Differences in the Evolution of the Ischemic Penumbra in Stroke-Prone Spontaneously Hypertensive and Wistar-Kyoto Rats

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Background and Purpose—Stroke-prone spontaneously hypertensive rats (SHRSP) are a highly pertinent stroke model with increased sensitivity to focal ischemia compared with the normotensive reference strain (Wistar-Kyoto rats; WKY). Study aims were to investigate temporal changes in the ischemic penumbra in SHRSP compared with WKY.

Methods—Permanent middle cerebral artery occlusion was induced with an intraluminal filament. Diffusion- (DWI) and perfusion- (PWI) weighted magnetic resonance imaging was performed from 1 to 6 hours after stroke, with the PWI-DWI mismatch used to define the penumbra and thresholded apparent diffusion coefficient (ADC) maps used to define ischemic damage.

Results—There was significantly more ischemic damage in SHRSP than in WKY from 1 to 6 hours after stroke. The perfusion deficit remained unchanged in WKY (39.9 ± 6.6 mm$^2$ at 1 hour, 39.6 ± 5.3 mm$^2$ at 6 hours) but surprisingly increased in SHRSP (43.9 ± 9.2 mm$^2$ at 1 hour, 48.5 ± 7.4 mm$^2$ at 6 hours; $P=0.01$). One hour after stroke, SHRSP had a significantly smaller penumbra (3.4 ± 5.8 mm$^2$) than did WKY (9.7 ± 3.8, $P=0.03$). In WKY, 56% of the 1-hour penumbra area was incorporated into the ADC lesion by 6 hours, whereas in SHRSP, the small penumbra remained static owing to the temporal increase in both ADC lesion size and perfusion deficit.

Conclusions—First, SHRSP have significantly more ischemic damage and a smaller penumbra than do WKY within 1 hour of stroke; second, the penumbra is recruited into the ADC abnormality over time in both strains; and third, the expanding perfusion deficit in SHRSP predicts more tissue at risk of infarction. These results have important implications for management of stroke patients with preexisting hypertension and suggest ischemic damage could progress at a faster rate and over a longer time frame in the presence of hypertension. (Stroke. 2009;40:3864-3868.)

Key Words: ischemic penumbra ▪ stroke ▪ hypertension ▪ magnetic resonance imaging

One of the most important considerations when treating acute stroke patients is to establish whether potentially salvageable (penumbral) tissue is still present within the brain. In this study, we used a pertinent rodent model to investigate whether recognized stroke risk factors such as hypertension influence the amount of penumbral tissue and the rate at which it is incorporated into the ischemic lesion.

The ischemic penumbra is a brain region surrounding the ischemic core that receives limited blood supply from the collateral circulation. Tissue perfusion lies between the blood-flow thresholds for functional impairment and morphological integrity, which means that the tissue may be capable of recovery, provided that perfusion is reinstated within a limited time window; beyond this time frame, the tissue becomes irreversibly damaged and is incorporated into the infarct. Ischemic penumbra can be assessed clinically with magnetic resonance imaging (MRI) by identifying the mismatch between the perfusion deficit (perfusion-weighted imaging, PWI) and injured tissue in which cell swelling has occurred (diffusion-weighted imaging, DWI).2

The stroke-prone spontaneously hypertensive rat (SHRSP) is a relevant model of human stroke. As its name implies, SHRSP develop hypertension between 8 and 12 weeks of age and have an increased incidence of spontaneous stroke.3,4 They are an ideal model for studying ischemic stroke because of similarities in terms of their cerebrovascular architecture and risk factors compared with stroke in humans.5 SHRSP have an increased sensitivity to experimental stroke compared with normotensive strains,6-9 which is thought to be due to genetically determined hypertension and additional genetic factors influencing the cerebral vessels and levels of oxidative stress.8,10 The Wistar-Kyoto rat (WKY), the nor-
motensive strain from which the SHRSP was derived, is used as the control strain for the SHRSP. To our knowledge, differences in the amount of penumbral tissue available between SHRSP and WKY have not previously been investigated, nor has the serial in vivo evolution of acute ischemic damage or the fate of the penumbra.

The aims of the present study were to (1) identify and follow the fate of penumbral tissue 6 hours after permanent middle cerebral artery occlusion (MCAO) with the use of DWI and PWI MRI and (2) compare the size and lifespan of penumbral tissue in SHRSP and WKY.

Materials and Methods

Animal Preparation
Experiments were carried out under license from the UK Home Office and were subject to the Animals (Scientific Procedures) Act, 1986. SHRSP and WKY rats were obtained from inbred colonies within the University of Glasgow, Division of Cardiovascular and Medical Sciences.

Male WKY and SHRSP (220 to 390 g, 12 to 16 weeks old, n=8 per strain) were anesthetized (4% to 5% isoflurane) in an induction chamber, then intubated, and artificially ventilated (with 1.5% to 2% isoflurane in 70:30 N₂O/O₂). Body temperature was monitored throughout surgery with a rectal thermocouple and maintained at 37°C. The femoral artery was cannulated with PE-50 tubing for continuous monitoring of arterial blood pressure and heart rate (Biopac) and for measurement of arterial blood gases (Bayer, Rapidlab 248).

