Arundic Acid Ameliorates Cerebral Amyloidosis and Gliosis in Alzheimer Transgenic Mice

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ABSTRACT

Like microglia, reactive astrocytes produce a myriad of neurotoxic substances in various brain pathologies, such as Alzheimer’s disease (AD), trauma, and cerebral ischemia. Among the numerous products of reactive astrocytes, attention has recently been directed toward the possible detrimental role of S100B, because the protein has been shown to be highly expressed along with the progression of brain damage and to exert neurotoxic effects at high concentrations. The present study aimed to examine the possible role of astrocyte-derived S100B in the progression of cerebral amyloidosis and gliosis in transgenic mice overproducing mutant amyloid precursor protein (Tg APPsw mice, line 2576). For this purpose, arundic acid (Ono Pharmaceutical Co., Ltd., Mishima, Osaka, Japan), which is known to negatively regulate astrocyte synthesis of S100B, was orally administered to Tg APPsw mice for 6 months from 12 months of age, and the effects of the agent on the above parameters were examined. Here, we report that β-amyloid deposits along with amyloid-β peptide/S100B levels, as well as β-amyloid plaque-associated reactive gliosis (astrocytosis and microgliosis), were significantly ameliorated in arundic acid–treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice at 19 months of age. Based on the above results, arundic acid is considered to deserve further exploration as a promising therapeutic agent for AD.

Accumulating evidence indicates that bystander neurotoxicity from glial inflammatory mechanisms plays a critical role in the pathogenesis of Alzheimer’s disease (AD) (Griffin et al., 1998b; Akiyama et al., 2000). Past research has focused on β-amyloid plaque-associated activated microglia because of their well documented roles in exacerbating or mitigating AD pathology (Akiyama et al., 2000). However, recent evidence suggests that a myriad of biologically active substances, which are produced by activated astrocytes, may play dichotomous roles in various brain pathologies, including AD and stroke (Ridet et al., 1997; Akiyama et al., 2000; Mrak and Griffin, 2001; Nederengaard and Dinnagl, 2005). Among these substances, increasing attention has been directed toward S100B on account of the following findings. 1) S100B at micromolar concentrations acts to kill neurons by causing overexpression of inducible nitric-oxide synthase and subsequent release of nitric oxide (Hu et al., 1997). 2) S100B production is up-regulated in a variety of neurodegenerative diseases, including AD (Mrak and Griffin, 2001). 3) Overexpression of S100B precedes the appearance of neuritic β-amyloid plaques in a transgenic mouse model of AD (Sheng et al., 2000). 4) S100B activates nuclear factor-κB, a key transcription factor in inflammatory responses (Lam et al., 2001).

With regard to stroke, we have shown that the occurrence of delayed infarct expansion after permanent focal ischemia in rats was associated with increased S100B production by activated astrocytes (Matsumi et al., 2002). Pharmacological inhibition of astrocytic S100B synthesis by a novel agent, arundic acid [(R)-(−)-2-propyloctanoic acid (ONO-2506); Ono Pharmaceutical Co., Ltd., Osaka, Japan], led to significant amelioration of both astrocytic activation and delayed infarct expansion.

ABBREVIATIONS: AD, Alzheimer’s disease; Aβ, amyloid-β peptide; CAA, cerebral amyloid angiopathy; ONO-2506, [(R)-(−)-2-propyloctanoic acid; GFAP, glial fibrillary acidic protein; ELISA, enzyme-linked immunosorbent assay; Tg APPsw, transgenic mouse overproducing mutant amyloid precursor protein; PBS, phosphate-buffered saline; GABA_A R, GABA_A receptor.
(Tateishi et al., 2002). Hereby, it is important to note that, as far as our past studies are concerned, the primary action of arundic acid seems to be inhibition of mRNA expression of S100B in activated astrocytes. In addition, arundic acid has been shown to exert additional effects both in vitro and in vivo on other biomolecules, such as inhibition of the expression of nerve growth factor β, inducible nitric-oxide synthase, and cyclooxygenase-2, as well as augmentation of the mRNA expression of glutamate transporters (glutamate transporter subtype 1 and glutamate/aspartate transporter) and GABA receptors (GABA_A β1, GABA_A β2, and GABA_A β3) in activated (reactive) astrocytes. It is, of course, difficult to know whether arundic acid modulates these other substances directly or indirectly via its effect on S100B. From available evidence, we hypothesized that the additional effects of the compound are secondary to suppression of astrocytic S100B synthesis, as we have discussed previously (Asano et al., 2005).

