Peripheral Administration of Antisense Oligonucleotides Targeting the Amyloid-β Protein Precursor Reverses AβPP and LRP-1 Overexpression in the Aged SAMP8 Mouse Brain

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Abstract. The senescence accelerated mouse-prone 8 (SAMP8) mouse model of Alzheimer's disease has a natural mutation leading to age-related increases in the amyloid-β protein precursor (AβPP) and amyloid-β (Aβ) in the brain, memory impairment, and deficits in Aβ removal from the brain. Previous studies show that centrally administered antisense oligonucleotide directed against AβPP can decrease AβPP expression and Aβ production in the brains of aged SAMP8 mice, and improve memory. The same antisense crosses the blood-brain barrier and reverses memory deficits when injected intravenously. Here, we give 6 μg of AβPP or control antisense 3 times over 2 week intervals to 12 month old SAMP8 mice. Object recognition test was done 48 hours later, followed by removal of whole brains for immunoblot analysis of AβPP, low-density lipoprotein-related protein-1 (LRP-1), p-glycoprotein (Pgp), receptor for advanced glycation endproducts (RAGE), or ELISA of soluble $A\beta_{40}$. Our results show that AβPP antisense completely reverses a 30% age-associated increase in AβPP signal ($p < 0.05$ versus untreated 4 month old SAMP8). Soluble $A\beta_{40}$ increased with age, but was not reversed by antisense. LRP-1 large and small subunits increased significantly with age (147.7%, $p < 0.01$ and 123.7%, $p < 0.05$ respectively), and AβPP antisense completely reversed these increases ($p < 0.05$). Pgp and RAGE were not significantly altered with age or antisense. Antisense also caused improvements in memory ($p < 0.001$). Together, these data support the therapeutic potential of AβPP antisense and show a unique association between AβPP and LRP-1 expression in the SAMP8 mouse.

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INTRODUCTION

A common difficulty in development of central nervous system (CNS) therapeutics is promoting their access to the brain. Due to the presence of the blood-brain barrier (BBB), the chemical nature of drugs to treat CNS disease is generally limited to small, non-polar molecules which can diffuse across cell membranes [1]. Given that the BBB is also a dynamic regulatory interface for bidirectional peptide transport [2, 3], there is much interest in utilizing these transport systems for drug delivery to the brain. Previously, our lab and others have used phosphorothioated antisense oligonucleotides to modify protein expression *in vivo* [4–7]. We have also demonstrated that these antisense oligonucleotides have a saturable BBB transport system which allows them to access the CNS and alter expression of their protein targets [5, 8, 9]. Therefore, in addition to their use in studying protein function *in vivo*, antisense also shows potential as a therapeutic for CNS disease.

One example of the therapeutic value of antisense oligonucleotide involves studies done in the SAMP8 mouse model. This mouse strain arose from a spontaneous mutation which results in a phenotype of accelerated aging [10], age-associated increases in brain levels of AβPP and Aβ [11–13] as well as impairments in learning and memory [14]. Therefore, the SAMP8 mouse has been studied as a model for Alzheimer's disease (AD) [15]. We have previously shown that intracerebroventricular (ICV) administration of phosphorothioated antisense oligonucleotide directed against AβPP in aged SAMP8 mice results in decreased levels of AβPP and Aβ in brain and reverses deficits in learning and memory [6]. The same antisense can cross the BBB when administered peripherally and exerts similar behavioral effects as ICV antisense [8]. The latter two findings demonstrate the potential of AβPP antisense as a possible treatment for AD, and warrant further investigation of neurochemical changes in reference to pathways resulting in Aβ accumulation in the brain.

