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Journal

Cerebrovascular and Brain Metabolism Reviews, 36(2)

ISSN

1040-8827

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Publication Date

2016-02-01

DOI

10.1177/0271678x15606720

Peer reviewed

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Brief Communication

JCBFM

Journal of Cerebral Blood Flow & Metabolism
2016, Vol. 36(2) 399–404
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DOI: 10.1177/0271678X15606720
jcbfm.sagepub.com

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Experimental hypertension increases spontaneous intracerebral hemorrhages in a mouse model of cerebral amyloidosis

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Abstract

Hypertension and cerebral amyloid angiopathy (CAA) are major risk factors for intracerebral hemorrhage (ICH); however the mechanisms of interplay between the two are not fully understood. We investigated the effect of hypertension in a transgenic mouse model with Alzheimer's-like pathology (Tg2576) treating them with angiontensin II and L-N^G-nitroarginine methyl ester. A similar increase in systolic blood pressure was observed in both Tg2576 and control mice; however Tg2576 mice developed signs of stroke with a markedly shorter latency. Cerebral deposition of amyloid beta promotes the hypertension-induced ICH, thus supporting the notion that hypertension is a risk factor for ICH among patients with CAA.

Keywords

Alzheimer's disease, cerebral amyloid angiopathy, hypertension, intracerebral hemorrhage, risk factors

Received 9 April 2015; Revised 7 July 2015; Accepted 11 August 2015

Introduction

Intracerebral hemorrhage (ICH) is a subtype of stroke with high morbidity and mortality, accounting for about 15% of all deaths from stroke.[1] Chronic hypertension is a major risk factor for ICH, and development of an acute hypertensive episode superimposed on chronic hypertension may trigger ICH.[2] However, the mechanisms underlying hypertension-induced ICH are poorly understood. In addition to hypertension, another major cause of ICH is cerebral amyloid angiopathy (CAA).[3] CAA, which is characterized by the deposition of amyloid beta (Aβ) peptides in the blood vessels of the cortex and leptomeninges, is a common finding in the elderly and especially prominent in patients with Alzheimer's disease (AD).[4] CAA induces degeneration of the tunica media, loss of smooth muscle and endothelial cells[5] in cerebral vasculature and is often associated with cognitive impairment in AD.[6]

Recently, it has been demonstrated that lowering of blood pressure reduces the risk of CAA-related ICH, suggesting that hypertension could be an ICH-promoting factor in patients with CAA.[7] To gain insight on how cerebral Aβ pathology affects the development of hypertension-induced ICH, we evaluated the susceptibility to ICH produced by acute, superimposed on chronic, hypertension in a transgenic mouse model of cerebral amyloidosis with progressive age-related accumulation of Aβ plaques and CAA.[8]

Methods

Animals and ICH model

Fifteen-month-old male and female Tg2576[8] mice and nontransgenic (nTg) littermates (C57Bl6/SJL background) were used in the experiments. The methods to produce spontaneous ICH and to assess signs of experimental stroke are described elsewhere.[9] Briefly, mice were treated with angiontensin II (AngII;

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1000 ng/kg per min; Bachem, Torrance, CA) via subcutaneously (s.c.) placed osmotic pumps (Durect Corporation, Cupertino, CA), and L-N^G-nitroarginine methyl ester (L-NAME; 100 mg/kg per day; Sigma-Aldrich, St. Louis, MO) in drinking water to produce chronic hypertension. After one week, transient acute hypertension was induced by daily AngII injections (0.5 μg/g, s.c., twice daily from day 7 to day 28 after pump implantation). Control mice had a vehicle (phosphate-buffered saline)-filled osmotic pump implanted and received vehicle injections at the same time points. Blood pressure was assessed by the tail cuff technique (CODA, Kent Scientific, Torrington, CT) at least 2 h after AngII injections ($n = 8$–21). Signs of stroke including circling behavior, contralateral forelimb extension, or other motor dysfunction were assessed twice daily, as previously described.[9] After appearance of stroke symptoms, mice were transcardially perfused with ice-cold phosphate-buffered saline, left hemispheres were collected for microhemorrhage detection and fixed in 4% paraformaldehyde, while right hemispheres were snap frozen on dry ice and processed for biochemical analysis. All procedures followed the ''Principles of Laboratory Animal Care'' from NIH publication No. 85-23, were approved by the University of California, Irvine, Institutional Animal Care and Use Committee, and followed the ARRIVE Guidelines for animal experiments reporting.

