Photothrombotic Middle Cerebral Artery Occlusion in Spontaneously Hypertensive Rats
Influence of Substrain, Gender, and Distal Middle Cerebral Artery Patterns on Infarct Size

Hong Cai, MD; Hiroshi Yao, MD; Setsuro Ibayashi, MD; Hideyuki Uchimura, MD; Masatoshi Fujishima, MD

Background and Purpose—To analyze the effects of substrain and gender differences in spontaneously hypertensive rats (SHR) and distal middle cerebral artery (MCA) branching patterns on infarct size, we compared infarct volumes produced by photothrombotic distal MCA occlusion using SHR/Kyushu and SHR/Izumo (Izm).

Methods—Twenty-four male and 8 female SHR/Kyushu, 15 male and 5 female SHR/Izm, and 6 male Wistar-Kyoto rats (WKY)/Izm (5 to 7 months old) were subjected to photothrombotic distal MCA occlusion, and infarct volumes were determined.

Results—Although blood pressure levels were essentially the same between the two substrains of hypertensive rats, infarct volumes were significantly larger in the SHR/Kyushu substrain than in SHR/Izm of either sex (P < 0.001); infarct volumes in male and female SHR/Kyushu were 83.8 ± 11.7 and 58.5 ± 9.2 mm³, and those in male and female SHR/Izm were 61.5 ± 10.7 and 34.8 ± 7.9 mm³, respectively (values are mean ± SD). Male SHR/Kyushu that had simple Y-shaped MCA showed smaller infarcts (75.8 ± 14.6 mm³, n = 11) than those with more branching (regular) MCA (93.2 ± 19.1, n = 13), the difference being significant (P = 0.022). Male SHR/Izm with simple distal MCA also produced smaller infarctions than those with regular MCA (51.0 ± 3.7 versus 68.9 ± 8.7 mm³, P = 0.0004).

Conclusions—Photothrombotic occlusion of distal MCA in hypertensive rats provides a simple and reproducible model of focal ischemia. Most importantly, this study emphasizes the substantial variabilities in infarct sizes caused by the differences in substrains of SHR, gender, and distal MCA patterns. (Stroke. 1998;29:1982-1987.)

Key Words: stroke, experimental cerebral ischemia, focal photochemistry models, animal

Reproducible animal models of stroke or focal ischemic infarction are crucial to the study of the pathophysiology of ischemic brain injury. Focal ischemia models are relevant to the human clinical setting, because ischemic stroke is the predominant type of cerebrovascular disease. The subtemporal approach technique of Tamura et al,1,2 occluding the proximal part of the middle cerebral artery (MCA), has been established as a standard focal cerebral ischemia model in the rat. Occlusion of the proximal MCA in the rat is technically feasible, but this model is surgically demanding. Chen et al3 used a method of more distal occlusion of MCA above the rhinal fissure combined with permanent ipsilateral and temporary contralateral common carotid artery (CCA) occlusions. Brint et al4 also used a distal MCA occlusion with permanent occlusion of the ipsilateral CCA and found great variability in cortical infarct volumes in normotensive rat strains. However, the infarcts were large and fairly consistent in spontaneously hypertensive rat(s) (SHR).

The results of Duverger and MacKenzie5 revealed considerable differences in lesion size and location, depending on such factors as strain, arterial blood pressure, and blood glucose concentration. Proximal MCA occlusion causes a small and variable infarction in Wistar-Kyoto rat(s) (WKY), a large but equally variable infarction in Sprague-Dawley rats, and the largest and most consistent infarction in Fisher-344 rats, among normotensive strains of each. In hypertensive rats, MCA occlusion gives rise to much larger infarcts than in normotensive strains. Distal MCA occlusion also produces the largest and most reproducible infarcts in stroke-prone SHR (SHRSP).6,7 Recently, a thrombotic distal MCA occlusion model of rats was established in earlier work and our previous study.8-11 Photothrombotic infarcts produced by distal MCA occlusion without CCA manipulation in SHR were moderate-sized and localized reproducibly with an acceptable coefficient of variation.11,12
Hypertensive rats are relevant to stroke research and are used widely for studies of hypertension-related cerebrovascular complications. However, one of the most confounding problems is that SHR or the normotensive control WKY from different sources are genetically heterogeneous. SHR/Izm, breed established at the Izu National Institute of Technology, have been used widely for studies of hypertension-related cerebrovascular complications. However, one of the most confounding problems is that SHR or the normotensive control WKY from different sources are genetically heterogeneous. SHR/Izm (Izm) have emerged as a new “prototype” of SHR that is different from original SHR. SHR/Izm and its control WKY/Izm have a common genetic marker pattern, a major histocompatibility antigen RT-1k type, and are now commercially available. In the present study, we attempted to assess the effects of strain and gender differences in hypertensive rats and of distal MCA anatomy on infarct size. The present data are the first to show significant differences in infarct volumes between two substrains of SHR.