In addition to Aβ accumulation, the SAMP8 mouse also develops deficits in Aβ transport across the BBB with age [16]. According to the neurovascular hypothesis of AD [17], this finding suggests that BBB impairment in this model promotes Aβ accumulation in the CNS and likely contributes to its AD phenotype. Three predominant transporters have been identified at the BBB which play important roles in Aβ transport and have been implicated in AD. These are low-density lipoprotein-related protein-1 (LRP-1), p-glycoprotein (Pgp), and receptor for advanced glycation endproducts (RAGE) [18, 19]. LRP-1 mediates the brain-to-blood transport (efflux) of Aβ [20] and plays a major role in Aβ clearance from the brain [21]. Alterations in brain levels of LRP-1 have been observed in normal aging and AD, and these alterations depend on cell type. Immunoblots using midfrontal cortex homogenates of AD individuals versus age-matched controls demonstrated that total LRP-1 expression in brain gradually decreases with age, and is downregulated to an even greater extent in AD [22]. Another study showed that increased oxidative modifications to LRP-1 were observed in AD hippocampus when compared to age-matched controls [23]. Immunohistochemical studies elucidated that LRP-1 is downregulated in the microvasculature of AD frontal cortex, and that microvascular LRP-1 expression was inversely correlated with degree of Aβ immunoreactivity [20]. A subsequent study found that in hippocampi from aged non-AD individuals, strong staining of LRP-1 was evident in the microvasculature, whereas neurons were weakly stained. This pattern was found to be reversed in AD [24]. Interestingly, evidence supports that through an interaction with AβPP, LRP-1 enhances amyloidogenic AβPP processing [25]. Together, these findings suggest that changes in LRP-1 observed in AD favor increased Aβ production and decreased Aβ clearance from the brain. RAGE has been shown to participate in blood-to-brain transport (influx) of Aβ [26], and is upregulated in brain microvessels with age [27, 28]. Therefore, circulating Aβ can contribute to the Aβ pool in brain, and may also contribute to changes in CNS Aβ observed in AD. Interestingly, the same study by Donahue et al. showing opposing changes in LRP-1 in neurons versus vasculature found that in addition to increased microvascular RAGE expression in AD, neuronal expression of RAGE was decreased compared to age-matched controls. This finding suggests that expression of LRP-1 and RAGE might

be inversely coupled both in normal aging and AD. Pgp is a multi-drug transporter of the ATP-binding cassette family, whose primary function is to restrict circulating components from entering the brain [29]. Pgp has also been identified to play a role in Aβ transport, both by increasing efflux [30, 31] and restricting influx [32] of Aβ. Downregulation of Pgp has been demonstrated in AD brain microvasculature compared to age-matched controls [33], suggesting that a Pgp deficiency may also contribute to Aβ accumulation in AD. Given the association of changes in all three proteins in AD, and the defect in BBB transport of Aβ in the SAMP8 model, an important question is whether expression of these transporters are altered in SAMP8 mice with age. Recently, we have shown that the Aβ efflux deficit in the aged SAMP8 mouse is reversed with AβPP antisense [34], suggesting that AβPP/Aβ pathology in this model may play a role in the regulation of Aβ transport out of the brain. Therefore, it is also possible that Aβ itself plays a role in the regulation of its transport in and out of the brain.

In this study, our first goal was to determine whether peripherally administered AβPP antisense reverses pathological increases in AβPP and Aβ in the brains of aged SAMP8 mice. Our second goal was to determine whether altered expression of the Aβ transporters LRP-1, Pgp, and RAGE were associated with alterations in AβPP/Aβ due to age and antisense in the SAMP8 model. Our findings were 1) that peripherally administered AβPP antisense is effective in reversing age-associated AβPP increases in SAMP8 mouse brains, but does not reduce total soluble Aβ$_{40}$, and 2) that LRP-1 follows a similar pattern of expression as AβPP with age and antisense in this model.

MATERIALS AND METHODS

Animal use

All animal protocols were performed in an AALCC (International Association for Assessment and Accreditation) accredited facility and approved by the animal committee of the VA and St Louis University Medical Centers. SAMP8 mice from in-house colonies were kept on a 12/12 hour light/dark cycle with food and water freely available.

