Cerebral Amyloid Angiopathy Increases Susceptibility to Infarction After Focal Cerebral Ischemia in Tg2576 Mice

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Background and Purpose—We and others have shown that soluble amyloid β-peptide (Aβ) and cerebral amyloid angiopathy (CAA) cause significant cerebrovascular dysfunction in mutant amyloid precursor protein (APP) mice, and that these deficits are greater in aged APP mice having CAA compared with young APP mice lacking CAA. Amyloid β-peptide in young APP mice also increases infarction after focal cerebral ischemia, but the impact of CAA on ischemic brain injury is unknown.

Methods—To determine this, we assessed cerebrovascular reactivity, cerebral blood flow (CBF), and extent of infarction and neurological deficits after transient middle cerebral artery occlusion in aged APP mice having extensive CAA versus young APP mice lacking CAA (and aged-matched littermate controls).

Results—We found that aged APP mice have more severe cerebrovascular dysfunction that is CAA dependent, have greater CBF compromise during and immediately after middle cerebral artery occlusion, and develop larger infarctions after middle cerebral artery occlusion.

Conclusions—These data indicate CAA induces a more severe form of cerebrovascular dysfunction than amyloid β-peptide alone, leading to intra- and postischemic CBF deficits that ultimately exacerbate cerebral infarction. Our results shed mechanistic light on human studies identifying CAA as an independent risk factor for ischemic brain injury. (Stroke. 2014;45:3064-3069.)

Key Words: Alzheimer disease ■ amyloid β-peptide ■ amyloid angiopathy ■ brain ischemia ■ mice, transgenic

Alzheimer disease and vascular dementia are the 2 most common forms of cognitive impairment in the elderly. For decades, each was considered a distinct disorder; recent results, however, show that they share many characteristics, including risk factors, neuropathology, and hemodynamics.1 This has led to a new conception that Alzheimer disease and vascular dementia are at the extremes of a spectrum of pathologies in which vascular and nonvascular factors coexist to varying degrees,2 a shift in thinking that has led to prioritization of investigating vascular contributors to dementia by the American Heart Association and American Stroke Association3 as well as by the National Institute on Neurological Disorders and Stroke.4 In vascular dementia, cerebrovascular dysfunction and ischemic brain injury drive cognitive impairment;2 multiple lines of evidence indicate that these 2 factors also contribute to Alzheimer disease.2 One common link between these 3 entities, cerebrovascular dysfunction, ischemic brain injury, and Alzheimer disease, is cerebral amyloid angiopathy (CAA).

CAA is characterized by cerebrovascular accumulation of amyloid-β peptide (Aβ) in a fibrillar form. Common in the elderly (=30% in those >60 years),6 it is also almost universally found in patients with Alzheimer.7,8 Experimental evidence in animals9-13 and humans14,15 shows that CAA significantly perturbs cerebral arteriole function and cerebral blood flow (CBF). In human autopsy studies, CAA is a strong and independent risk factor for both ischemic infarction16-18 and cognitive impairment.19,20 Taken together, these results have led many to postulate that CAA contributes to Alzheimer disease by compromising cerebral hemodynamics and promoting infarctions throughout the cerebral hemispheres.17,21 Yet, experimental evidence that CAA contributes to ischemic infarction is currently lacking, as is underlying mechanism.

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Previously, 2 groups showed that amyloid precursor protein (APP) mice having elevated Aβ (but no CAA) develop larger infarcts after focal cerebral ischemia compared with littermate controls. One study implicated Aβ-induced cerebrovascular function, whereas the other linked it to Aβ-induced inflammation. Neither examined the effect of CAA on cerebral infarction, as both examined APP mice at ages where CAA is not present. In the current study, we sought to determine the effect of CAA on cerebral ischemia by examining pre-CAA and post-CAA APP mice. We also examined whether CAA-induced alterations in cerebrovascular function and CBF might underlie the observed effect.