**Materials and Methods**

All procedures were done in accordance with the Animal Care Guidelines of Kyushu University.

**Materials**

A total of 58 rats (24 male and 8 female SHR/Kyushu, 15 male and 5 female SHR/Izm, and 6 male WKY/Izm) aged 5 to 7 months were used in this study. SHR/Kyushu and SHR/Izm are two substrains of SHR. SHR/Kyushu were maintained in the Kyushu University Animal Center under specific pathogen-free conditions and fed regular rat chow (CLEA rodent diet CE-2, containing 25% protein, Na+ 2.6 mg/g, and K+ 10.6 mg/g per pellet) and tap water ad libitum. SHR/Izm and WKY/Izm were obtained from a commercial vendor (Funabashi Farm, Chiba, Japan) and commissioned by the Disease Model Cooperative Research Association (Chiba, Japan) at the age of 3 months. Four groups of rats (male and female SHR/Kyushu and SHR/Izm) were randomly assigned to photothrombotic MCA occlusion ($n=5$ to 10 per group).

**Surgical Setup**

Rats were anesthetized with halothane (4% for induction; 1.5% during the surgical preparation, with a face mask; 0.75% after intubation; and 0.5% for maintenance) in a mixture of 70% nitrous oxide/30% oxygen. After 3 days, the rat was decapitated under amobarbital anesthesia (100 mg/kg IP), and the brain was rapidly removed. The entire brain was cooled in ice-cold saline for 10 minutes and cut into 2-mm-thick coronal sections in a cutting block; the brain slices were then immersed in 2% 2,3,5-triphenyltetrazolium chloride (Wako Pure Chemical Industries Ltd) at 37°C for 30 minutes in the dark. The posterior surface of each section was imaged via video camera, and the infarct areas indicated by the lack of staining were calculated using NIH Image software (version 1.56). The infarct volume of each rat was calculated by the following formula (trapezoidal rule): $V = \frac{d}{2} \left( Y_1 + Y + Y + \cdots + Y + Y_{n-1} \right)$, where $V$ indicates volume; $d$, distance between the sections; and $Y_i$, cross-sectional area of the $i$th section, and where the ends ($Y_1$ and $Y_n$) are equal to zero. Statistical Analysis

The values were expressed as mean±SD. Differences in physiological variables and infarct volume were analyzed with the unpaired $t$ test. The levels of significance were set at $P<0.0125$ (Figure 2) and $P<0.025$ (Figure 3) according to the number of multiple comparisons (ie, Bonferroni’s principle).

**Results**

Physiological variables in experimental groups are shown in Table 2. Arterial gases were in the normal range. MABP averaged 183 mm Hg in male SHR/Kyushu, 180 in male SHR/Sprague-Dawley, and 148 in male WKY/Izm. Therefore, the lower half of distal MCA of SHR can be enveloped with an elliptical, almost linear, laser beam.

### Table 1. Variations in Distal MCA of 3 Strains of Rats

<table>
<thead>
<tr>
<th>Distal MCA Patterns</th>
<th>SHR (n=85)</th>
<th>Sprague-Dawley Rats (n=127)</th>
<th>Wistar Rats (n=42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple (I), %</td>
<td>44</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Regular (II, III, IV, V), %</td>
<td>49</td>
<td>66</td>
<td>45</td>
</tr>
<tr>
<td>Complicated (VI, VII, VIII), %</td>
<td>7</td>
<td>14</td>
<td>48</td>
</tr>
</tbody>
</table>

**Figure 1.** The 8 branching patterns of distal MCA suitable for male SHR, Sprague-Dawley rats, and Wistar rats (adapted from Figure 2 of reference 8). P, F, T, and Py indicate parietal, frontal, temporal, and pyriform branches, respectively.
Figure 2. Infarct volume in two substrains of SHR (male and female SHR/Kyushu and SHR/Izm). Values are mean±S.D. * P<0.001 vs SHR/Izm; # P<0.001 vs female SHR/Kyushu and female SHR/Izm. According to Bonferroni’s principle, P<0.0125 was considered to be significant.