Antisense regimens

Phosphorothioate oligodeoxynucleotide antisense directed at Aβ$_{17-30}$ (5′-(P=S)GGCGCCTTTGTTCG AACCCACATCTTCAGCAAAGAACACCAG-3′) or a random antisense sequence (5′-(P=S) GATCA CGTACACATCGACACCAGTCGCCATGACTGAG CTT-3′) was synthesized by the Midland Certified Reagent Company (Midland, TX). Male 12 month only SAMP8 mice were given 3 tail vein injections of 6 μg AβPP or control antisense (n = 10 per group) suspended in 0.2 ml normal saline at 2 week intervals, followed by behavioral studies at 48 h or two weeks after the final injection. Mice from the 48-h group were anesthetized with 0.15 ml 40% urethane 24 h later (72 h after the final antisense injection) and the upper circulatory system was perfused with Lactated Ringer's solution to clear blood from the vascular space. Whole brains (cerebrum and cerebellum) were harvested, cut in half along the sagittal suture, and each half snap-frozen and stored at −70°C immediately following removal. As an additional control, brains from 4 month old untreated SAMP8 mice (n = 10) were identically harvested for comparison.

Behavioral assessment

Memory assessment was conducted using the object recognition test as previously described [5]. This method tests hippocampal-dependent memory [35] and was chosen over other behavioral tests because it is low-stress and reveals similar cognitive impairments as those observed in AD [36–39]. Prior to testing, mice were habituated for three consecutive days to the testing apparatus (a 58 × 66 × 11 cm white, plastic box). During habituation, each mouse was allowed to freely explore the testing apparatus for 5 min. On the first day of training, mice were placed in the testing apparatus for 5 min and allowed to explore a pair of identical objects (objects A and B; both were 7 × 6.3 × 5.1 cm). On the second day of training, one of the original objects was replaced with a new, or novel, object (Object C; 8.2 × 3.8 × 7.4 cm). Mice were placed in the testing apparatus for 5 min and the amount of time each mouse spent sniffing or touching of the novel object was recorded. Results were expressed as the percent of time spent investigating the novel object.

Protein extraction from brains

Protein extraction for immunoblot analysis was done by adding a 10x volume of extraction buffer (20 mM Tris-HCl, 0.15 M NaCl, 2 mM EDTA, 1% Triton X-100, 1 mM PMSF, Protease Inhibitor cocktail) to each hemibrain, and homogenizing using a Tissue Tearor (Cole-Parmer) homogenizer. Homogenates were shaken at 4°C for 30 min and then centrifuged

twice at 20,000 g to pellet debris. Protein estimates on supernatants were done using a BCA assay (Pierce). Protein extraction for measurement of $A\beta_{40}$ was done using the same protocol in extraction buffer containing 0.2% Triton X-100, which extracts soluble extracellular and intracellular Aβ [40].

Immunoblotting and densitometric analysis for AβPP, LRP-1, Pgp, and RAGE

Prior to analyte measurement in SAMP8 brain homogenates, all experimental conditions were optimized for each antibody to ensure protein loading amounts were within the linear range of signal. The AβPP and LRP-1 antibodies were also validated for their antigen specificity using either knockout brain tissue (AβPP; a generous donation from the lab of Dr. David Cook), or knockout cell lines (LRP-1; MEF-1 and PEA-13 cell lines, ATCC). For measurement of AβPP, 15 μg of denatured and reduced brain protein extract was run on a 4–12% Bis-tris gel (Invitrogen). For measurement of LRP-1, 15 μg of denatured brain protein extract was run on a 3–8% Tris-acetate gel (Invitrogen). For measurement of Pgp, 15 μg of denatured brain protein extract was run on a 4–12% Bis-tris gel (Invitrogen), and for measurement of RAGE, 25 μg of denatured and reduced brain extract was run on a 4–12% Bis-tris gel. Proteins from all gels were then transferred onto nitrocellulose membranes for 8 min at 20 V using an iBlot transfer device (Invitrogen). Portions of the membrane not containing the antigen of interest were either SYPRO stained [41] (Invitrogen) or blocked with 5% milk/PBS-T and probed for β-tubulin (Epitomics, 1 μg/ml in 5% milk, 1 h room temperature (RT)) to confirm even loading. Portions of the membrane containing the antigens of interest were blocked with either 5% milk/PBS-T (AβPP, LRP-1, Pgp) or 5% BSA/PBS-T for 1 h RT and probed for the following: 1) N-terminal LRP-1 subunit (Sigma, 2 μg/ml 5% milk/PBS-T 1 h RT); 2) C-terminal LRP-1 subunit (Epitomics, 0.1 μg/ml 5% milk/PBS-T 1 h RT); 3) AβPP (Epitomics, 1 μg/ml 5% milk/PBS-T 1 h RT); 4) Pgp (Calbiochem, 3 μg/ml 5% milk/PBS-T overnight 4°C); or 5) RAGE (Thermo Scientific, 2 μg/ml 5% BSA/PBS-T overnight 4°C). Membranes were then washed and incubated with anti-rabbit (LRP-1, AβPP, Tubulin, RAGE) or anti-mouse (Pgp) secondary antibodies (Santa Cruz, 1 : 5000 1 h RT), then washed again. Bands were visualized using SuperSignal West Pico chemiluminescent reagents (Thermo Scientific) and images (including SYPRO signal) captured on an ImageQuant LAS 4000 CCD camera (GE Health-