Hemorrhage detection and quantification

Brain hemorrhages were detected using Prussian blue staining, as previously described.[10] Briefly, 4% paraformaldehyde-fixed coronal hemi-brain sections from three different planes (with coordinates −1.2 mm, −2.2 mm and −3.2 mm posterior to bregma) from each mouse were used ($n = 7$–13). Staining was performed using 5% potassium hexacyanoferrate trihydrate and 5% hydrochloric acid, followed by counterstaining with Nuclear Fast Red after 30 min (all Sigma-Aldrich). Hemorrhages were counted by three blinded observers, using an Olympus BX40 microscope at 20x magnification; and the average number and size of Prussian Blue positive deposits were calculated. A scale of 1 to 4 was used to semiquantitatively determine the size of microbleeds (where 1 = 1–5 grains of iron or small microvessel involvement; 2 = multiple grains of iron and microvessel involvement; 3 = several positive microvessels in one area; 4 = large blood vessel involvement).

Biochemical assay

Brain extracts prepared from right hemisphere ($n = 7$–13) were analyzed for endothelial activation/adhesion proteins pro-matrix metalloproteinase-9 (pro-MMP-9) and P-selectin as a part of Cardiovascular Disease multiplex assay Panel I kit (Millipore, Billerica, MA) according to manufacturer's recommendations and read on the Bio-Plex™ 200 (Bio-Rad, Hercules, CA) based on Luminex 200™ platform. Cerebrovascular endothelial activation/adhesion protein levels were normalized by the total protein concentrations in brain extracts detected with Bradford method.

Statistical analysis

The primary study endpoint was time-to-stroke, estimated using the Kaplan–Meier method. Tests for differences in the distribution of time-to-stroke were based upon the logrank test, which is sensitive to constant relative differences in the risk of stroke over time, and the Gehan–Breslow–Wilcoxon test, which applies greater weight to early differences in the risk of stroke.[11] The Cox proportional hazards model was used to estimate the hazard ratio comparing nTg and Tg2576 mice and the corresponding Wald-based 95% confidence interval was computed. Secondary study endpoints included systolic blood pressure, number and size of microhemorrhages, and pro-MMP-9 and P-selectin expression, summarized by the mean ± standard error of the mean (SEM). Group means were tested using two-way analysis of variance. A Bonferroni procedure was used to account for multiple comparisons in the form of adjusted p values. A probability value of $p < 0.05$ was considered significant.

Results

The Tg2576 model expresses human APP harboring the Swedish familial double mutation K670N, M671L, and is characterized by the development of parenchymal plaque pathology and CAA.[8] To assess the influence of Aβ pathology in hypertension-induced ICH, mice were subjected to an experimental model of spontaneous ICH.[9] The induction of acute and chronic hypertension resulted in a significant increase in systolic blood pressure in both Tg2576 (mean ± SEM, 191 ± 15 mmHg; $p < 0.01$) and nTg (179 ± 6 mmHg; $p < 0.01$) littermates, compared with vehicle-treated mice (137 ± 8 mmHg and 121 ± 6 mmHg, respectively), when evaluated 14 days after angiotensin treatment (Figure 1(a)). Although the incidence of signs of stroke was similar in both nTg and Tg2576 mice after acute and chronic hypertension induction (77% and 81%, respectively), Tg2576 mice developed signs of stroke with shorter latency (Figure 1(b)). Median time to stroke was estimated to be 11 days in the Tg group versus 24 days in the nTg group (hazard ratio 1.764, 95% confidence interval 0.779–3.995;