We have further examined the effect of arundic acid on astrocytic activation and delayed infarct expansion after permanent focal ischemia in apolipoprotein E knock-in mice, because the apolipoprotein E4 isoform is a salient risk factor for not only AD but also for stroke (Sorbi et al., 1995; Leung et al., 2002). The apolipoprotein E4 knock-in mice exhibited significant aggravation of astrocytic activation and delayed infarct expansion after permanent focal ischemia relative to apolipoprotein E2 or apolipoprotein E3 knock-in mice (Mori et al., 2004). Because the above detrimental effect of the apolipoprotein E4 isoform was abolished by administration of arundic acid, it is considered to be mediated by enhanced expression of S100B, most likely derived from astrocytes (Mori et al., 2005). Based on the above evidence, we hypothesized that a similar pathogenetic mechanism as above may operate in AD, whereby astrocytic activation may worsen the progression of AD pathology. To clarify this hypothesis, we sought to investigate whether suppression of S100B-associated astrocytic activation by arundic acid may attenuate β-amyloid plaque burden and amyloid-β peptide (Aβ) levels, as well as β-amyloid plaque-associated reactive gliosis (astrocytosis and microgliosis), in arundic acid-treated aged transgenic mice overproducing mutant amyloid precursor protein (Tg APPsw mice, line 2576) by comparing with vehicle-treated aged Tg APPsw mice or vehicle-treated age-matched wild-type littersmates. In the present study, all of the above parameters were significantly ameliorated in arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice. The above amelioration of AD-like pathology was accompanied by significant decreases in both S100B tissue levels and S100B burden by image analysis. The results of the present study thus bolster the view that pharmacological modulation of S100B-associated astrocytic activation by arundic acid may confer a novel therapeutic strategy against AD.

Materials and Methods

Mice. Male Tg APPsw mice (line 2576, C57BL/6×SJL background) and their wild-type littermates of the same gender were purchased from Taconic (Germantown, NY). This transgenic mouse model of AD overproduces human Aβ_{40} and Aβ_{42} and develops progressive β-amyloid deposits and learning and memory impairment beginning at 9 to 10 months of age (Hsiao et al., 1996). Animals were housed and maintained in a specific pathogen-free barrier facility under a 12-h/12-h light/dark cycle, with ad libitum access to food and water. All experiments were performed in accordance with the guidelines of the Animal Use Ethics Committee of the Saitama Medical School and National Institutes of Health (NIH) guidelines [Department of Health and Human Services (NIH) Publication No. 85-23, revised 1985].

Experimental Design. The inhibitory actions of arundic acid on astrocytic S100B synthesis, as well as its general pharmacological actions, have been described elsewhere (Asano et al., 2005). Tg APPsw mice were randomly assigned to the following two experimental groups: 1) the arundic acid-treated group (n = 14) or 2) the vehicle control group (n = 15). In addition, wild-type littersmates were assigned to the vehicle control group (n = 14). Arundic acid (lot number 206008; 10 mg/kg/vehicle: 0.1% v/v Tween 80) was orally administered once a day. At a dose of 10 mg/kg, arundic acid was shown to exhibit pronounced inhibitory actions on delayed infarct expansion, peri-infarct astrocytosis, and S100B overexpression after focal ischemia in rats (Tateishi et al., 2002) and mice (Mori et al., 2005). Treatment was started at 12 months of age and continued for 6 months. One month after withdrawal of drugs (19 months of age), animals were euthanized for examining the effect of arundic acid on cerebral amyloidosis, β-amyloid plaque-associated reactive astrocytosis/microgliosis, and expression of S100/S100B tissue levels in brains from each of the Tg APPsw or wild-type mice described above.

