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Therapeutic Modulation of Cerebral Microhemorrhage in a Mouse Model of Cerebral Amyloid Angiopathy

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Abstract

Background and Purpose—The aging brain demonstrates frequent MRI and pathological evidence of cerebral microbleeds, which are often associated with cerebral amyloid angiopathy. In order to develop new therapeutic strategies for this disorder, we studied cerebral microhemorrhage in a well-characterized mouse model of cerebral amyloid angiopathy.

Methods—Tg 2576 mice were studied at ages ranging from 2 to 21 months. Spontaneous and induced microscopic bleeding was analyzed with and without passive anti-amyloid immunization regimen and dietary supplementation of ischemic stroke prevention medication dipyridamole.

Results—Areas of microhemorrhage were easily demonstrated, and were significantly more prominent in oldest mice and in animals treated with anti-amyloid immunotherapy. Dipyridamole supplementation in diet generated plasma levels greater than 790ng/ml, within the range seen clinically. Dipyridamole treatment did not worsen frequency and size of cerebral microscopic bleeding.

Conclusions—The Tg2576 mouse is a useful model to study progression and modification of spontaneous and immunotherapy-induced cerebral microhemorrhage. Absence of microhemorrhage worsening with dipyridamole treatment suggests a potential therapeutic role of this agent when ischemic and microhemorrhagic lesions coexist.

Keywords

Alzheimer's; Amyloid angiopathy; Animal models; Antiplatelet drugs; Basic science; Experimental; Intracerebral hemorrhage; Microcirculation

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Disclosures

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INTRODUCTION

There is increasing recognition of the high prevalence of cerebral microbleeds in the aging population. Over the age of 60 years, MRI evidence of cerebral microbleeds is present in approximately 20% of the population; this number rises to nearly 40% for individuals over the age of 80 (1). Moreover, post-mortem pathology studies demonstrate microscopic brain hemorrhages occurring at the capillary level in the vast majority of individuals over the age of 70 (2,3).

There are at least two key issues that emanate from these microbleed observations. First, there is the necessity for establishing animal models of this disorder that will enable investigators to delineate pathophysiological mechanisms of microbleed formation. Second, new treatments and treatment modifications are needed to address the microbleeds themselves.

The issue of microbleeds treatment is of necessity complex. Microbleeds are not only common, but they do not occur in a neurovascular vacuum. Microbleeds may coexist with other forms of cerebrovascular disease. Cerebral white matter disease, which has at least a partial microvascular origin (4), has a strong association with cerebral microbleeds in the presence of cerebral amyloid angiopathy (5). Moreover, ischemic stroke may coexist with cerebral microbleeds, leading to a profound challenge in stroke neurology: How to protect the patient against ischemic stroke while not worsening the brain hemorrhage potential of cerebral microbleeds? The concept of “mixed cerebrovascular disease” has thus been established in order to encompass the wide spectrum of ischemic and hemorrhagic cerebrovascular disease, both clinical and subclinical (6).

It is against this background that we initiated the current study in which we analyzed microhemorrhage in a well-characterized mouse model of Alzheimer’s disease and cerebral amyloid angiopathy. The Tg2576 transgenic mouse has been extensively studied in the course of investigations of Alzheimer’s disease-like amyloid deposition (7). In the current study, we have used this mouse model to investigate the development of brain microhemorrhage, as well as initiate new therapeutic strategies directed at the microhemorrhages themselves. We thus utilized the Tg2576 mouse model, alone and modified by passive immunization (8,9), with and without the ischemic stroke prevention medication dipyridamole (10). The establishment of reliable animal models of microhemorrhage and related therapeutic interventions should have translational value in the clinical efforts directed against mixed cerebrovascular disease.

METHODS

All animal studies were performed in accordance with IACUC-approved protocols at UC Irvine.

Animals and Treatment Protocols

Experiment 1—In order to determine achievable plasma levels with dipyridamole supplementation, 2-month-old female Tg2576 mice (n=6) were treated with dipyridamole-supplemented high fat diet (HFD, 40% fat, 40% carbohydrate and 20% protein, with dipyridamole 5g/kg chow) for 2 weeks. Age-matched **female** wild-type (WT) littermates were used as controls. HFD was used due to poor oral intake by mice when bitter tasting dipyridamole was incorporated into a standard diet.