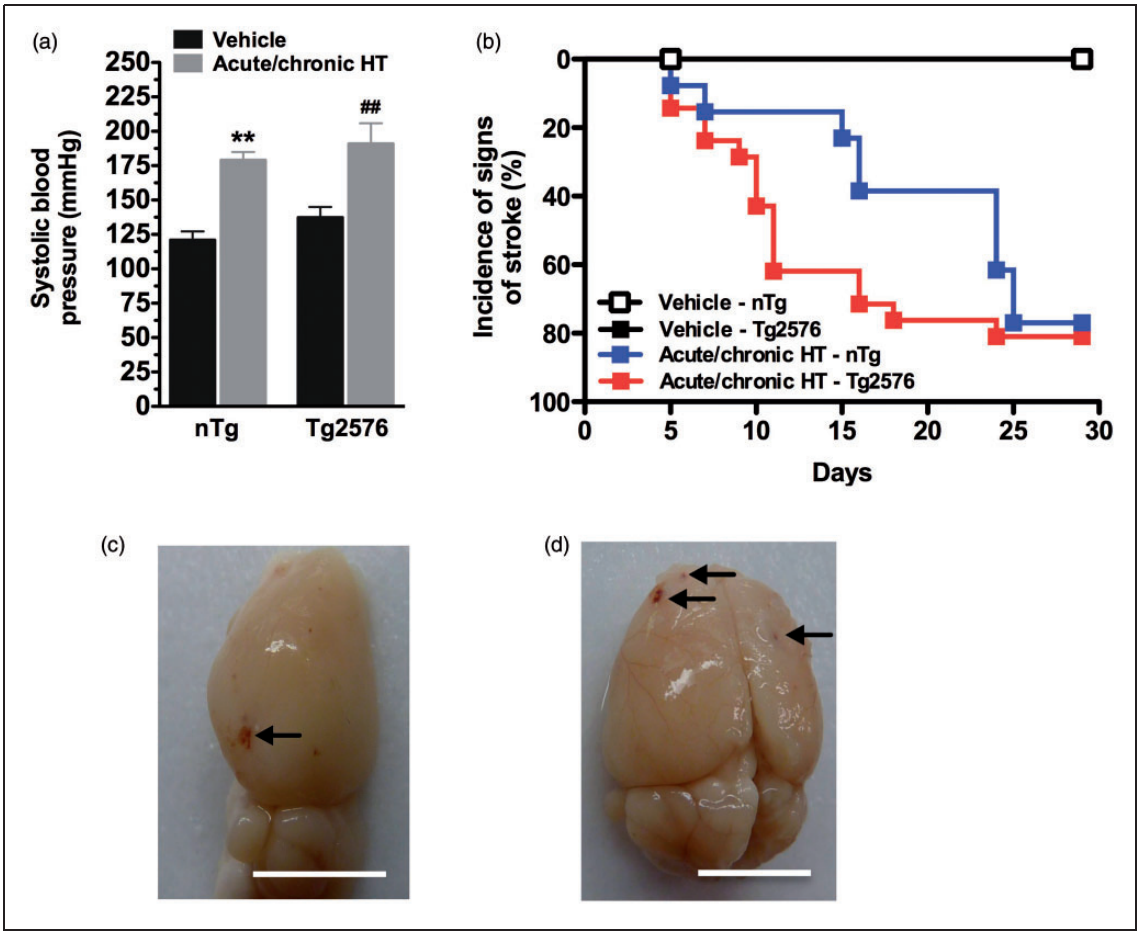

Figure 1. Acute and chronic hypertension-induced spontaneous intracerebral hemorrhages in Tg2576 mice. (a) Systolic blood pressure in Tg2576 mice and nTg littermates subjected to acute/chronic hypertension (HT), evaluated 14 days after initiation of hypertension. (b) Kaplan–Meier plot of signs of stroke ($n = 8$–21). (c, d) Spontaneous intracerebral hemorrhage (arrows) in Tg2576 brains subjected to acute/chronic HT. Scale bar, 5 mm. The values represent the mean ± SEM ($n = 8$–21). $^{**}p < 0.01$ compared to vehicle-nTg group. $^{\#\#}p < 0.01$ compared to vehicle-Tg2576 group.

$p = 0.173$ (log-rank) and $p = 0.076$ (Gehan–Breslow–Wilcoxon)). Finally, the induction of acute and chronic hypertension resulted in multiple ICHs in Tg2576 mice (Figure 1(c) and (d)).

Prussian blue staining revealed that the induction of acute and chronic hypertension significantly increased the number of microscopic hemorrhages in Tg2576 brains, but not in nTg brains (Figure 2(a) and (b)), while the size of microscopic hemorrhages was found to be significantly larger in both Tg2576 and nTg mice, compared with respective vehicle-treated groups (Figure 2(a) and (c)). Notably, the number (8.82 ± 0.65 vs. 5.17 ± 0.52; $p < 0.01$) and size (1.64 ± 0.04 vs. 1.47 ± 0.06; $p < 0.05$) of microscopic hemorrhages observed in Tg2576 mice are significantly higher compared to those observed in nTg mice (Figure 2).