Brain Tissue Preparation. At 19 months of age, animals were anesthetized with sodium pentobarbital (25 mg/kg) and euthanized by transcardial perfusion with ice-cold physiological saline containing heparin (10 U/mL). Brains were rapidly isolated and quartered (sagittally at the level of the longitudinal fissure of the cerebrum and then coronally at the level of the anterior commissure) using a mouse brain slicer (Muromachi Kikai, Tokyo, Japan). The left and right anterior cerebral quarters were quick-frozen at −80°C. The left anterior cerebral quarters were used for guanidine-soluble Aβ ELISA, and the right anterior cerebral quarters were used for SDS-soluble Aβ ELISA, S100B ELISA, and Western blot analyses. The left posterior cerebral quarters were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer at 4°C overnight, and routinely processed in paraaffin for immunohistochemical analyses. The right posterior cerebral quarters were quick-frozen at −80°C for immunohistochemical analyses.

Immunohistochemistry. For paraffin and frozen sectioning, five coronal sections (per set) with a 100-μm interval were made at a thickness of 5 μM in each separate region [for cingulate cortex (CC) bregma −0.10 mm to −0.82 mm; for entorhinal cortex (EC) and hippocampus (H), bregma −2.92 mm to −3.64 mm]. Three sets of sections in each separate region were prepared for analyses of Aβ, S100, or glial fibrillary acidic protein (GFAP) (astrocytosis) burden as well as β-amyloid plaques (number and diameter (maximal length)). One set of sections in each separate region was prepared for Iba-1 (microgliosis) burden analysis. Immunohistochemical staining was done according to the manufacturer’s protocol using a Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA) coupled with the diaminobenzidine reaction, with the exception that the biotinylated secondary antibody step was omitted for Aβ immunohistochemical staining. The following primary antibodies were used for immunohistochemical staining: a biotinylated human amyloid-β monoclonal antibody (4G8; 1:100; Signet Laboratories, Dedham, MA), GFAP and S100 polyclonal antibodies (1:500 and undiluted, respectively; DAKO, Carpinteria, CA), and an Iba-1 polyclonal antibody (1:1000; Wako, Osaka, Japan). The S100 antibody used for immunohistochemical staining is polyclonal and does not discriminate among different S100 isoforms; hence, we adapted the designation of “S100” to express the corresponding results. Normal rabbit or normal mouse serum (isotype control) or phosphate-buffered saline (PBS, 0.1 mM, pH 7.4) was used instead of primary antibody or ABC reagent as a negative control.

Image Analysis. Quantitative image analysis was done based on a previous method (Tan et al., 2002), with modifications. Images were acquired as digitized tagged-image format files to retain maximal resolution using an Olympus BX60 microscope with an attached digital camera system (DP-70; Olympus, Tokyo, Japan), and digital images were routed into a Windows PC for quantitative analyses using SimplePCI software (Compix, Inc. Imaging Systems, Cranberry Township, PA). The cingulate region was captured from the
image of the cortex adjacent to the sagittal fissure, and the entorhinal region was captured from the image of the cortex ventral to the rhinal fissure. In images from cingulate and entorhinal regions, the cortical edge was not included in order to capture the full anatomic region of interest. The hippocampal region was captured from between a portion of the CA1 subfield of the pyramidal cell layer and the lacunosum molecular layer. The anatomical locations and boundaries of the regions analyzed were based on those defined by Franklin and Paxinos (2001). Images of five 5-μm sections through each anatomic region of interest were captured, and a threshold for optical density was obtained that discriminated staining from background. Each anatomic region of interest was manually edited to eliminate artifacts. For β4, GFAP (astrocytosis), Iba-1 (microgliosis), and S100 burden analyses, data are represented as percentage of immunolabeled area captured (positive pixels) relative to the full area captured (total pixels). For β-amyloid plaque morphometric (number and diameter [maximal length]) analyses, diameters (maximal length) of β-amyloid plaques were measured, and numbers of β-amyloid plaques falling into three diameter categories (<25, 25–50, or ≥50 μm) were calculated. Each analysis was done by a single examiner blinded to sample identities.