SHR/Izm, 161 in female SHR/Kyushu, and 155 in female SHR/Izm. Photothermalbottic distal MCA occlusion led to a consistent pattern of cortical infarction; the coefficients of variation (SD divided by mean value) were 14% to 23%.

Infarct size in four different groups of rats were compared (Figure 2). Although the levels of blood pressure were essentially the same between male SHR/Kyushu and SHR/Izm and between female SHR/Kyushu and SHR/Izm, mean infarct volume was larger in male SHR/Kyushu (83.8±11.7 mm^3) than in SHR/Izm (61.5±10.7 mm^3) (P<0.001) and in female SHR/Kyushu (58.5±9.2 mm^3) than in SHR/Izm (34.8±7.9 mm^3) (P<0.001). In female SHR/Kyushu and SHR/Izm, the MABP levels were lower, and infarct volumes were smaller than those in males (P<0.001). The difference of infarction volumes was 30% between male and female SHR/Kyushu. Mean hemispheric size determined on the fourth brain section in female SHR/Kyushu (58.1±2.2 mm^3) was 5% less than in males (61.1±3.3 mm^3) (P<0.05). Average-sized cortical infarction in each group is presented in Figure 4.

Figure 3. Infarct volume in male SHR/Kyushu (n=24) and SHR/Izm (n=15) with simple or regular MCA patterns. * P=0.022, ** P=0.004 vs SHR/Kyushu (n=13) or SHR/Izm (n=9) with regular MCA pattern. According to Bonferroni’s principle, P<0.025 was considered to be significant.

Pooled data of male hypertensive rats (24 SHR/Kyushu and 15 SHR/Izm) were used to investigate the relationship between branching patterns of distal MCA and infarct size. Infarct volume in SHR/Kyushu with simple, Y-shaped MCA was 75.8±14.6 mm^3, which was <93.2±19.1 mm^3 in other SHR/Kyushu with more branching (regular) MCA (P=0.022) (Figure 3). In male SHR/Izm, simple distal MCA also produced smaller infarction than did regular distal MCA (51.0±3.7 vs 68.9±8.7 mm^3, respectively, P=0.0004). In these analyses, physiological variables (MABP, body weight, arterial gases, hematocrit, blood glucose, and head temperature) were not different between the groups of rats with simple or regular MCA patterns.

Discussion

Although blood pressure levels were not different between the two substrains of hypertensive rats (ie, SHR/Kyushu and SHR/Izm), infarct volumes were significantly larger in the SHR/Kyushu substrain than in SHR/Izm of either sex. In female SHR/Kyushu and SHR/Izm, MABP was lower and infarcts were smaller than in male rats. Infarct volumes in male SHR/Kyushu and SHR/Izm with simple Y-shaped MCA were smaller than those with more branching (regular) MCA.

The lower limit of CBF autoregulation is shifted to a higher level and cerebrovascular resistance is increased in SHR compared with normotensive rats.18 Furthermore, markedly decreased cerebral perfusion pressure after bilateral carotid occlusions in SHR, as represented by lowered carotid back pressure, resulted in ischemic brain energy metabolism19,20 and ischemic infarction.21 These changes associated with long-standing hypertension are considered to be major factors for the susceptibility to global cerebral ischemia in SHR.22 In focal ischemia, the narrower anastomoses between MCA and anterior or posterior cerebral artery at cortical arterial boundary zones in SHRSP compared with normotensive rats restrict blood flow into the territory of the occluded MCA, resulting in large infarcts in hypertensive rats.6 Our present results were consistent with above studies, showing 2.7 to 3.7 times larger infarctions in hypertensive rats (SHR/Izm and SHR/Kyushu) than in normotensive WKY/Izm (22.7±3.6 mm^3, n=6).

