels of 12mR SAMP8 brain protein were significantly increased compared to those in brain from 4-month-old mice (118 ± 3.4%, $p < 0.001$). However, after injection of the antisense against APP, the 3-NT levels were significantly decreased to 99.6 ± 2.7%

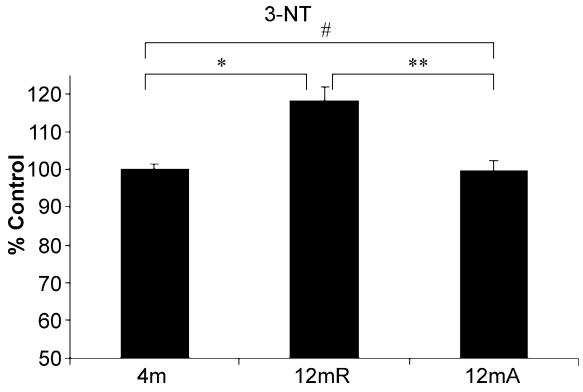

Fig. 5. 3-Nitrotyrosine level. Results represent the average protein 3-NT level of 4-month-old SAMP8 mice treated with vehicle (4m), 12-month-old SAMP8 mice treated with random AO (12mR), and 12-month-old SAMP8 mice treated with AO directed to the Aβ region of APP (12mA). Error bars indicate the SEM for six animals in each group. Measured values are normalized to the 4m values. *$p<0.001$; **$p<0.005$; #$p>0.8$.

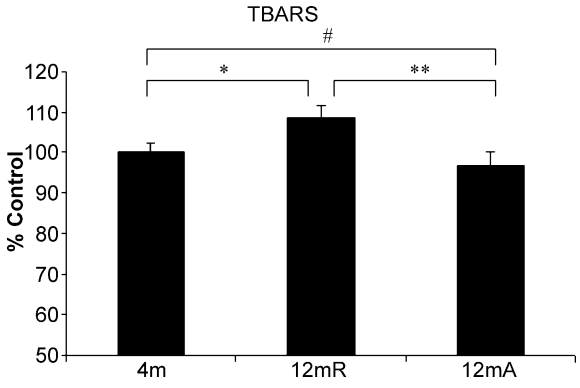

Fig. 7. TBARS level. Results represent the average TBARS level of 4-month-old SAMP8 mice treated with vehicle (4m), 12-month-old SAMP8 mice treated with random AO (12mR), and 12-month-old SAMP8 mice treated with AO directed to the Aβ region of APP (12mA). Error bars indicate the SEM for six animals in each group. Measured values are normalized to the 4m values. *$p<0.05$; **$p<0.05$; #$p>0.4$.

($p<0.005$), a level similar to that of brain from 4m mice ($p>0.8$).

3.3. Lipid peroxidation

3.3.1. HNE

HNE is a reactive product of lipid peroxidation [21] that is elevated in AD brain [28,73,81]. Fig. 6 shows that in the 12mR SAMP8 mice brain, the protein-bound HNE levels were 114 ± 0.8% of the level found in 4-month-old mice, a statistically significant increase ($p<0.00005$). However, the HNE levels for brain protein from 12-month-old mice was decreased significantly to 106 ± 1.9% by the AO injection ($p<0.005$), a value only slightly elevated from that in 4m mice brain ($p=0.04$).

3.3.2. TBARS

Consistent with the increase in HNE levels, Fig. 7 shows that TBARS levels in 12mR were increased to 108.6 ± 3.0% of that found in 4-month-old mice, a statistically significant increase ($p<0.05$). However, with the injection of AO, the TBARS levels were significantly decreased to 96.7 ± 3.5% ($p<0.05$), a level that was not significantly different from that in 4m mice brain ($p>0.4$).