ELISA. For ELISA analyses of SDS-soluble AB1-40 and AB1-42, brain Aβ species were extracted in 0.15% SDS solution containing 2 mM EDTA and centrifuged at 15,000g for 5 min. To measure SDS-soluble AB1-40 and AB1-42, 1:1000 and 1:500 dilutions of SDS-soluble extracts were made, respectively. SDS-soluble AB1-40 and AB1-42 were subsequently measured using ELISA kits (catalog numbers 477731 and 477711; IBL, Gunma, Japan), which rely on combinations of AB1-40 (1A10; capture) or AB1-42 C-terminal polyclonal antibodies (capture) with an Aβ middle region-specific monoclonal antibody (12B2; detection) (Horikoshi et al., 2004). Standard curves were generated using human synthetic β4 (5.81–500 pg/ml; IBL) or β4 (12.5–800 pg/ml; IBL) peptides, and all samples fell within the linear range of the standard curve. For ELISA analyses of guanidine-soluble AB1-40 and AB1-42, brain Aβ species were acid-extracted in a buffer containing 5 M guanidine and 50 mM Tris-HCl, pH 8.0, by gentle shaking at room temperature for 4 h followed by centrifugation at 15,000g for 5 min (Johnson-Wood et al., 1997). To measure guanidine-soluble AB1-40 and AB1-42, 1:50,000 and 1:8000 dilutions of guanidine-soluble extracts were made, respectively. Guanidine-soluble Aβ species were subsequently measured using the above-mentioned ELISA kits. For ELISA analysis of S100B, supernatants extracted in 0.15% SDS were used. S100B was measured by ELISA using a S100B monoclonal antibody (SH-B4; capture, 1:1000; Sigma, St. Louis, MO) with a S100B polyclonal antibody (detection, 1:1000; DAKO) (Revesz et al., 2005). Standard curves were generated using bovine S100B protein (0.03–300 ng/ml; Calbiochem, La Jolla, CA), and all samples fell within the linear range of the standard curve. All ELISA values were reported as ng/mg of total protein. Each analysis was done by a single examiner blinded to sample identities.

Western Blot. For Western blot analysis of full-length (holo) APP, supernatants extracted in 0.15% SDS were used. Aliquots of the homogenate containing 1 μg of total protein were electrophoretically separated using 7.5% Tris-glycine gels according to molecular weights of the target molecules. Electrophoresed proteins were then transferred to polyvinylidene difluoride membranes (Bio-Rad, Richmond, CA), and all samples fell within the linear range of the standard curve. The target molecules. Electrophoresed proteins were then transferred to polyvinylidene difluoride membranes (Bio-Rad, Richmond, CA), and all samples fell within the linear range of the standard curve. Western blots were then washed in PBS, and blocked 1 h at ambient temperature in Block Ace (Dainippon Sumitomo Pharma, Osaka, Japan). After blocking, membranes were hybridized for 1 h at ambient temperature with an N-terminal APP antibody (10D1; 1:1000; IBL). Membranes were then washed three times for 15 min each in PBS containing 0.05% Tween 20 and incubated for 1 h at ambient temperature with the horseradish peroxidase-linked anti-mouse IgG (1:5000; Amersham Biosciences, Piscataway, NJ). Blots were developed using ECL Plus Western Blotting Detection Reagents (Amersham Biosciences).

Statistical Analysis. Data were presented as the mean ± 1 S.E.M. Histograms were generated to evaluate the normality of the data. If data were not found to be normally distributed, the nonparametric Kruskal-Wallis H test was performed following by post hoc testing using the Mann-Whitney U test. If data were found to be normally distributed, statistical analysis was performed using parametric one-way ANOVA, followed by post hoc comparison of the means using Bonferroni’s or Dunnett’s T3 methods (where appropriateness was determined using Levene’s test for equality of the variance). P values of less than 0.05 were considered to be significant. All analyses were performed using the Statistical Package for the Social Sciences, release 12.0 (SPSS, Inc., Chicago, IL).