care). Densitometric analysis was done using IQTL software (GE Healthcare). For analysis of SYPRO signal, bands which were detectable across all lanes were quantified, and the sum of signal from each lane was used to normalize the protein signal of interest. This method of normalization was used because housekeeping proteins either changed with treatment, or did not produce linear signal within the range of protein loading amounts under the conditions specified and therefore were unsuitable.

Quantification of $A\beta_{40}$

Colorimetric high-sensitivity kits from WAKO were used to quantify $A\beta_{40}$ in our extracts. Samples were diluted 1 : 3 in standard diluent provided with the kit, and standards were diluted in buffer resembling sample matrix. Samples were then measured for $A\beta_{40}$ according to kit instructions. We have extensively tested this kit for reliable quantification of Aβ levels, and have found that signal is linear over a wide range of brain tissue dilutions, spike recovery is approximately 100%, and signal is specific as demonstrated by lack of signal in AβPP knockout brain tissue (unpublished results). Others have also found that Aβ levels measured by Western blot correlate with Aβ levels measured using this kit [42].

Statistical analysis

All data were analyzed using GraphPad Prism 5 software. The number of mice used in each study is indicated, with error bars representing the standard error of the mean (SEM). Student's *t*-test was used for
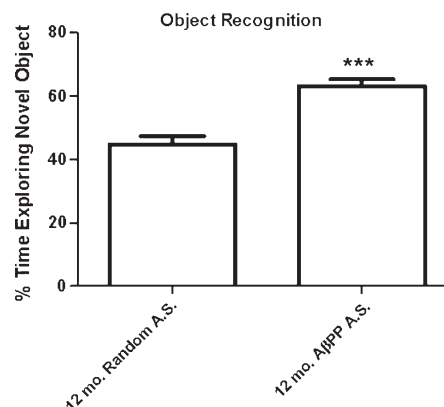

Fig. 1. Object recognition test performance of 12 month old SAMP8 mice 48 h following a regimen of peripherally administered AβPP antisense or random control antisense. Data analyzed by Student's *t*-test ($n = 10$, ***$p < 0.001$).

comparison of two groups. More than two groups were compared by analysis of variance (ANOVA) followed by Newman-Keuls multiple comparisons test.

RESULTS

Assessment of memory following systemic administration of AβPP antisense

48 h after the last injection of the antisense treatment regimen, 12 month old SAMP8 mice were tested for hippocampal-dependent memory based on their ability to recognize a novel object [35]. Figure 1 shows that peripherally administered AβPP antisense improves performance in the object recognition test. There was no difference in the total amount of time spent exploring the objects on either the acquisition or the retention phase of the test. The mean time in seconds spent exploring the objects during acquisition was 25.36 ± 3.36 for random antisense and 23.80 ± 2.97 for AβPP antisense. Because previous studies have examined effects of AβPP antisense 2 weeks after the last injection, a second group of mice was tested for learning and memory 2 weeks after an antisense treatment regimen. In this group, a similar albeit less robust trend was found for AβPP antisense (data not shown). Therefore, all studies of protein expression were performed on the 48-h antisense group.