Sources of potential experimental variability in focal cerebral ischemia models have been emphasized.23 Strain-related variables affect outcome in models of MCA occlusion.24,25 We found intrainstrain differences in infarct volume in our distal MCA occlusion model. Because MABP levels were the same between SHR/Kyushu and SHR/Izm, factors other than hypertension probably account for different lesion sizes. Recent linkage analyses have identified that blood pressure–independent genetic factors, genetic foci on chromosome 1, termed STR 1,26 or on chromosome 4,27 determine susceptibility to spontaneous stroke. Interestingly, the former study showed that genetic foci on chromosomes 4 and 5 were protective against stroke. Furthermore, a genetic focus (the QTL) on chromosome 5 has been demonstrated to account for 67% of the variance in infarct volume in SHRSP after occlusion of the MCA; there was no linkage between this QTL and any known blood pressure phenotypes.28 Such genetic factors, in addition to long-standing hypertension, play critical roles in deteriorating ischemic stroke.
The female SHRSP have a lower incidence of stroke and a longer life span compared with males.29 In our experience, female SHR have consistently lower blood pressure than male SHR and milder disturbance in cerebral energy metabolism after bilateral CCA occlusions.30 In the present study, infarct size after MCA occlusion was smaller in female SHR than in males. Lower blood pressure in female hypertensive rats may partly explain the smaller infarcts in female than in male SHR. Although female body weights are much less than those of males in SHR/Kyushu, there was only a 5% difference in brain size between male and female SHR/Kyushu. That is not enough to explain the 30% difference in infarction volume. Ovarectomy deteriorates and estrogen attenuates focal ischemic injury.31,32 Estrogen modulates the vascular dysfunction by preserving nitric oxide synthesis in female SHR.33 Therefore, two factors (ie, lower blood pressure levels and gonadal hormones in female rats) may be responsible for the gender difference in susceptibility to cerebral ischemia in SHR.

During the course of establishing a criterion for laser irradiation of the MCA in the distal field distribution, which is combined with an infusion of photosensitizing dye rose bengal (ie, photothrombosis), we found approximately one half of the distal MCAs in SHR are simple.8–11 Infarct volume of SHR with simple Y-shaped (Type I) MCA was smaller than that of the regular pattern (Types II, III, IV, and V). Menzies et al34 failed to find any relationship between the anatomical pattern of MCA and the surface size of the brain infarction in Sprague-Dawley rats. The reason for the discrepancy between the results by Menzies et al and our present results is not clear, but our data suggest an important caveat to pharmacoprotection studies; biased selection of SHR with simple or regular distal MCA may generate misleading treatment effects.

In conclusion, this study demonstrates the critical importance of several variables, such as differences in the substrain and gender of hypertensive rats and arterial patterns of distal MCA, in determining infarct volume in the simple model of photothrombotic occlusion of distal MCA in SHR.

Acknowledgments
This study was supported in part by the Social Insurance Agency Contract Fund commissioned by the Japanese Health Sciences Foundation. We thank Toru Nabika, MD, and Brant D. Watson, PhD, for their valuable advice during the course of this study.

References

\[ \text{TABLE 2. Physiological Variables} \]

<table>
<thead>
<tr>
<th>Experimental Groups</th>
<th>No.</th>
<th>BW, g</th>
<th>MABP, mm Hg</th>
<th>PaCO(_2), mm Hg</th>
<th>PaO(_2), mm Hg</th>
<th>pH</th>
<th>Hct, %</th>
<th>Glucose, mmol/L</th>
<th>Head Temperature, °C</th>
<th>Body Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male SHR/Kyushu</td>
<td>8</td>
<td>409±6</td>
<td>183±2</td>
<td>37±1</td>
<td>115±13</td>
<td>7.45±0.01</td>
<td>45±1</td>
<td>5.11±0.61</td>
<td>36.2±0.1</td>
<td>37.3±0.2</td>
</tr>
<tr>
<td>Male SHR/Izm</td>
<td>10</td>
<td>376±15</td>
<td>180±7</td>
<td>37±2</td>
<td>128±10</td>
<td>7.44±0.01</td>
<td>43±1</td>
<td>5.66±0.94</td>
<td>36.1±0.1</td>
<td>37.1±0.1</td>
</tr>
<tr>
<td>Female SHR/Kyushu</td>
<td>8</td>
<td>231±6</td>
<td>161±5</td>
<td>38±2</td>
<td>128±12</td>
<td>7.42±0.04</td>
<td>41±1</td>
<td>5.22±0.61</td>
<td>36.0±0.1</td>
<td>37.0±0.2</td>
</tr>
<tr>
<td>Female SHR/Izm</td>
<td>5</td>
<td>247±3</td>
<td>155±5</td>
<td>36±2</td>
<td>115±14</td>
<td>7.43±0.01</td>
<td>43±2</td>
<td>6.38±1.11</td>
<td>36.3±0.2</td>
<td>37.5±0.2</td>
</tr>
</tbody>
</table>

Values are mean±SD. BW indicates body weight; Hct, hematocrit.