Results

Arundic Acid Mitigates β-Amyloid Pathology and Aβ Levels in Tg APPsw Mice. Aβ (4G8) immunohistochemistry in Tg APPsw mice disclosed pronounced β-amyloid deposits both in the cerebral parenchyma and in cerebral blood vessels at 19 months of age. Vehicle-treated-Tg APPsw mice had a typical high percentage of Aβ burden in the three brain regions examined. Aβ burden was significantly reduced by 45 to 66% in arundic acid-treated Tg APPsw mice (P < 0.001 for CC, EC, and H) (Fig. 1, A and E). To further evaluate which subsets of β-amyloid plaques were responsible for the observed Aβ burden reduction, morphometric analysis of β-amyloid plaques was performed in the hippocampus and other cortical regions as mentioned above. Arundic acid-treated Tg APPsw mice showed a significant reduction in large (≥50 μm), medium (25–50 μm), and small-sized (<25 μm) β-amyloid plaque subsets (percentage reduction in large, medium, and small subsets: CC: 63%, P < 0.01; 45%, P < 0.01; and 48%, P < 0.01; EC: 82%, P < 0.001; 56%, P < 0.01; and 40%, P < 0.01; H: 68%, P < 0.001; 41%, P < 0.05; and 27%, P < 0.05) (Fig. 1, B–D). It is interesting that, among each of the brain regions examined, the large β-amyloid plaque subset showed the greatest reduction.

It has been reported that Tg APPsw mice are more prone to vessel pathology (i.e., cerebral amyloid angiopathy; CAA, similar to that observed in AD patients) relative to other AD mouse models [e.g., platelet-derived growth factor B promoter-driving mutant APP(V717F) transgenic mice (Fryer et al., 2003)]. We also attempted to determine whether arundic acid might alter cerebral vascular amyloidosis in Tg APPsw mice. We found that CAA-immunolabeled area ranged between 0.3 and 0.4% in each region examined; however, we did not detect a statistically significant difference between arundic acid-treated and vehicle-treated Tg APPsw mice. One possibility is that arundic acid does not alter CAA levels. It is also possible that the small percentage of the CAA-immunolabeled area we observed is partly due to the fact that we have excluded the brain surface containing the pial arteries from each anatomic region of interest; therefore, our technique is not sensitive enough to detect a difference if one was in fact present. In any event, such a hypothetical difference would likely be of small effect size.

The above findings of reduced 4G8-positive β-amyloid deposits were further validated by Aβ ELISA analysis. Specifically, this assay disclosed that Aβ levels in both SDS-soluble and guanidine-soluble fractions were significantly decreased in arundic acid-treated Tg APPsw mice. In the SDS-soluble fraction, the reduction of Aβ1-40 and Aβ1-42 levels was 24% (P < 0.01) and 28% (P < 0.01), respectively, whereas in the guanidine-soluble fraction, the corresponding reductions were 41% (P < 0.01) and 39% (P < 0.01) (Fig. 2, A and B). To ensure that such reductions in β-amyloid pathology and Aβ levels were not due to arundic acid-mediated down-regulation of the mutant human APP transgene under regulatory control of the hamster...
Fig. 1. Quantitative analysis of β-amyloid plaques in arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice at 19 months of age. Percentage reduction of Aβ burden in arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice is indicated for cingulate cortex (CC), entorhinal cortex (EC), and hippocampus (H). A, Aβ burden (%) was quantified by image analysis. Significant differences were noted in each brain region examined between arundic acid-treated and vehicle-treated Tg APPsw mice (* * * P < 0.001 for each comparison). Morphometric analysis (mean plaque subset number per mouse) is shown for CC (B), EC (C), and H (D). There were significantly fewer large (>50 μm), medium (between 25 and 50 μm), and small-sized (<25 μm) β-amyloid plaques in arundic acid-treated Tg APPsw mice (+, P < 0.05; **, P < 0.01; *** P < 0.001 for each comparison) relative to vehicle-treated Tg APPsw mice in each brain region examined. E, at 19 months of age, mouse brain sections from the indicated brain regions were stained with 4G8 antibody. Vertical photo sets are for the individual mouse groups indicated at the bottom. Scale bar denotes 50 μm. F, Western blot analysis using antibody 10D1 against the N terminus of APP showed comparable full-length APP expression in brain homogenates of arundic acid-treated and vehicle-treated Tg APPsw mice. Western blots shown are of individual brain homogenates that were representative of each treatment group.
prion promoter, Western blot analysis was performed and showed that full-length holo APP expression did not change in brain homogenates of arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice (Fig. 1F).