Effects of antisense on AβPP and Aβ40 expression and on Aβ transport proteins

Following antisense treatment and behavioral assessment, protein expression of AβPP, LRP-1,

RAGE, and Pgp was measured. Preliminary immunoblot studies were carried out using brain tissue extracts to optimize experimental conditions for each antibody, testing signal linearity over a range of protein concentrations (supplementary Figure 1; available online: http://www.j-alz.com/issues/28/vol28-4.html# supplementarydata10). Importantly, AβPP signal was found to saturate at loading amounts higher than $25 \, \mu g$, and Pgp signal was linear up to $25 \, \mu g$, but paradoxically decreased at a loading amount of $50 \, \mu g$. Therefore, caution was taken to ensure that the amount of protein loaded for each immunoblot produced signal within the linear range of detection. The LRP-1 and AβPP antibodies were tested for antigen specificity in knockout tissue, and no immunoreactive bands were observed in knockouts (supplementary Figure 2). AβPP antisense treatment reversed the age-associated increase of AβPP expression to levels comparable to 4 month old SAMP8 mice (Fig. 2a and b). Levels of soluble $A\beta_{40}$ increased with age in the SAMP8 mice, but this was not reversed by antisense (Fig. 2c). We were unable to measure $A\beta_{42}$ in our samples, as levels were either below the detectable range of commercially available assays which can measure murine Aβ, or commercially available kits claiming enhanced sensitivity did not show specificity for Aβ (unpublished findings). Immunoblot analysis showed that SAMP8 mice have an age-dependent increase in both the large and small subunits of LRP-1, and that AβPP antisense reverses this increase in LRP-1 expression (Fig. 3). No significant changes in RAGE were found with age or antisense treatment (Fig. 4a and b). Pgp showed a trend towards an increase with age in the SAMP8 mouse (Fig. 4 c and d), although this
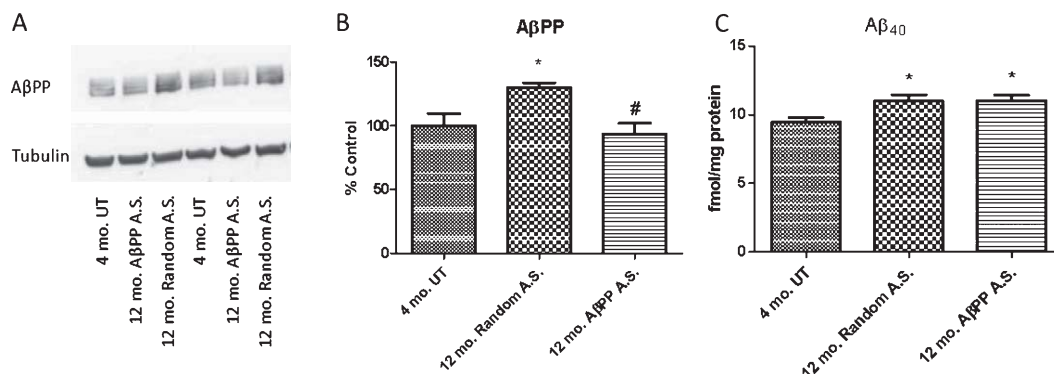