Experiment 2—12-month-old female Tg2576 mice were divided into 2 groups: 1) Tg2576-Control (Control) mice (n=9) fed HFD; 2) Tg2576- + dipyridamole (DIP) mice

(n=11) fed HFD supplemented with dipyridamole (5 g/kg of chow). Animals were treated for 3 months.

Experiment 3—21-month-old Tg2576 were divided into 4 groups: 1) Tg2576-Control mice (n=9) fed HFD; 2) Tg2576 +DIP mice (n=11) fed HFD supplemented with dipyridamole (5 g/kg of chow); 3) Tg2576- mice (n=9) on HFD were treated with anti-A β 40 antibodies clone 1313 (10mg/kg, i.p., weekly) (Abody); 4) Tg2576 +DIP mice (n=9) treated with anti-A β 40 antibodies clone 1313 (10mg/kg, i.p., weekly) (DIP+Abody). There were two males with seven or nine female mice in each of the four groups. Mice were pretreated with the diets for 2 weeks before the antibody injections, which continued for 9 weeks together with the diet supplement.

Behavioral Test and Blood Pressure Monitoring

Cognition was assessed at end of treatment using the Novel Object Recognition task as described (11). Blood pressure was assessed noninvasively in conscious restrained mice by the tail cuff technique using the CODA System (Kent Scientific) for 3 days at the end of the experiment.

Immunohistochemistry

PBS perfused 4% paraformaldehyde fixed brain sections were immunostained for A β using 6E10 and 4G8 mouse monoclonal antibodies (Signet Laboratories, Dedham, MA) at 1:1,000 dilution, as described (11, 12).

Hemorrhage Detection

Determination of hemorrhages was performed using Prussian blue staining of ferric acid, as previously described (12). Coronal sections of experimental and control mouse brains were collected from 3 different planes of the brain (about 2.5 mm apart). The sections were mounted on Superfrost Plus microscopic slides (Fisher Scientific, Pittsburg, PA). Staining was performed using Prussian blue solution, ie, freshly made 5% potassium hexacyanoferrate trihydrate and 5% hydrochloric acid (Sigma, MO). 30 minutes later, sections were rinsed in water, and counterstained with Nuclear Fast Red (Sigma, MO), dehydrated and covered using DPX (BDH Laboratory Supplies, England). Hemorrhage profiles were counted by three independent observers using Olympus BX40 microscope at 20x original magnification. For each brain section, the average number and size of Prussian blue-positive deposits was calculated. We used a scale of 1 to 4 to determine the size of microhemorrhage, where 1 was 1–5 grains of iron or small microvessel involvement; 2 was multiple grains of iron and microvessel involvement; 3 was several positive microvessels in one area; and 4 was large blood vessel involvement. Results were averaged between investigators and presented as a mean \pm SEM.

Beta Amyloid (A β) ELISA

ELISA of detergent soluble and 70% formic acid soluble A β brain fractions was performed using our established assay, as previously described (11, 12)

Statistical Analysis

All data are expressed as mean \pm SEM. The statistical evaluation was carried out using Student's t-test or analysis of variance (ANOVA). Following significant ANOVAs, multiple post hoc comparisons were performed using Bonferroni's test.

RESULTS

Tg 2576 mice showed progressive accumulation of microhemorrhages with aging, which ranged from 1.76 ± 0.25 microhemorrhages per brain section in 15-month old mice to 4.54 ± 0.6 microhemorrhages in 24-month old animals (Fig. 1A). The average size of spontaneous microhemorrhage also significantly increased with age (Fig 1B). Dipyridamole supplementation in high fat diet was well tolerated by mice. After 2 weeks, plasma levels of dipyridamole reached 794 ± 246 ng/ml and 793 ± 97 ng/ml in WT and Tg2576 mice, respectively. We then studied the effects of three months dipyridamole-supplemented diet on blood pressure, object recognition, and A β 40 and A β 42 in 15 month old mice. In these studies, there were no effects of dipyridamole on novel object recognition, blood pressure, and A β 40 and A β 42 levels (data not shown). The latter were measured in both detergent-soluble and detergent-insoluble brain fractions. In addition, there was no significant difference in spontaneous microhemorrhage size or number observed in mice receiving dipyridamole-supplemented diet (Figure 2). There was also no significant difference in microhemorrhage size and number between animals fed high fat diet and animals (N=9) fed standard (10% fat, 70% carbohydrate, and 20% protein) diet for three months (data not shown).