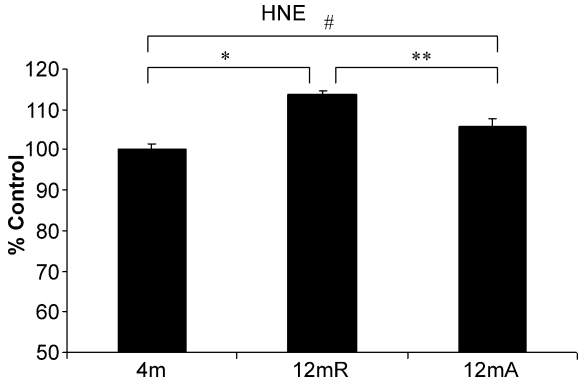

Fig. 6. HNE level. Results represent the average HNE level of 4-month-old SAMP8 mice treated with vehicle (4m), 12-month-old SAMP8 mice treated with random AO (12mR), and 12-month-old SAMP8 mice treated with AO directed to the Aβ region of APP(12mA). Error bars indicate the SEM for six animals in each group. Measured values are normalized to the 4m values. *$p<0.00005$; **$p<0.005$; #$p=0.04$.

4. Discussion

We previously showed that AO is able to cross blood–brain barrier (BBB) and reduce APP protein levels in aged SAMP8 mice brains [11,70]. We report here that AOs successfully cross cell membranes and enter neurons (Fig. 1). Others reported that cells in culture can take up antisense oligonucleotides against APP [1], and neuronal cells in culture appear to internalize AOs relatively efficiently [72]. Consistent with these other studies, we show here that small amount of neurons are able to internalize AOs. The low amount of neuronal uptakes of AOs was possibly due to the short incubation time (2 h). Nonetheless, AOs were shown to enter the cells. Although no direct evidence here shows that AO binds to APP mRNA, APP level reduction was previously reported [70], which in turn reduced Aβ production. Others reported that AO against APP can reduce Aβ production in rats to approximately 37% [82]. Consistent with this result, we find that AOs reduced Aβ level by approximately 40% in SAMP8 mice brains. This indicates that the AO is able to cross the BBB [11] and enter neurons, then reduce the protein levels of APP [70], consequently reducing the level of Aβ (Fig. 2).

Consistent with previous findings [27,47], our current results show that the 12mR SAMP8 mice have increased brain lipid peroxidation and protein oxidation compared to those in brain from 4m SAMP8 mice. Therefore, the random AO treatments had no effect on lipid peroxidation or protein oxidation observed in aged SAMP8 mice brain. Our findings also show that brain lipid peroxidation, protein oxida-

tion, and Aβ levels of 12-month-old SAMP8 mice are reduced by injection of AO.

Lipid peroxidation is an important mechanism of neurodegeneration in AD brains. TBARS and protein-bound HNE levels, commonly used as lipid peroxidation markers [21], are significantly elevated in AD brains [10,19,26,75,78,81,90,93,99]. HNE is able to diffuse to sites distant from that of its formation because of its comparatively long half-life [21]. HNE elevation has been described in multiple brain regions and in ventricular cerebrospinal fluid (CSF) in AD [81]. Aβ leads to HNE formation [73,79]; this alkenal can alter the conformation of membrane proteins [100]. It is, therefore, a reasonable hypothesis that reducing the production of Aβ should decrease lipid peroxidation. Unlike other antioxidant studies which decrease lipid peroxidation by blocking the Aβ-associated radical insult [9,15,22,29,39,59,79,80,113], our current study shows that Aβ-mediated lipid peroxidation can be reduced by decreasing the production of Aβ through AO injection into brain.

Protein oxidation is another important factor in aging and age-related neurodegenerative disorders [19,21,24,30,47,61,98]. Protein carbonyls, 3-NT levels, and GS assay are commonly used as markers of brain protein oxidation[21]. A significant increase in protein carbonyls was reported in AD brains [6,20,31,32,61,76]. 3-NT is formed by oxidation of tyrosine by peroxynitrite. 3-NT levels were significantly increased in the hippocampus and cortical regions of AD as well as CSF [33,62,103]. Other studies have demonstrated that oxidative processes often result in decreased activity of key enzymes [5,57,61,63]. AD is characterized by the loss of GS activity [61], which can also be caused by Aβ [3,4,23]. Antioxidants can inhibit brain protein oxidation induced by Aβ [17,25,26,60,104,105,110,112,113,114], and here we show that antisense directed against APP reduce levels of Aβ and of brain-resident oxidative stress.