Arundic Acid Ameliorates Aβ-Associated Reactive Gliosis and S100(B) Production in Tg APPsw Mice. At 19 months of age, vehicle-treated Tg APPsw mice exhibited pronounced Aβ-amyloid plaque-associated reactive astrocytosis and microgliosis, as evidenced by enhanced expression of GFAP/Iba-1 in glial somata and processes, respectively. Reactive gliosis was typically located in close proximity to β-amyloid plaques as described previously (Benzing et al., 1999; Stalder et al., 1999; Lim et al., 2000; Tan et al., 2002). S100 immunohistochemistry revealed faint staining of activated astrocytic processes, disclosing delicate structures. Numerous minute S100-positive granules, which were probably within the astrocytic processes, were dispersed between neurons. Wild-type littermates generally showed much less GFAP/S100 and Iba-1 immunostaining relative to vehicle-treated Tg APPsw mice, although a tendency toward increased GFAP burden was noted in the hippocampus (Figs. 3–5). Compared with wild-type littermates, the magnitude of astrocytosis as assessed by GFAP burden in each of the three brain regions examined was significantly increased in vehicle-treated Tg APPsw mice, whereas it was significantly reduced by 42 to 56% in arundic acid-treated mice.

**Fig. 2.** ELISA measurement of 0.15% SDS-soluble Aβ species (A), 5 M guanidine-soluble Aβ species (B), and S100B (C) in arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mouse brain homogenates at 19 months of age. Percentage reduction of 0.15% SDS-soluble Aβ species, 5 M guanidine-soluble Aβ species, and S100B in arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice is indicated. Data are expressed as nanogram/milligram protein (**, P < 0.01; ***, P < 0.001 for each comparison).

**Fig. 3.** Astrocytosis is reduced in arundic acid-treated Tg APPsw mice. A, at 19 months of age, mouse brain sections from the indicated brain regions were stained with GFAP antibody. Vertical photo sets are for the individual mouse groups indicated at the bottom. CC, cingulate cortex; EC, entorhinal cortex; H, hippocampus. Scale bar denotes 50 μm. B, at 19 months of age, GFAP burden (%) was calculated by quantitative image analysis. Percentage reduction of GFAP burden in arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice is indicated for each brain region. Significant attenuation of astrocytosis was noted in arundic acid-treated Tg APPsw mice in each brain region examined relative to vehicle-treated Tg APPsw mice (***, P < 0.001 for each comparison).
Tg APPsw mice relative to vehicle-treated Tg APPsw mice (P < 0.001 for CC, EC, and H) (Fig. 3B). Likewise, the magnitude of microgliosis as assessed by Iba-1 burden in the three brain regions examined was significantly enhanced in vehicle-treated Tg APPsw mice relative to wild-type littermates, whereas it was significantly reduced by 40 to 46% in arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice (P < 0.001 for CC, EC, and H) (Fig. 4B). S100 burden, which was typically observed in close proximity to β-amyloid plaques, was significantly increased in vehicle-treated Tg APPsw mice in the three brain regions examined relative to wild-type littermates. It is noteworthy that S100 burden was significantly reduced by 39 to 45% in arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice (P < 0.001 for CC, EC, and H). In each brain region examined, there was no significant difference in the magnitude of S100 burden between arundic acid-treated Tg APPsw mice and wild-type littermates (Fig. 5B).

Consistent with results from the immunohistochemical analysis, ELISA from brain homogenates revealed that the S100B levels in arundic acid-treated Tg APPsw mice were significantly reduced by 24% (P < 0.001) relative to vehicle-treated Tg APPsw mice, whereas these levels were not significantly different from wild-type littermates. Thus, if one subtracts out “background” levels of S100B in vehicle-treated, wild-type littermates from the other two mouse groups, S100B levels in arundic acid-treated Tg APPsw mice were reduced overall by as much as 74% (Fig. 2C).