Figure 4. Diagrams showing the representative infarcts in male and female SHR/Kyushu and SHR/Izm.
1986

Thrombotic MCA Occlusion Model

15. Okamoto K, Aoki K. Development of a strain of spontaneously hyper-
17. Rosen GD, Harry JD. Brain volume estimation from serial section mea-
19. Fujishima M, Sugi T, Morotomi Y, Omae T. Effects of bilateral carotid artery ligation on brain lactate and pyruvate concentrations in normoto-
21. Ogata J, Fujishima M, Morotomi Y, Omae T. Cerebral infarction follow-
 ing bilateral carotid artery ligation in normotensive and spontaneously hyper-
25. Oliff HS, Coyle P, Weber E. Rat strain and vendor differences in col-

Editorial Comment

By means of the photothrombotic method developed by Yao et al (see reference 11), in which a 2-mm-long segment of the distal MCA is occluded (in the absence of concurrent carotid artery occlusion), Cai and coauthors in the accompanying article confirmed for the first time that MCA territory infarct volume in SHR (Kyushu and Izumo strains) is sensitive to gender1 and strain and, strikingly, also sensitive to the MCA branching pattern but was not influenced by differences in blood pressure between strains. The single branch (“simple”) Y-shaped MCA distribution yielded smaller infarcts than the multiple branch (“regular”) MCA pattern in each strain-matched case, and this aspect (as the authors have suggested) must now be taken into account to evaluate properly the results of drug therapies on lesion development. Evaluation of drugs should also be more feasible with the moderate infarct volumes produced by the present method, because the resultant ischemia is submaximal in severity (see reference 11, Cai et al). As long as infarct volume remains the universal standard for such evaluations, the findings of Cai and coworkers constitute an important contribution to research in stroke development and its mitigation.

Why the simple MCA branching pattern should be less susceptible to infarct development than the regular, multiple-branched pattern is most intriguing. At first glance, this result would seem counterintuitive, inasmuch as the more complicated regular pattern should provide more avenues for retrograde blood infiltration via collat-
eral channels. However, as the authors have recognized, anastomoses between neighboring territories in stroke-prone SHR are narrower compared with normotensive rats (see reference 6, Cai et al). Possibly this deficiency is compounded in SHR with the regular branching pattern compared to SHR with the simple pattern, owing to the presence of a larger proportion of higher-generation ves-
sels (with narrower diameters) in the former variety. Also, vasculature reflecting the more complicated regular pattern may provide more vascular space for blood-brain barrier breaches, which are known to result from secretions produced during thrombosis.2 The fact that these anasto-
moses are nonetheless effective to some degree is sug-
gested by noting that infarct volume resulting from the present method of inducing a long MCA occlusion (refer-
ence 11, Cai et al) is about twice that obtained by occluding the equivalent segment in three different spots (reference 10, Cai et al).

Finally, it would be quite interesting to speculate which branching pattern would be more susceptible to reperfu-

Downloaded from http://stroke.ahajournals.org/ by guest on January 15, 2018
sion injury, if in fact reperfusion could be elicited in this model or in another one utilizing a normotensive strain. In the case of SHR, this may be difficult to observe inasmuch as ischemia alone confers near-maximal damage to cerebral tissue; evidently, reperfusion injury can be seen only in the context of intermediate ischemia, as moderated by the presence of sufficient collateral channels. From this, one would predict that rats exhibiting the single-branch simple MCA pattern would evince reperfusion injury at early times, while those with more complicated MCA patterns would likely already display irreversible damage at these same times.

References
Photothrombotic Middle Cerebral Artery Occlusion in Spontaneously Hypertensive Rats: Influence of Substrain, Gender, and Distal Middle Cerebral Artery Patterns on Infarct Size

Hong Cai, Hiroshi Yao, Setsuro Ibayashi, Hideyuki Uchimura and Masatoshi Fujishima

doi: 10.1161/01.STR.29.9.1982

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 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/29/9/1982

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/