Vascular Permeability Precedes Spontaneous Intracerebral Hemorrhage in Stroke-Prone Spontaneously Hypertensive Rats

Jin-Moo Lee, MD, PhD; Guihua Zhai, PhD; Qingwei Liu, MS; Ernesto R. Gonzales, BSN; Kejie Yin, MD, PhD; Ping Yan, PhD; Chung Y. Hsu, MD, PhD; Katie D. Vo, MD; Weili Lin, PhD

Background and Purpose—Stroke-prone spontaneous hypertensive rats (SHRsp) fed a high-salt diet develop malignant hypertension, blood–brain barrier breakdown, and spontaneous intracerebral hemorrhage (ICH). The precise spatial and temporal relationship between these events has not been well-delineated.

Methods—Ten SHRsp male rats, fed a high-salt diet, were imaged weekly using MRI, starting at 12 weeks of age. Arterial blood pressure increased rapidly after 8 weeks of age in salt-loaded SHRsp rats (n=8), reaching a peak of 250/195 mm Hg.

Results—Seven SHRsp rats had spontaneous ICH develop before death. Five of the 7 rats had focally increased vascular permeability up to 2 weeks before spontaneous ICH. These rats, when fed a high-salt "Japanese-style diet," develop accelerated hypertension and early spontaneous ICH, and then die. ICH permeability at the site of the ICH; 3 of these rats had vascular permeability 1 to 2 weeks before spontaneous ICH. Salt-loaded SHRsp rats have increased vascular permeability up to 2 weeks before ICH, predicting hemorrhage both in space and time. These results suggest that hypertensive ICH is preceded by focal vasculopathy detectable by Gd leak. (Stroke. 2007;38:3289-3291.)

Key Words: hypertension ■ intracerebral hemorrhage ■ MRI

The most significant risk factor for intracerebral hemorrhage (ICH) is hypertension, which is found in up to 89% of patients with hemorrhage.1,2 The pathophysiological link between chronic hypertension and spontaneous ICH is poorly understood. Stroke-prone spontaneous hypertensive rats (SHRsp), a substrain of spontaneously hypertensive rats (SHRs) derived from Wistar Kyoto rats, are an animal model for hypertension-related spontaneous ICH. These rats, when fed a high-salt “Japanese-style diet,” develop accelerated hypertension and early spontaneous ICH, and then die.3 ICH in these rats appears to be hypertension-related, because antihypertensive agents4,5 and normal-salt diets3 prolong ICH latency and longevity. Although blood–brain barrier (BBB) breakdown and spontaneous hemorrhage has been well-described, the spatial and temporal relationship between these events has not been well-defined. In this study, we serially imaged SHRsp rats using MRI to examine the spatial and temporal evolution of BBB breakdown (using permeability maps) and the development of spontaneous hemorrhage (using the T2* sequence).

Subjects and Methods

Animals

SHRsp/A3N male rats from the NIH Autoimmune Rat Model Repository (Bethesda, Md) and Wistar Kyoto rats from Taconic (Germantown, NY) were fed a high-salt low-protein “Japanese-style” diet (Zeigler Bros) after weaning (3 to 4 weeks of age). Femoral artery blood pressure measurements were obtained at various times after birth. Rats were euthanized at the first sign of obvious neurological deficit, in compliance with regulations of the animal studies committees at Washington University and University of North Carolina.

MRI

Rats were imaged weekly under isoflurane anesthesia on a 3-T SIEMENS Allegra scanner with a 4.3-cm birdcage coil beginning at 12 week of age. T2-weighted (0.25 mm; repetition time [TR]=6790 ms; echo time [TE]=98 ms; 30 slices), T2*-weighted (3-dimensional gradient echo: TR=35 ms; TE=25 ms; 256×256; slice thickness [TH]=0.4 mm; field of view [FOV]=43×43 mm²), and turbo-FLASH sequences were used. Gd-DTPA (0.15 mL/100 grams) was injected via tail vein for permeability measurements, and the Look-Locker technique was used, using the T1 by multiple read-out pulses for pixel-by-pixel estimates of T1 (TI of 40 ms was used; 20 and 9 echoes were acquired before and after the injection of contrast, respectively, matrix size=128×64, FOV=32 mm, and 4 2-mm slices). T-one by multiple read-out pulses method was repeated 10 times after contrast, and the PATLAK approach was used for obtaining permeability maps for each rat.

Results

Arterial blood pressure increased rapidly after 8 weeks of age in salt-loaded SHRsp rats (n=8), reaching a peak of 250/195 mm Hg.

Received April 19, 2007; final revision received May 11, 2007; accepted May 18, 2007.

From Department of Neurology (J.-M.L., E.R.G., K.Y., P.Y.), Department of Radiology (K.D.V.), Washington University School of Medicine, St. Louis, Mo; Department of Radiology (Q.L., W.L.), University of North Carolina at Chapel Hill, NC; Topnotch Stroke Center (C.Y.H.), Taipei Medical University, Taipei, Taiwan; Department of Radiology (G.Z.), University of Chicago, Chicago, Ill.