**Discussion**

The present study aimed to examine whether pharmacological suppression of astrocytic S100B synthesis by administration of arundic acid might ameliorate the progression of AD-like pathology in Tg APPsw mice. Our previous studies...
have shown that arundic acid negatively regulates S100B synthesis by suppressing mRNA expression in activated astrocytes (Asano et al., 2005). In accordance with the above finding, arundic acid reduced astrocytic expression of S100B in Tg APPsw mice. Moreover, arundic acid significantly suppressed cerebral amyloidosis (Aβ levels/β-amyloid deposits) and β-amyloid plaque-associated reactive gliosis (astrocytosis and microgliosis). In particular, Aβ plaques of all three plaque size subsets (<25, 25–50, or >50 μm) in each brain region examined were significantly decreased in arundic acid-treated Tg APPsw mice. The concurrent decrease in brain levels of both SDS-soluble and guanidine-soluble Aβ corroborates these data. To our knowledge, this is the first demonstration that pharmacological suppression of astrocytic S100B synthesis confers therapeutic effects for AD-like pathology in Tg APPsw mice; hence, the possible mechanisms of action of arundic acid on the interplay among increased astrocytic S100B synthesis, formation of β-amyloid plaques, and increased brain Aβ levels in Tg APPsw mice are discussed below.

Inflammatory responses incurred by microglial and astrocytic activation in addition to abnormalities in the neuronal Aβ synthetic pathway and in brain Aβ clearance are thought to contribute to AD pathogenesis (Akiyama et al., 2000). Formation of β-amyloid plaques is closely associated with activation of astrocytes and microglia, and the subsequent occurrence of inflammatory responses (Benzing et al., 1999; Stalder et al., 1999; Lim et al., 2000; Tan et al., 2002). Among the many biologically active substances that activated microglia produce, interleukin-1 has been shown to activate astrocytes, thereby promoting S100B synthesis. S100B released from activated astrocytes in turn causes further activation of microglia, which culminates in the establishment of the vicious “cytokine cycle” and aggravation of inflammatory responses (Griffin et al., 1998b). The cytokine cycle leads to the release of large amounts of pro-inflammatory cytokines and toxic substances by activated microglia and astrocytes, such as oxygen radicals and nitric oxide, resulting in further enhancement of brain inflammation and bystander injury in AD (Griffin et al., 1998b; Akiyama et al., 2000). In fact, it has been shown that S100B production is up-regulated in patients with AD (Mrak and Griffin, 2001), and overexpression of S100B precedes the appearance of neuritic β-amyloid plaque pathology in a transgenic mouse model of AD (Sheng et al., 2000). Moreover, neuritic changes in β-amyloid plaques in Down’s syndrome were shown to be closely associated with astrocytic overproduction of S100B (Griffin et al., 1998a). Thus, the above findings may be interpreted as S100B being an important perpetrator in the progression of diffuse, nonfibrillar β-amyloid plaques to neuritic β-amyloid plaques, thereby exacerbating the progression of AD pathology (Mrak et al., 1996; Mrak and Griffin, 2001).

Our results showing that arundic acid significantly attenuated cerebral amyloidosis (Aβ levels/β-amyloid deposits) along with β-amyloid plaque-associated reactive astrocytosis are in full agreement with the above view. Importantly, microgliosis was also significantly inhibited, suggesting that the feed-forward cytokine cycle allowing for astrocytic activation of microglia was suppressed by arundic acid. It deserves mentioning that glial activation is proportional to β-amyloid plaque load (Benzing et al., 1999; Stalder et al., 1999; Lim et al., 2000; Tan et al., 2002). However, this does not necessarily imply a unidirectional relationship between the two events. Clearly, it is the formation of Aβ plaques (and/or the increase in soluble Aβ levels) that takes place first. However, the succeeding growth and maturation of the plaques are in all likelihood incurred by the mutual interactions of the two events. Therefore, the putative action of arundic acid needs to be discussed in relation to the mechanisms underlying both glial activation and amyloidosis.

Firstly, it is possible that arundic acid might act to alter APP expression in Tg APPsw mice, either by affecting endogenous murine APP levels or by modulating the mutant human APP transgene that is under regulatory control of the hamster prion promoter. To clarify this point, we examined APP expression in brain homogenates of arundic acid-treated Tg APPsw mice relative to vehicle-treated Tg APPsw mice by Western blot analysis. Because APP expression in brain homogenates was similar between these two groups, it seems improbable that reduction in APP expression was responsible for reduction of amyloidosis induced by arundic acid.