Materials and Methods

Animals

All experiments were approved by the Animal Studies Committee at Washington University. All data were collected by experimenters blinded to age and genotype. Tg2576 mice, originally a generous gift from Dr K. Ashe (University of Minnesota, MN), were bred to B6/SJJ wild-type (WT) mice (Tacomin Farms, Germantown, NY) and genotyped as described. Male Tg2576 mice were used at 6 months (having elevated Aβ but no CAA) and at 15 months (having elevated Aβ and extensive CAA); WT littersmates served as controls.

Cerebrovascular Reactivity

Cerebrovascular reactivity was assessed as previously described. Briefly, mice were anesthetized with isoflurane and a 4-mm right parietal craniotomy was performed. After 15 hours of recovery, mice were reanesthetized and ventilated with a rodent ventilator (Harvard Apparatus; Holliston, MA). Core body temperature was maintained at 37±0.1°C by a thermoregulated heating pad. Arterial blood pressure and gases were assessed via femoral catheterization. Leptomeningeal arterioles were visualized using Nikon Eclipse ME600 microscope (Nikon Instruments Inc, Melville, NY) and MetaMorph Image Analysis (Molecular Devices, Sunnyvale, CA). The endothelium-dependent vasodilator acetylcholine (100 μmol/L) and the endothelium-independent vasodilator S-nitroso-N-acetyl-penicillamine (SNAP; 500 μmol/L) were infused, followed by artificial CSF until baseline vessel diameter returned. Vessel diameters were quantified using Diamtrak (Tin Neild, Monash University, Melbourne, Australia).

Transient Focal Cerebral Ischemia Model

Focal ischemia was performed as described. Briefly, mice were anesthetized with isoflurane and a 4-mm right parietal craniotomy was performed. After 15 hours of recovery, mice were reanesthetized and ventilated with a rodent ventilator (Harvard Apparatus; Holliston, MA). Core body temperature was maintained at 37±0.1°C by a thermoregulated heating pad. Arterial blood pressure and gases were assessed via femoral catheterization. Leptomeningeal arterioles were visualized using Nikon Eclipse ME600 microscope (Nikon Instruments Inc, Melville, NY) and MetaMorph Image Analysis (Molecular Devices, Sunnyvale, CA). The endothelium-dependent vasodilator acetylcholine (100 μmol/L) and the endothelium-independent vasodilator S-nitroso-N-acetyl-penicillamine (SNAP; 500 μmol/L) were infused, followed by artificial CSF until baseline vessel diameter returned. Vessel diameters were quantified using Diamtrak (Tin Neild, Monash University, Melbourne, Australia).

Measurement of CBF

CBF was measured before, during, and after MCAO via laser Doppler flow as described. Probes were placed through burr holes over the ischemic core (3.5 mm lateral, 1 mm caudal to bregma) and penumbra (1.5 mm lateral, 1.7 mm rostral to lambda). Data are presented as a percentage of the preocclusion value.

Infarct Volume Quantification

Infarction was assessed via 2,3,5-triphenyl/tetrazolium chloride (TTC) staining 72 hours post MCAO as previously described. Briefly, mice were transcardially perfused with heparinized PBS. Brains were sectioned coronally using a 1.5-mm matrix and stained in 2% TTC; after fixation in 10% formalin, imaging was performed with a desktop scanner. Infarct area was assessed using ImageJ (National Institutes of Health, Bethesda, MD) and used to calculate infarct volume via the indirect method.

Neurobehavioral Tests

Neurobehavioral outcome was assessed daily using 8-point sensorimotor scoring as previously described.