Fig. 2. AβPP and soluble $A\beta_{40}$ protein levels in untreated 4 month old and antisense treated 12 month old SAMP8 mouse brains. A) Representative immunoblot of AβPP. B) Densitometric analysis of AβPP band intensities normalized to β-tubulin ($n = 6$ per group). C) Quantification of soluble $A\beta_{40}$ by ELISA ($n = 8$–10 per group). All data analyzed by one way ANOVA and Newman-Keuls multiple comparisons test, $*p < 0.05$ compared to 4 month old UT, $^{\#}p < 0.05$ compared to 12 month old Random.
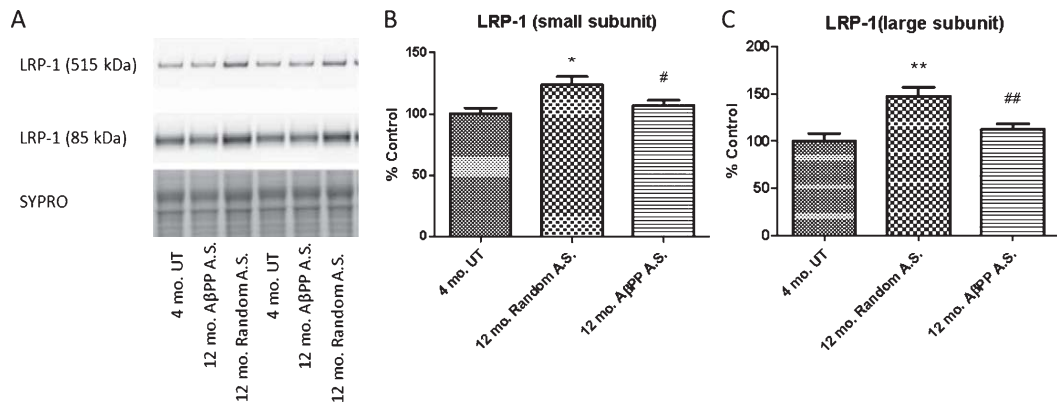
Fig. 3. Protein levels of LRP-1 in untreated 4 month old and antisense treated 12 month old SAMP8 mouse brains. A) Representative immunoblots of LRP-1 large and small subunits. B, C) Densitometric analysis of small and large subunits respectively, band intensities normalized to SYPRO stain ($n = 6$ per group). All data analyzed by one way ANOVA and Newman-Keuls multiple comparisons test, $*p < 0.05$, $**p < 0.01$ compared to 4 month old UT, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared to 12 month old Random.

was not significant due to the high variability of Pgp expression in all samples. To confirm that this trend was not due to Pgp levels in some samples exceeding the linear range of detection, immunoblotting of Pgp was repeated using 5 μg of sample instead of 15. This second blot produced a similar trend in Pgp expression of these samples (data not shown) and therefore the source of variability observed is likely biological.

DISCUSSION

In this study, we have shown for the first time that peripheral administration of antisense which specifically targets AβPP reduces its expression in SAMP8 mouse brains. This was associated with improved performance in the object recognition test, which is a low-stress assessment of hippocampal-dependent memory [35–37]. Individuals with AD also have deficiency in recognizing novel objects, supporting that this test may reveal analogous impairments in murine models [38, 39]. Improvement of cognitive defects in aged SAMP8 mice highlights the therapeutic potential of antisense in AD. This particular antisense molecule has been well-characterized for its ability to cross the BBB by a saturable mechanism, suggesting that a specific transport system exists which facilitates its entry into the brain [43]. Furthermore, this study by Banks et al. showed that uptake of this molecule was highest in hippocampus, one of the predominant brain regions affected in AD. Although the transport system for antisense has not yet been identified, this latter

finding suggests that an increased density of this transporter is also present in hippocampus, offering the advantage of selective delivery to this region.