Finally, we studied the effects of eleven weeks dipyridamole-supplemented diet on aged (21 months old) mice, with and without passive immunization to A β 40. The latter was achieved by nine weekly injections beginning two weeks after the start of the experiments. There were no significant effects of dipyridamole on novel object recognition and biochemical levels of A β 40 and A β 42, and there were no significant male-female differences in dipyridamole effects (data not shown). There was a significant increase in the number and size of microhemorrhages in antibody-treated mice on control diet (Figure 3). Antibody-treated mice fed dipyridamole-supplemented diet demonstrated significant increase in number, but not size, of microhemorrhages (Figure 3).

DISCUSSION

Cerebral microscopic bleeding was easily demonstrable in this transgenic mouse model of cerebral amyloid angiopathy, with microhemorrhages more than twice as frequent in elderly compared to younger adult mice. These microhemorrhages tended to be exacerbated by our passive immunotherapy regimen. Treatment with dipyridamole did not worsen microhemorrhages, and the significantly increased microhemorrhage size of immunized mice was not seen after dipyridamole treatment. Plasma dipyridamole levels achievable were within the range encountered clinically, ie, 0.5–2.0ug/ml (13).

The Tg2576 mouse expresses mutant amyloid precursor protein (APP) from which pathogenic beta-amyloid fragments 1–40 and 1–42 are easily generated (7). Tg2576 is characterized by predictable progression of band-like vascular amyloid deposits in leptomeningeal and cortical arteries and arterioles, notable by age 12 months (14,15). Immunotherapy directed at beta-amyloid fragments tends to lessen parenchymal beta-amyloid but may worsen vascular amyloid deposition and exacerbate microhemorrhages, perhaps by direct antibody targeting of vascular wall (9,16).

The relative ease of demonstrating cerebral microhemorrhages makes the Tg2576 mouse an attractive model to study progression and treatment of these lesions. The latter issue is of particular importance where there is coexistence of ischemic and microhemorrhagic lesions, the typical picture of mixed cerebrovascular disease (6). Use of platelet agents, particularly aspirin, has been associated with higher prevalence of microbleeds on MRI (17) and increased risk of intracerebral hemorrhage in patients with cerebral amyloid angiopathy

(18). Dipyridamole, shown to be effective as monotherapy for prevention of ischemic stroke (10), has therapeutic actions largely directed at the vessel wall, rather than platelets alone (19); these vessel wall effects include vasodilation via cGMP potentiation, anti-oxidant and anti-inflammatory actions, and enhancement of endothelial nitric oxide (19). Moreover, acute treatment with dipyridamole at the time of reperfusion tends to reduce hemorrhagic conversion of ischemic stroke in a rodent model of experimental stroke (20). Findings of our study are limited by lack of aspirin treatment arm. Nevertheless, absence of microhemorrhage worsening with dipyridamole treatment in this model suggests a potential role for this agent in patients with coexisting ischemic and microhemorrhagic disease.