CAA and Neuritic Plaque Quantification

In animals undergoing cerebrovascular reactivity assessment, CAA was quantified as previously described. Briefly, mice were administered the Congo red derivative methoxy-X04 (10 mg/kg IP) at installation of the cranial window. Percent CAA coverage was determined for eight 25-μm segments per animal using MetaMorph. In a subset of aged Tg2576 mice (n=4), CAA and neuritic plaque load were quantified postmortem as per our established protocol. Briefly, mice were euthanized 72 hours post MCAO, followed by brain extraction, fixation (4% paraformaldehyde), equilibration (30% sucrose), and coronal sectioning (50 μm). Brain sections were stained with 1 μmol/L resorufin (which selectively stains CAA) and 2 μmol/L methoxy-X34 (which stains both CAA and neuritic plaques). Fluorescent staining was visualized using Nikon Eclipse ME600 microscope and MetaMorph. CAA and neuritic plaque load were assessed as percent coverage of MCA-territory parietal cortex, contra- and ipsilateral to MCAO, using ImageJ.

Statistical Analyses

Data are expressed as means±SEM. After determination of normality, comparisons between 2 groups were performed with a Student t test; among multiple groups, with a 1-way omnibus ANOVA followed by Dunnett’s multiple comparison method or with a omnibus repeated measures ANOVA followed by Newman–Keuls method. STATISTICA (StatSoft, Inc, Tulsa, OK) was used. Statistical significance was set at P<0.05.

Results

Aged Tg2576 Mice Develop Extensive CAA and More Severe Cerebrovascular Dysfunction

Young Tg2576 mice had no CAA, whereas aged Tg2576 mice had extensive CAA covering most leptomeningeal arterioles without interruption (Figure 1A). In young mice, no significant difference in baseline vessel diameter was noted. In aged mice, baseline vessel diameter was significantly greater in Tg2576 mice (Figure 1C; P<0.05, Student t test), which is consistent with our28 and other10,29 past results. This difference is consistent with a hypocontractile vascular phenotype, likely the result of CAA-induced smooth muscle cell dysfunction and death. In young Tg2576 mice, responses to acetylcholine and SNAP were not significantly different. In aged Tg2576 mice, responses to acetylcholine and SNAP were severely impaired (Figure 1B; P<0.05, omnibus ANOVA). These deficits were CAA dependent, as acetylcholine-induced dilation was normal in vessel segments from aged WT controls, moderately reduced in vessel segments from aged WT controls, moderately reduced in vessel segments from aged Tg2576 mice having mild CAA (<20%), and absent in vessel segments from aged Tg2576 mice having extensive CAA (>20%; Figure 1D; P<0.05, omnibus ANOVA).

Aged Tg2576 Mice Develop Worse CBF Deficits During and Immediately After Focal Cerebral Ischemia

In young Tg2576 mice, no significant differences in CBF, in core or penumbra, intra- and postischemia, were noted...
Aged Tg2576 Mice Have Increased Susceptibility to Focal Cerebral Ischemia

Young Tg2576 mice had infarct volumes that were 46% larger than littermate controls (Tg2576: 62±9 mm³ versus control: 42±7 mm³; P<0.05; omnibus ANOVA). The difference in infarct volumes was statistically significant in aged versus young Tg2576 mice, but not in WT mice (Figure 3A and 3B).

Aged and Young Tg2576 Mice Develop Comparable Neurological Deficits After Focal Cerebral Ischemia

At baseline (before MCAO), no difference in sensorimotor scoring was noted between young Tg2576 and littermate
controls, whereas a small but significant difference was noted between aged Tg2576 and littermate controls (Figure 3D; $P<0.05$; Student t test). After MCAO, deficits were greater in Tg2576 mice (Figure 3C and 3D; $P<0.05$; omnibus repeated measures ANOVA); however, no significant age-related difference was noted.

**Focal Cerebral Ischemia Does Not Affect CAA or Neuritic Plaque Load**

In aged Tg2576 mice, no significant differences in CAA load (0.50±0.16% versus 0.48±0.13%) or neuritic plaque load (1.37±0.15% versus 1.24±0.09%; both $P>0.05$, Student t test) were noted between ischemic and nonischemic motor cortex 3 days after MCAO (Figure 4).