Results from this study agree well with previous findings that both AβPP and Aβ increase with age in SAMP8 mice [6]. We did not, however, find that AβPP antisense also reduces brain levels of Aβ. Although this does raise the question of whether AβPP itself plays a role in memory impairment in this model, the complexity of Aβ biology and methodology may also explain this finding. It has been extensively shown that the method used for Aβ extraction from brain is reflective of its cellular compartmentalization and aggregation state [40, 44]. Our goal in this study was to measure soluble forms of Aβ, as these (monomeric forms, in particular) are available for transport across the BBB or for cellular uptake [45]. For this reason, our extraction method enriched for soluble forms of Aβ present in both intra- and extracellular compartments. This extraction method may not detect redistribution of compartmentalization and/or aggregation state of Aβ which likely occur following decreases in its production. Given that aggregation status [46–48] as well as cellular localization [49] in the brain are both important in dictating Aβ neurotoxicity, a redistribution of Aβ pools could explain improvement of learning and memory in this model. Because we only measured Aβ40 in our samples, we also cannot rule out the possibility that soluble Aβ42 levels are decreased by antisense. Aβ42 is more prone to aggregation and oligomer formation [50–52], and therefore likely plays an important role in age-associated pathology in the SAMP8 model. This highlights the importance of
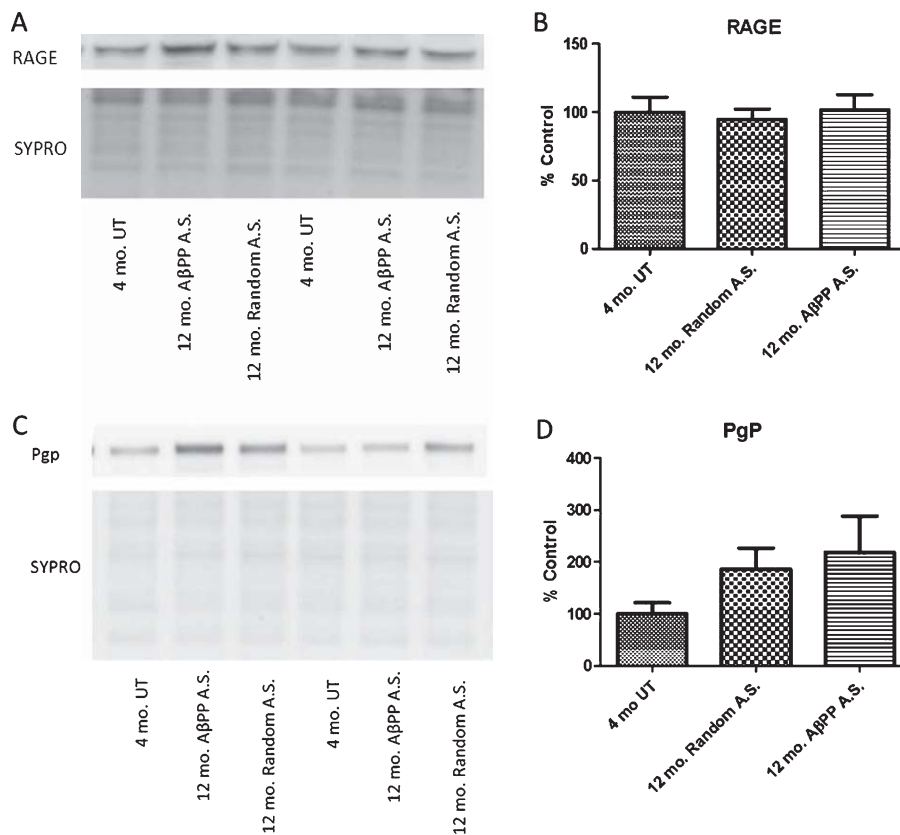
Fig. 4. Protein levels of Pgp and RAGE in untreated 4 month old and antisense treated 12 month oldSAMP8 mouse brains. A, C) Representative immunoblots of Pgp and RAGE respectively. B, D) Densitometric analysis of Pgp and RAGE respectively, band intensities normalized to SYPRO stain ($n = 6$ per group). All data analyzed by one way ANOVA and Newman-Keuls multiple comparisons test.

developing a highly sensitive and specific assay to measure the murine form of $A\beta_{42}$ that is compatible with a variety of extraction methods. Future studies correlating cognitive function to soluble versus total pools of Aβ over a time curve following AβPP antisense administration would provide much information on Aβ biology as well as therapeutic mechanisms of AβPP antisense.