Secondly, it has been suggested that activation of nuclear factor-κB, which mediates inflammatory responses, might play a cardinal role in the progression of amyloidogenesis in Tg APPsw mice. This suggestion arises because long-term administration of an inhibitor of nuclear factor-κB, indomethacin, significantly reduces brain Aβ burden and Aβ levels in Tg APPsw mice (Sung et al., 2004). Nuclear factor-κB is downstream of S100B (Lam et al., 2001), and in the present study, enhancement of astrocytic S100B synthesis in aged Tg APPsw mice was significantly inhibited by arundic acid. In this regard, we have observed that lipopolysaccharide-induced activation of nuclear factor-κB in cultured astrocytes was significantly inhibited by arundic acid (T. Mori, T. Shimoda, and N. Tateishi, unpublished data). Therefore, it seems likely that inhibition of nuclear factor-κB at least partly participated in arundic acid-induced reduction of Aβ levels. To validate the role of S100B per se in promoting AD-like pathology and to further explore the possible pathogenic interplay among S100B, nuclear factor-κB, and amyloidosis, we are now in the process of crossing S100B-overexpressing mice with Tg APPsw mice.

Thirdly, our results showing arundic acid-induced significant decrease in brain levels of SDS-soluble as well as guanidine-soluble Aβ in the face of unaltered APP production in Tg APPsw mice indicate that arundic acid affected amyloidogenesis in these mice. Although it is unknown whether arundic acid directly inhibits β-secretase, the reduction in the expression of S100B as well as nuclear factor-κB induced by arundic acid is likely a pertinent mechanism as discussed above. However, this interpretation is further complicated by the following issue. Whereas Aβ synthesis has classically been attributed to altered APP processing at neuronal synapses, it has recently been demonstrated that reactive astrocytes surrounding β-amyloid plaques express β-secretase in AD patients and in Tg APPsw mice (Hartner-Lubsamen et al., 2003; Rossner et al., 2005). It is noteworthy that this finding provides a straightforward explanation for arundic acid-induced reduction of amyloidogenesis, because the agent simultaneously reduced the magnitude of reactive astrocytosis. Therefore, it is tempting to speculate that suppression of S100B expression induced by the agent led to inhibition of astrocyte-derived β-secretase through generalized suppression of reactive astrocytosis on one hand and to inhibition of
synapse-derived β-secretase through reduction of brain levels of S100B and other cytokines, such as interleukin-1, on the other hand. Resolution of this issue is beyond the scope of the present study, but we intend to address it in our next study.

Finally, it is tempting to speculate that, in addition to the pro-inflammatory effects of astrocytic S100B, its effects on neural reparative processes might be related to the formation of β-amyloid plaques. In humans, synaptic dysfunction occurs in both the prodromal and the clinical phases of AD (Selkoe, 2002), and excessive neuroplastic burden has been postulated to be a prime mover for the disease process (Mesulam, 1999). There is a host of evidence indicating that astroglial filodia in tripartite synapses sense alterations in synaptic transmission, leading to activation of astrocytes (Haydon, 2001). Activated astrocytes confer both reparative and destructive actions, which are considered to be at least partially mediated by altered levels of S100B (McAdory et al., 1998).

Thus, pharmacological suppression of astrocytic S100B synthesis by arundic acid may have dual significance in slowing the progression of the disease process; in AD cerebral regions demarked by relatively low levels of S100B, arundic acid might inhibit neurite extension in response to cerebral amyloidosis, thereby decreasing neuroplastic burden. In AD brain regions with high levels of S100B, the agent may attenuate the damaging cytokine cycle, hence suppressing the autotoxic loop and reducing β-amyloid plaque formation (Mrak et al., 1996; Sheng et al., 2000; Mrak and Griffin, 2001).

In conclusion, we demonstrate that arundic acid acts to negatively regulate reactive astrocyte-associated S100B levels in a transgenic mouse model of AD-like cerebral amyloidosis. Furthermore, we show that the agent remarkably attenuates cerebral amyloidosis along with β-amyloid plaque-associated reactive gliosis in Tg APP/△71 mice. If AD-like pathology in these Alzheimer model mice is indeed representative of the clinical syndrome, these data suggest that pharmacological inhibition of S100B biosynthesis may be a novel and valuable therapeutic target, particularly to slow disease progression and/or to delay its onset.

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References


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