**Discussion**

The main findings of our study are (1) aged Tg2576 mice develop more severe cerebrovascular dysfunction than young Tg2576 mice, and that this is because of the presence of CAA; (2) aged Tg2576 mice develop more severe CBF deficits than young Tg2576 mice during and immediately after focal cerebral ischemia; and (3) infarct volumes (but not neurological deficits) are exacerbated to a greater degree in aged versus young Tg2576 mice. In combination, these findings implicate CAA, via its deleterious effects on cerebrovascular function and CBF, in the heightened susceptibility to cerebral infarction observed in aged Tg2576 mice. They also corroborate numerous human studies identifying CAA as a strong and independent risk factor for ischemic infarction and lend mechanistic insight into the pathophysiological underpinnings.

Two previous studies have examined the susceptibility of APP mice to ischemic brain injury. The first used 3- to 4-month-old Tg2576 mice, which have elevated $\beta\varepsilon$ but no CAA or neuritic plaques. They found that young Tg2576 mice have reduced CBF in the ischemic penumbra immediately after MCAO and develop infarct volumes 32% larger than littermate controls. The second used 8- and 20-month-old APP751 transgenic mice, which also have elevated $\beta\varepsilon$ but no CAA or neuritic plaques. They found that APP751 mice develop infarct volumes 34% to 41% larger than littermate controls. Both studies causally linked $\beta\varepsilon$ to the increased infarct volume noted in APP mice, although the former implicated the deleterious effect of $\beta\varepsilon$ on cerebrovascular function and the latter its impact on inflammation. Neither, however, ruled out the potential effect of APP overexpression (as opposed to $\beta\varepsilon$) as the underlying cause, and neither examined the potential effect of CAA on ischemic brain injury. Our study used 2 distinct ages of Tg2576 mice, 1 with elevated $\beta\varepsilon$ alone and 1 where elevated $\beta\varepsilon$ is accompanied by extensive CAA, to examine this potential CAA effect.

First, we confirmed what we and others have reported that Tg2576 mice develop age-dependent cerebrovascular dysfunction, with the most severe deficits noted in aged Tg2576 mice having extensive CAA. We also found that the severity of cerebrovascular impairment in aged Tg2576 mice depends on the extent of CAA, consistent with our and others’ results that CAA is toxic to vascular smooth muscle cells, causing dysfunction and eventually death in a dose-dependent fashion, a process that contributes to the impairment of microvascular function. Second, we found that posts ischemia CBF was modestly but significantly compromised in the ischemic core of aged Tg2576 mice as compared with young Tg2576 mice and littermate controls. We also found that intrainschemic and posts ischemic CBF were significantly reduced in the ischemic penumbra of aged Tg2576 mice as compared with young Tg2576 mice and littermate controls. These results are consistent with previous studies in AD mice documenting neurovascular uncoupling: astrocyte endfeet swell and retract from CAA-laden vessels (but not from CAA-free vessels) in Arc$\beta\varepsilon$ mice, and greater disruption is seen after cerebral ischemia in 3xTg mice than in age-matched WT controls. Third, we documented that CAA and neuritic plaque load in aged Tg2576 mice were not acutely altered by MCAO. Taken together, these data strongly indicate that CAA’s pathological effect on cerebrovascular function produced worse CBF deficits during and immediately after MCAO, which likely relates to the inability of nearby CAA-laden cerebral arterioles to provide collateral CBF through autoregulatory vasodilation.
Next, we examined what effect CAA-induced cerebrovascular dysfunction has on infarct volume and neurological outcome after MCAO. Consistent with past reports, we found that young Tg2576 mice develop larger infarct volumes and worse neurological deficits compared with littermate controls. In addition, we found that aged Tg2576 mice also develop greater infarct volumes and more severe neurological deficits compared with controls, but found that infarct volumes were significantly larger in aged versus young Tg2576 mice. No age-dependent effect on sensorimotor scoring was noted, likely because of baseline neurological deficits associated with aged Tg2576 mice, a floor effect in the postischemic deficits seen in this model, or both. Taken together, these data strongly suggest that the heightened vulnerability to ischemic brain injury observed in aged versus young Tg2576 mice is the direct result of CAA and its deleterious effect on cerebrovascular function and CBF.