In addition to AβPP, LRP-1 was also found to be overexpressed with aging in brains of SAMP8 mice, and this increase was reversed by AβPP antisense. Although we did find a trend toward an increase in Pgp expression with age which agrees with a previous report from another group [53], we found no significant changes in Pgp or RAGE following AβPP antisense administration. This indicates that AβPP may play a unique role in the regulation of LRP-1 expression. LRP-1 can bind KPI domains of AβPP on its N-terminus [54], and can also interact with its C-terminus via the adaptor protein Fe65 [55, 56]. The latter interaction has been shown to increase AβPP

endocytosis, which increases the amount of AβPP available for BACE cleavage and promotes production of Aβ [57, 58]. Therefore, in addition to overexpression of AβPP, increased expression of LRP-1 which is observed with age in the SAMP8 mouse could contribute to increased Aβ load. LRP-1 has also been implicated in neuronal uptake of Aβ [59]. Because intraneuronal Aβ can be toxic through a variety of mechanisms [49], increased LRP-1 in neuronal tissue in the SAMP8 mouse could play an important role in its age-related pathology. Interestingly, total LRP-1 levels has been found to decrease in both mild cognitive impairment [60] and in AD [22]. Immunohistochemical studies have found that LRP-1 stains predominantly in the microvasculature compared to neurons in aged non-AD individuals, and that expression patterns are reversed in AD, such that predominant LRP-1 expression is in neurons [24] and LRP-1 is downregulated in the brain microvasculature [20, 24]. Furthermore, increases of neuronal Aβ are an early sign of AD [61, 62] and LRP-1 has been shown to mediate neuronal

Aβ uptake [59]. Therefore, a pattern of LRP-1 upregulation in neurons and downregulation in the brain microvasculature would both contribute to Aβ accumulation in the brain. The fact that we observed an increase in AβPP expression in the SAMP8 mouse may reflect a mechanistic difference in SAMP8 and human AD pathology, and/or a temporal mismatch in LRP-1 related changes in brain. One disadvantage to our study is that the use of whole brain homogenate does not provide information about changes in protein expression for specific cell types. However, given that the brain microvasculature makes up approximately 1% of brain tissue by weight, changes in microvascular LRP-1 may be masked by abundant LRP-1 expression in other brain cell types, such as neurons [63]. Therefore, it is possible that LRP-1 expression patterns in the SAMP8 mouse mimic immunohistochemical findings for AD, but that these changes are masked. This may also explain why we did not observe significant changes in total RAGE, even though its expression patterns are also altered in AD and normal aging [27, 28, 30, 64]. Future studies investigating both regional and cell-type specific changes in both AβPP and Aβ transporter expression would provide useful information both on the mechanism of Aβ accumulation in the aged SAMP8 mouse brain, and how well observed changes match pathological findings in AD.

Antisense reversal of LRP-1 overexpression further demonstrates that AβPP plays a regulatory role in LRP-1 expression. In a recent study, it has been shown that LRP-1 expression is increased in AβPP knockout mice due to the lack of transcriptional repression by AICD, an AβPP cleavage product [65]. Although we observed an opposite trend for LRP-1/AβPP expression, there are many differences in our study compared to that of Liu et al. which may explain this apparent conflict. First, AβPP manipulation was done using antisense instead of gene deletion, and knockdown in our study resulted in restoration of AβPP expression to physiological levels. Second, we compared LRP-1 levels in young and aged SAMP8 mice, whereas Liu et al. measured LRP-1 in newborn and 4 month old mice. Therefore, effects of aging, differences in mouse strain, and genetic manipulation could all contribute to conflicting results. Future studies investigating 1) AICD expression in the aged SAMP8 mouse, and 2) transcriptional regulation of LRP-1 by AICD when AβPP expression is decreased with antisense would provide more information on how LRP-1/AβPP interactions affect each other in context of AD. Results from studies mentioned in the previous paragraph, however, do suggest that LRP-1 overexpression in neurons may be

an early pathological event in AD. Therefore, this may be an additional mechanism by which AβPP antisense exerts its therapeutic effects in the SAMP8 mouse.

In summary, we have shown that peripherally administered AβPP antisense is effective in reducing expression of its target in brain. Our findings suggest, however, that the mechanism by which improvement of learning and memory occurs likely involves multiple systems which participate in Aβ homeostasis within the brain. This highlights the possibility that novel AD therapeutics that target established pathways which reduce Aβ toxicity and/or improve clearance in addition to reducing Aβ production may show improved efficacy.

ACKNOWLEDGMENTS

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