Correspondence to Weili Lin, PhD, University of North Carolina at Chapel Hill, Department of Radiology, CB #7515, Chapel Hill, NC 27599. E-mail weili_lin@med.unc.edu

© 2007 American Heart Association, Inc.

Stroke is available at http://stroke.ahajournals.org

DOI: 10.1161/STROKEAHA.107.491621
at 16 weeks (Figure 1A). Salt-loaded Wistar Kyoto rats (n = 8) maintained constant blood pressure in the range of 110/85 throughout this period of time. SHRsp rats developed significant neurological deficit (and were euthanized), beginning at 11 weeks of age. All rats were euthanized or died spontaneously by 18 weeks of age (Figure 1B), similar to previous findings.3 Another cohort of SHRsp (n = 10) was serially imaged by MRI at weekly intervals. Seven rats developed spontaneous ICH as detected by T2* sequences during at least one of the imaging sessions. ICH location varied (cortex, corpus callosum, striatum), as did size. Figure 2 illustrates a rat with a large hemorrhage in the frontal cortex (T2* images) that developed at 14 weeks of age, with extensive edema surrounding the hemorrhage (T2 images).

Increased vascular permeability was detected in a single focus in 5 of the 7 rats in which ICH developed; 3 of these rats demonstrated increased permeability 1 to 2 weeks before the detection of ICH (Figure 3A). Increased vascular permeability developed in rat 3 during week 14 (yellow arrow, Figure 3B), but ICH did not develop until week 16 (red arrow). In all cases, regions of increased permeability coincided with the area of subsequent hemorrhage. Increased vascular permeability was not detected in 2 rats, even in images acquired at the time of hemorrhage (Figure 3A). Hemorrhages were very small in these rats.

**Discussion**

Chronic hypertension induces well-described changes in arteries and arterioles6 similar to that seen in SHRsp rats, including smooth muscle cell disarray, structural remodeling,7,8 and BBB breakdown, especially around vessels with fibrinoid degeneration.9,10 In this study, we found that salt-loaded SHRsp rats spontaneously developed foci of increased vascular permeability. In many cases, these lesions were predictive of subsequent ICH both spatially and temporally. Increased vascular permeability preceded spontaneous hemorrhage in 3 of 7 rats by 1 to 2 weeks; furthermore, areas of increased vascular permeability coincided with ICH in 5 of 7 rats.

Two rats developed areas of increased permeability only at the time of ICH detection. Although it is possible that BBB breakdown in these 2 rats did not precede ICH, another possibility is that the poor temporal resolution of our imaging scheme (weekly imaging) did not capture BBB leak before ICH (which may have occurred days before the hemorrhage). An additional 2 animals did not show detectable areas of increased permeability even on the scan that demonstrated ICH (these hemorrhages were very small, similar to the microbleeds observed in human patients with amyloid angiopathy). It seems unlikely that ICH would occur in the absence of BBB leak; more plausible is the lack of sensitivity of GdDTPA to detect subtle BBB leak, perhaps better-accomplished by smaller molecular weight contrast. The small sample size of our study does not permit statistical analysis of the corelationship of BBB leak and ICH, but the spatial and temporal prediction of ICH development is certainly suggestive of a cause and effect relationship. Gd enhancement was reported to precede hemorrhagic transformation in a rodent embolic stroke model,11 and in acute stroke patients treated with intravenous tissue plasminogen activator.12,13 Although the pathogenesis of hemorrhagic transformation is likely different from hypertension-induced ICH, both may involve vascular degeneration and BBB breakdown, before frank hemorrhage. Based on our results, we raise the possibility that focal Gd enhancement may precede spontaneous ICH in patients with malignant hyper-
tension. The clinical significance of this potential predictor of ICH in patients with hypertension remains to be studied.

**Sources of Funding**

This work was supported by National Institutes of Health grants R01-NS48283 (to J.M.L.), P01-NS32636 (to J.M.L.), R01 NS054079 (to W.L.), an American Health Assistance Foundation grant (to J.M.L.), an American Heart Association SDG grant (to K.Y.), and a grant from the Ministry of Education Topnotch Stroke Center and the Department of Health Clinical Research Center of Excellence at Taipei-Medical University Wanfang Hospital (to C.Y.H.).

**Disclosures**

None.

**References**

Vascular Permeability Precedes Spontaneous Intracerebral Hemorrhage in Stroke-Prone Spontaneously Hypertensive Rats

Jin-Moo Lee, Guihua Zhai, Qingwei Liu, Ernesto R. Gonzales, Kejie Yin, Ping Yan, Chung Y. Hsu, Katie D. Vo and Weili Lin

*Stroke*. 2007;38:3289-3291; originally published online October 25, 2007; doi: 10.1161/STROKEAHA.107.491621

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/38/12/3289

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/