Our data have several important implications. First, they provide the first experimental support for multiple observational human studies identifying a role for CAA in inducing and exacerbating ischemic brain injury. Second, they provide an advanced experimental foundation for future studies designed to examine the efficacy of a growing number of novel CAA-directed therapeutics (eg, anti- \( \beta \)- immunotherapy, \( \beta \)-secretase inhibitors). Specifically, study end points could comprise not only CAA load and CAA-induced microhemorrhage but also cerebrovascular function, CBF, and ischemic brain injury. Third, they provide an opportunity to examine whether an entirely new therapeutic approach, targeting vessel function (rather than CAA itself), may reduce the deleterious effect of CAA on ischemic infarction.

Other interpretations of our results exist. One possibility is that elevated \( \beta \) (rather than CAA) underlies the heightened susceptibility to ischemic brain injury seen in aged versus young Tg2576 mice. This would be substantiated if \( \beta \) levels were higher in aged versus young Tg2576 mice; however, evidence using the most sophisticated techniques for specifically measuring \( \beta \) strongly argues against this. Initial studies examining this issue noted greater levels of biochemically extractable \( \beta \) with increasing age and greater amyloid pathology in APP mice. The extraction technique used, however, is confounded by its potential to liberate \( \beta \) from amyloid deposits with tissue homogenization, and therefore it likely overestimates the true level of \( \beta \) present in the extracellular space. To address this concern, several groups turned to in vivo microdialysis of APP mice to directly assess \( \beta \) in the interstitial fluid. Using this assay, we and others show that, in fact, absolute levels of \( \beta \) decrease with age in several APP mouse models of Alzheimer disease and CAA. Similar decreases in \( \beta \) are seen in CSF of aged APP mice, patients with Alzheimer, and patients with CAA. Based on these results, it is therefore highly unlikely that differences in extracellular \( \beta \) in aged versus young APP mouse account for the observed heightened susceptibility to ischemic brain injury.

Another possibility is that greater ischemic brain injury is seen in aged Tg2576 mice because of increased neuronal vulnerability that is CAA independent. It could be that overproduction of mutant human APP and exposure to elevated \( \beta \) throughout the lives of Tg2576 mice render the aging brain less capable of coping with ischemic injury, leading to increased infarction. The fact that extracellular levels of \( \beta \) are actually lower in aged versus young Tg2576 mice, however, argues against this. Finally, the enhanced vulnerability to ischemia could also relate to the presence of neuritic plaque pathology in aged Tg2576 mice. But our observation that aged Tg2576 mice have significant intra- and postischemic CBF deficits that appear CAA induced argues strongly that vascular, not parenchymal, pathology is the primary underlying driver of the heightened susceptibility. To completely exclude this possibility, however, additional experiments would be required, likely using APP Dutch mice that exclusively develop CAA, albeit at an extremely advanced age (22–25 months).

In summary, our work corroborates past results that APP mice having elevated \( \beta \) (but no CAA) have increased vulnerability to ischemic brain injury. In addition, we are the first to show that aged APP mice having extensive CAA and severe CAA-induced cerebrovascular dysfunction develop marked intra- and postischemic CBF deficits and greater infarct volumes after MCAO. These data strongly implicate CAA as a key contributor to ischemic brain injury, which substantiates the growing notion that CAA, and its attendant cerebrovascular and CBF deficits, is a key contributor to the ischemic infarcts and cognitive dysfunction found in Alzheimer disease and vascular dementia. Whether restoration of vasoreactivity via CAA-directed or vessel function–directed therapeutics ultimately improves CBF, reduces ischemic brain injury, and enhances cognitive function will require further investigation.

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Disclosures

Dr Holtzman is a co-founder of C2N Diagnostics, sits on advisory boards for Astra Zeneca and Genentech, and is a consultant for Eli Lilly, Forum Pharmaceuticals, and Neurophage. None of these relationships directly affect the results reported in this article. The other authors report no conflicts.

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