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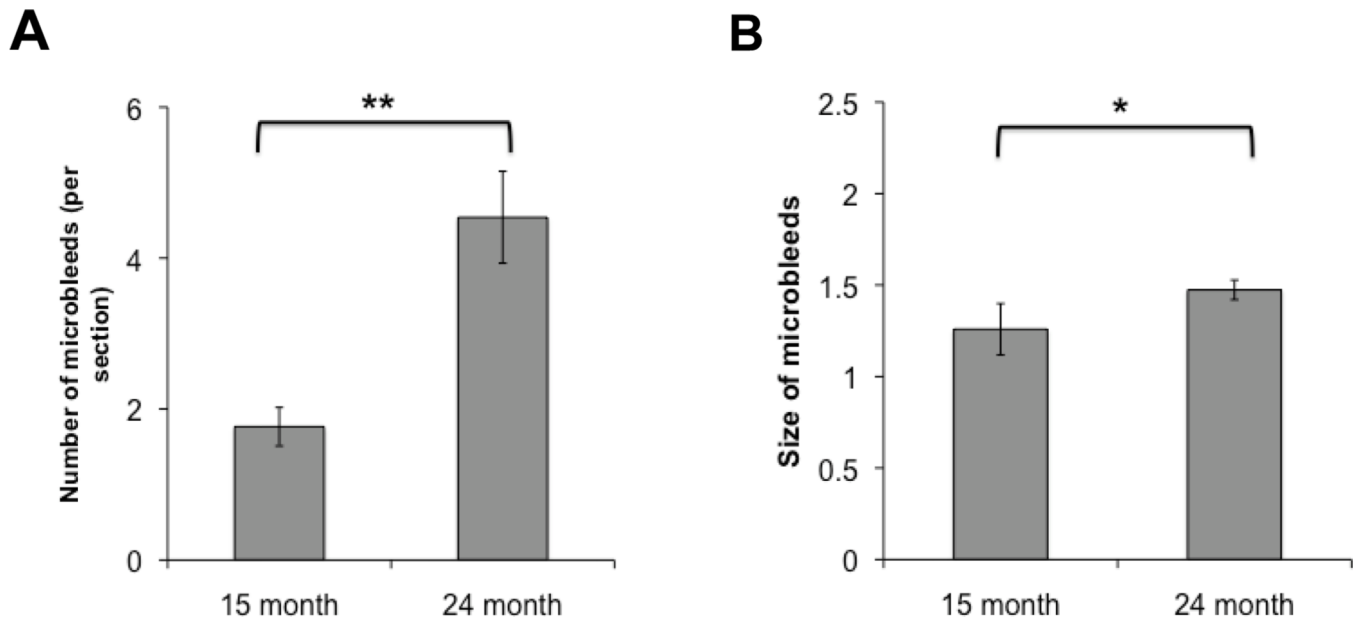


FIGURE 1. Tg2576 mice progressively accumulate microhemorrhages with aging. Number (A) and size (B) of microhemorrhages assessed in 15- and 24-month old Tg2576 mice fed regular mouse chow. (**- $P < 0.01$; * - $P < 0.05$)