Biochemical analysis of brain matrix metalloproteinase pro-MMP-9 showed that initial levels of immature protein are higher, though not significant, in Tg2576 mice, than in nTg littermates (10.01 ± 1.38 vs. 6.52 ± 0.41 pg/mg protein, respectively). Acute and chronic hypertension significantly increased the levels of pro-MMP-9 in Tg2576 compared with vehicle-treated animals (25.7 ± 6.85 vs. 10.01 ± 1.38, $p < 0.05$), and metalloproteinase levels were higher in hypertensive Tg2576 than in nTg mice (9.85 ± 2.82, $p < 0.05$) (Figure 2(d)). Cell adhesion molecule P-selectin was evenly and significantly increased in both nTg (13.99 ± 2.01 vs. 34.34 ± 3.79 pg/mg, $p < 0.05$) and in Tg2576 mice (16.13 ± 1.78 vs. 37.66 ± 7.57 pg/mg, $p < 0.01$) after experimental hypertension (Figure 2(e)).

Discussion

In this study, we demonstrate that Aβ deposition in Tg2576 mouse brain is associated with an increased susceptibility to spontaneous ICH, as indicated by a markedly shorter latency for the development of

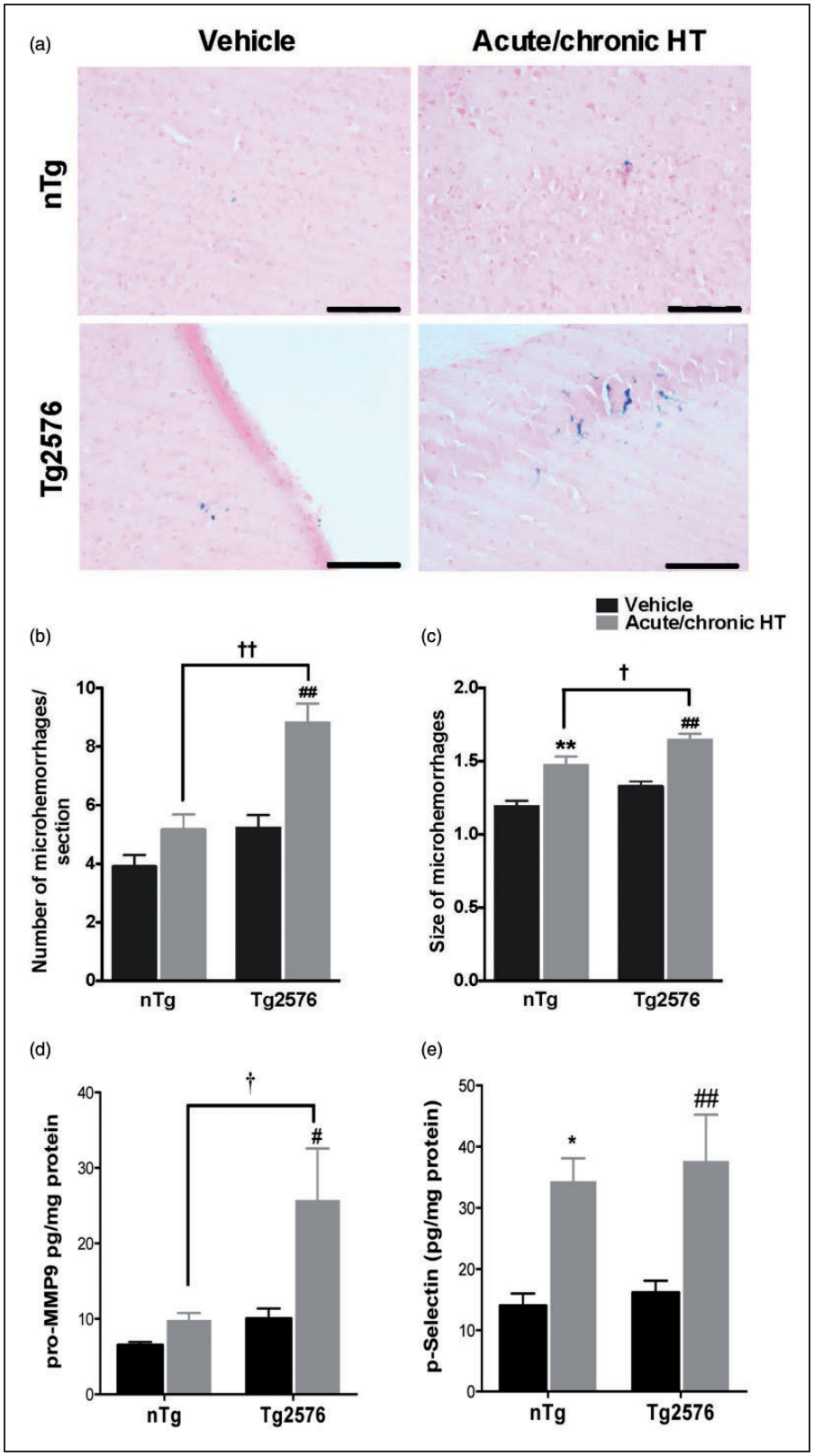

Figure 2. Increased microhemorrhages during acute and chronic hypertension (HT) in Tg2576 mice. Prussian blue staining demonstrating number (a and b) and size (a and c) of microhemorrhages in Tg2576 mice and nTg littermates subjected to acute/chronic HT. Changes in brain pro-MMP-9 (d) and P-selectin (e) protein levels after experimental HT. Representative photomicrographs were taken for frontal cortex or hippocampus. Scale bars = 100 μm. The values represent the mean ± SEM ($n = 7–13$). *$p < 0.05$, **$p < 0.01$ compared to vehicle-nTg group. #$p < 0.05$, ##$p < 0.01$ compared to vehicle-Tg2576 group. †$p < 0.05$, ††$p < 0.01$ compared to acute/chronic HT-nTg group.

stroke signs, as well as an increase in the number and size of cerebral microhemorrhages. Susceptibility to hypertension-induced stroke and ICH was paralleled by the increased levels of matrix metalloproteinase pro-MMP-9 in Aβ-depositing Tg mice. These findings suggest that cerebral Aβ can affect ICH advance in the presence of hypertension, possibly through the induction of MMP-9.

Spontaneous ICH has the highest mortality of all stroke subtypes, yet there are no pharmacological therapies currently available, and the role of surgery remains controversial.[12] The development of preclinical models that reproduce the underlying mechanisms of injury is a valuable tool for the understanding of ICH pathophysiology and may contribute to new therapeutic strategies. A model of ICH in hypertensive mice was developed using acute hypertensive episodes superimposed on chronic hypertension.[2,9] In this model, chronic hypertension is induced by joint administration of AngII and the nitric oxide synthase inhibitor L-NAME, and acute hypertension through daily AngII injections. The ICHs were associated with an induction of oxidative stress and MMP-9 activation.[9,13] Here, we evaluated the association between hypertension and CAA, two of the major causes of ICH.