Our laboratory has proposed a model for neurodegeneration in AD brains based on free radical oxidative stress associated with Aβ(1–42) [19,28,105]. This model predicts that chemistry associated with the single methionine residue of Aβ(1–42) [Met-35] would induce lipid peroxidation and protein oxidation in neuronal membranes. The role of Met-35 as a mediator of the toxicity of Aβ is most likely to involve an oxidative event at the sulfur atom [68,69,111]. However, the event that initiates the oxidation of Met-35 is not yet clear. It could involve one or more of the several reactive oxygen species present in biological systems [106] or redox metal ions [12,38]. Since the structure of Aβ from position 28 to position 42 is helical [36,96], the backbone oxygen atom of Ile-31 is within a van der Waals distance of the sulfur atom of Met-35. Such an interaction could increase the susceptibility for oxidation of the sulfur atoms of Met-35 in Aβ, leading to sulfuramyl free radicals [68,106]. APP in SAMP8 mice has 89.2% homology to the human protein. The amino sequence of Aβ in SAMP8 mice is highly similar to that of humans with the exception of glycine replacing arginine at position 5, phenylalanine instead of tyrosine at position 10, and arginine instead of histidine at position 13 [71]. The critical Met-35 and Ile-31 remain present in Aβ of SAMP8 mice. Moreover, Aβ can increase $O_2^{\cdot -}$ and NO production by stimulating NADPH oxidase and macrophages, respectively [67,83]. Aβ was also shown to mediate H_2O_2 production in cell culture [13], possibly through reduction of copper and iron [65,66]. Another Aβ-mediated reactive oxygen species (ROS) production is through its binding to the receptor of advance glycated end-products (RAGE) [109]. Therefore, it is reasonable that the 12mR mouse brains suffer higher Aβ-mediated oxidative stress than the 4m mouse brains, since the Aβ level was significantly higher in the brains from 12mR compared to that in 4m. Since APP expression was reduced by AO treatments in 12mA SAMP8 mice brain [70], there is less Aβ present in brain to induce direct and indirect oxidative insults. The current study suggests that decreased Aβ production contributed to the decreased protein oxidation and lipid peroxidation in 12-month-old AO SAMP8 mouse brains. Reduction of protein oxidation and lipid peroxidation conceivably could alleviate the mitochondrial dysfunction, redox metal imbalance, advanced glycation end-products, and excitotoxicity that interact with, and are exacerbated by Aβ [26]. Thus, this AO approach, which improves the learning and memory impairment observed in 12-month-old SAMP8 mice [70], points to the importance of Aβ in oxidative stress in this mouse strain, and presumably in AD patients as well.

Previously, we have shown that oxidative stress is closely related to lessening of learning ability and memory retention in SAMP8 mice [47]. It was also demonstrated that using AO against the Aβ region of APP to lower Aβ production could improve learning and memory in SAMP8 mice [70]. In the current study, we extended the previous research and investigated the role of Aβ in oxidative stress associated with learning and memory impairment. Lesion studies in other animals have shown that spatial learning is dependent on the integrity of the septohippocampal pathway [46,89]. Although Aβ may not directly inhibit cellular function, Aβ-mediated oxidative stress in the form of lipid peroxidation may do so [26,28]. It is possible that Aβ-mediated free radicals, possibly through the effects of the lipid peroxidation product HNE and acrolein, react with enzymes, receptors, and neurotransmitters (such as norepinephrine or dopamine), thereby disrupting the function of the septohippocampal pathway. As a result, the functioning of the whole septohippocampal pathway would be decreased. Pharmacological studies have shown that the memory defects in SAMP8 mice are dependent on the septohippocampal pathway [56].

Therefore, injecting AO directed to the Aβ region of APP in 12-month-old SAMP8 mice can alleviate the Aβ-mediated oxidative stress, likely contributing to the improved the learning ability and memory retention as reported [11,70]. Further studies on the effect of Aβ and protection by AO on

the septohippocampal pathway are in progress. These studies should provide a better understanding of the role in Aβ in learning and memory impairment with aging and in AD.

Acknowledgements

The authors would like to thank Marcia Butterfield and Susan Poon for assistance in the preparation of this manuscript. This work was supported in part by grants from NIH [AG-05119, AG-10836, D.A.B], Merit Review (WAB), R01 NS41863 (WAB), R01 AA12743 (WAB) NIA.

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