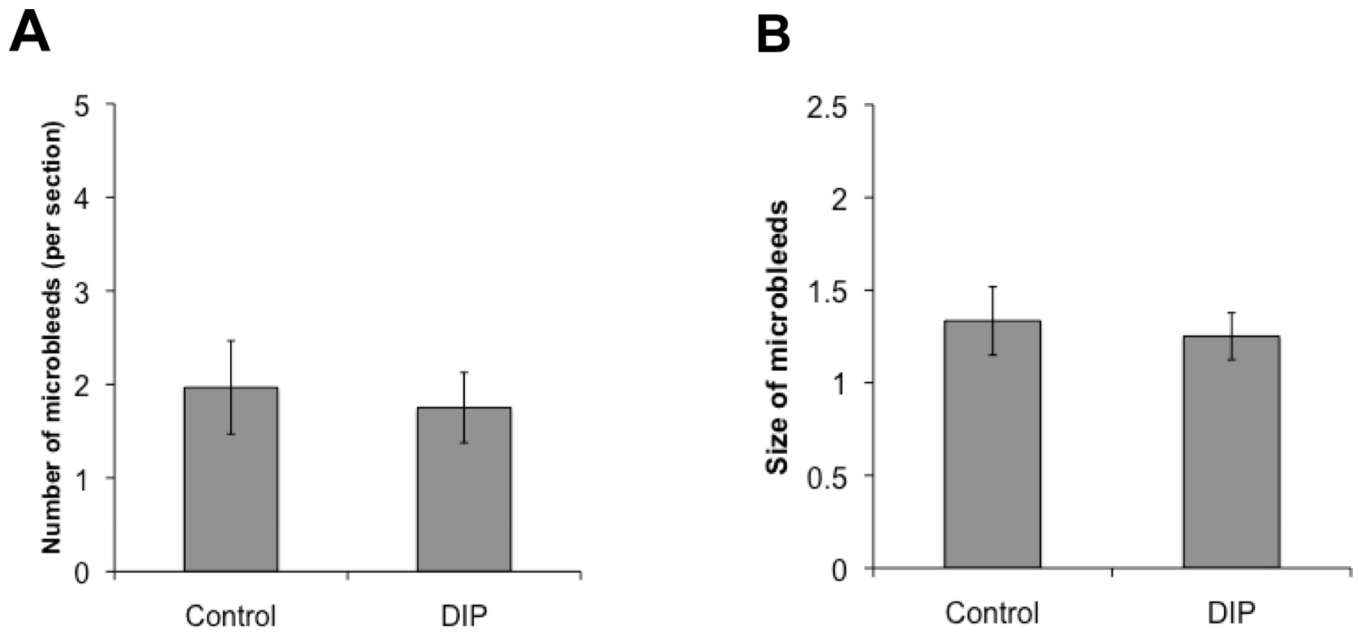
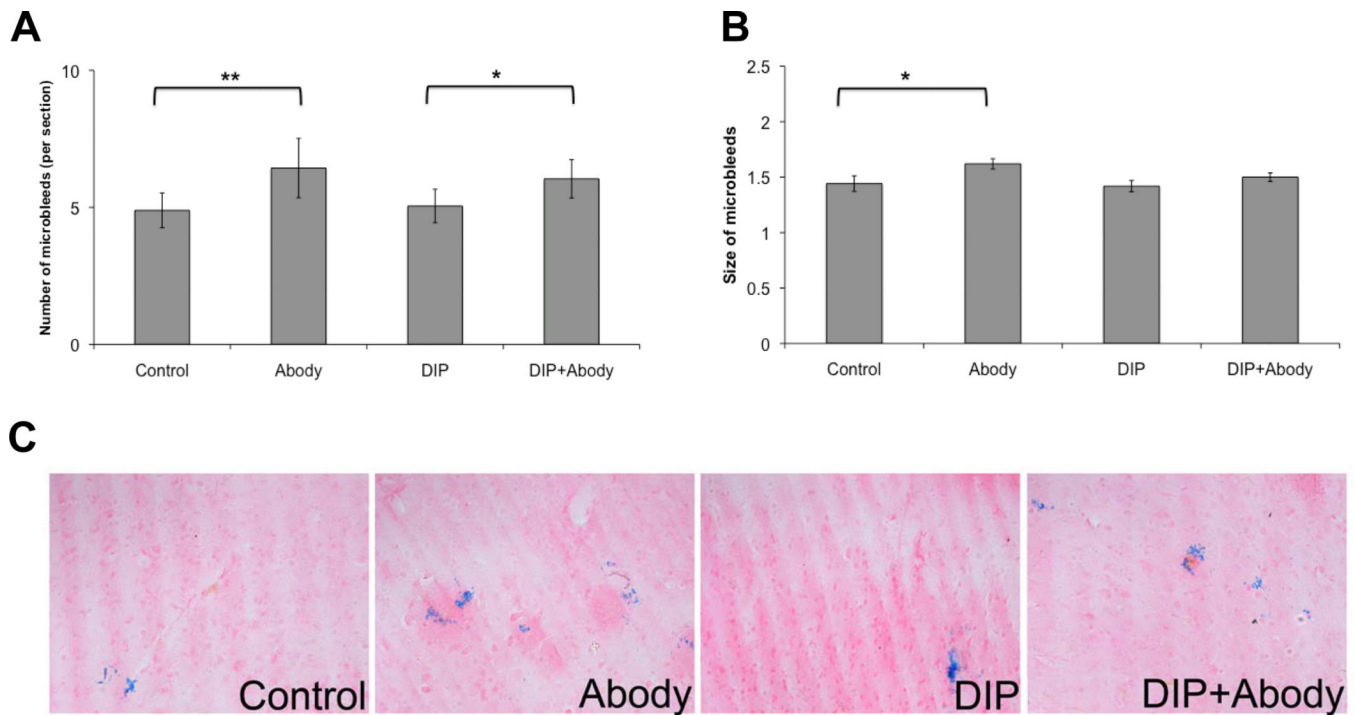


FIGURE 2. Dipyridamole diet (5 g/kg of chow) does not affect the number (A) nor the size (B) of spontaneous microhemorrhages in 15-month old Tg2576 mice receiving 3 months dipyridamole (DIP).

**FIGURE 3.**

Immunotherapy effects on number (A) and size (B) of microhemorrhages. Twenty-one-month-old mice were treated for 11 weeks with control (high fat) diet, or with diet supplemented with dipyridamole (DIP; 5 g/kg chow). Two weeks after initiation of the diet, mice were given anti-A β 40 antibody (Abody, 10mg/kg, i.p., weekly for 9 weeks). Significantly increased number of microhemorrhages after immunotherapy was seen with both diets, and significantly increased microhemorrhage size with control diet only. Representative photomicrographs were taken from cortex (C). Magnification: $\times 100$. (**- $P < 0.01$; * - $P < 0.05$).