[1] Although there was a similar increase in systolic blood pressure in hypertensive nTg and Tg2576, the Tg2576 mice were more prone to develop ICH. This is the first report establishing a direct interaction between Aβ pathology and hypertension in ICH. Furthermore, it has been shown previously that Aβ peptide induces the expression, release, and activation of MMP-9 in vitro, and extensive MMP-9 immunoreactivity was observed in CAA-affected vessels with evidence of microhemorrhage in Tg2576 mice,[14] thus suggesting that increased MMP-9 activation in response to human Aβ could further contribute to the development of acute/chronic hypertension-induced ICH. Corroborating this hypothesis, we demonstrated that the induction of hypertension was accompanied by a significant increase in pro-MMP-9 levels in Tg2576 animals. At the same time, the cell adhesion molecule P-selectin, an indicator of cerebrovascular endothelial cell activation, was evenly elevated in both Tg and nTg mice after experimental hypertension. Our data are also supported by previous reports evaluating the effect of blood pressure on the risk of CAA-related ICH in humans. An autopsy study found that cases with CAA and ICH had a history of hypertension more often than those without ICH.[15] In addition, recently randomized, placebo controlled studied with angiotensin-converting enzyme inhibitor perindopril clearly demonstrated that control of blood pressure reduces the risk of CAA-related ICH by 77% in patients diagnosed with cerebrovascular disease.[7]

Herein, we describe a model that combines hypertension in the presence of human Aβ, which develops accelerated ICH and stroke symptoms. This mixed pathology model may be a valuable tool for the identifying pathways through which hypertension and Aβ interact to contribute to ICH and microhemorrhages. Finally, this model replicates critical features of some of the human cerebrovascular disorders that comprise mixed cerebrovascular disease.

While there is no current therapy available for treatment of CAA, managing the blood pressure in the affected population may significantly reduce the incidence of ICH.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: NIH grants: NIA PO1 AG00538 and NINDS RO1 NSO20989 (DHC). Major support was provided by an Alzheimer's Association grants IIRG-11-204835 (DHC) and NIRG-12-242781 (VV). GFP holds a Postdoctoral Fellowship awarded through the Research Training Program II (TG2-01152) from the California Institute for Regenerative Medicine (CIRM).

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Authors' contributions

GFP designed and performed experiments, wrote the manuscript; KK performed the experiments and analysis; DLG performed the statistical analysis of the data; DHC designed the experiments and wrote the manuscript; VV designed and performed experiments, wrote the manuscript.

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