Permanent occlusion of the MCA was performed by intraluminal thread occlusion with a 3-0 nylon monofilament with a rounded tip (diameter=0.28 to 0.3 mm for rats 200 to 400 g) as previously described. After filament insertion, the animal was transferred to the magnet. Anesthesia was maintained at 1% to 2% isoflurane in 70:30 N₂O/O₂, and temperature was continuously monitored and maintained by an enclosed warm water circuit.

Magnetic Resonance Imaging
MRI was performed on a Bruker Biospec 7-T/30-cm system with a gradient coil (internal diameter=121 mm, 400 mT/m) and a 72-mm birdcage resonator. An actively decoupled linear surface receiver coil (2-cm diameter) was used for brain imaging. After surgery, animals were placed prone in a rat cradle, with the head restrained by ear and tooth bars to limit movement, with the surface coil placed above the head.

A pilot sequence was acquired first to determine the correct geometry, followed by a (RARE) T₂, effective echo time [TE]=47.2 ms, repetition time [TR]=5000 ms, 4 averages, matrix=256×256, field of view [FOV]=25×25 mm, 30 contiguous slices, 0.5-mm thickness) for anatomic reference. For quantitative determination of the apparent diffusion coefficient (ADC), a 4-shot spin echo planar imaging (EPI) DWI scan (TE=22.5 ms; TR=4000.3 ms; 4 averages; matrix=96×96; FOV=25×25 mm; 3 directions=x, y, z; B values=0, 200, 600, 1200, and 2000 s/mm²; slice thickness=2 mm) was also carried out on the same slice as used for the CBF measurements, which had the same image distortion as the CBF maps and allowed us to coregister the ADC and CBF maps for determination of the diffusion-perfusion mismatch area. DWI and PWI scans were started 1 hour after stroke and repeated every hour until 6 hours after MCAO.

Data Analysis
Quantitative ADC maps, in units of square millimeters per second, were calculated from the Stejskal-Tanner equation. ADC maps and rCBF maps were generated with Image J software (http://rsb.info.nih.gov/ij/). A 23% reduction in mean contralateral ADC was used to determine ischemic lesion volume from the multislice ADC maps and lesion area from the single-slice ADC map. Perfusion deficit area was calculated on the basis of a 57% reduction of mean contralateral CBF. Diffusion-perfusion mismatch was calculated as the difference between the perfusion deficit minus the ADC lesion area on the corresponding slice. All data analysis was carried out blind as to rat strain.

Statistical Analysis
Data are expressed as mean±SD. A Student’s paired t test was used to determine significance within strains. Sequential changes between strains were compared with 2-way ANOVA with a Bonferroni post test to correct for multiple comparisons. An unpaired t test was used to compare lesion progression between strains. A probability value of 0.05 or less was considered statistically significant.

Results

Physiologic Variables and 1-Hour Perfusion and Diffusion Scans
Physiologic variables are shown in the Table. All physiologic variables were well controlled throughout the 6-hour scanning period in both groups (P>0.05). The mean CBF reduction, taken from the ischemic core in the caudate putamen, was not significantly different between strains (9.7±6.7% and 10.5±6% of mean contralateral CBF for WKY and SHRSP, respectively), indicating a similar severity of ischemic insult. Figure 1 highlights the DWI-PWI mismatch at 1 and 6 hours after permanent MCAO in WKY and SHRSP.
Evolution of ADC-Derived Lesion Volume

In both strains, the ADC-derived lesion volume increased significantly, from 1 to 6 hours after MCAO (WKY, 171 ± 30 mm³ at 1 hour, 253 ± 43 at 6 hours; SHRSP, 275 ± 75 mm³ at 1 hour, 330 ± 57 mm³ at 6 hours; Figure 2). The ADC lesion was significantly larger in SHRSP than in WKY within 1 hour of stroke and at all subsequent time points (P < 0.05 for all time points). The 1- to 6-hour evolution of the ADC lesion was not significantly different between the 2 strains (increase of 77 and 54 mm³ in WKY and SHRSP, respectively; P > 0.05, unpaired t test). However, the SHRSP lesion volume was virtually maximal at 6 hours, covering the entire MCA territory.

Evolution of the Perfusion Deficit

Single-slice rCBF maps within the MCA territory revealed the persistence of MCAO for 6 hours. Figure 3 shows the area of perfusion deficit throughout the entire time course for WKY and SHRSP. The perfusion deficit area was unchanged in WKY throughout the 6-hour time course (from 39.9 ± 6.1 mm² at 1 hour to 39.6 ± 5.3 mm² at 6 hours after MCAO; P = 0.64, paired t test). In contrast, SHRSP displayed a significant increase in perfusion deficit area during the 6 hours (from 43.9 ± 9.2 mm² at 1 hour to 48.5 ± 7.4 mm² at 6 hours after MCAO; P = 0.01, paired t test). There was little difference in perfusion deficit area at 1 hour after MCAO between strains (39.9 ± 6.1 mm² in WKY vs 43.6 ± 9.2 mm² in SHRSP). However, by 5 hours (40.6 ± 5.6 vs 48.7 ± 6.2 mm²) and 6 hours (39.6 ± 5.3 vs 48.5 ± 7.5 mm²), there was a greater difference in perfusion deficit between strains, although this was not statistically significant.

Evolution of the Diffusion-Perfusion Mismatch

The DWI image from the same coronal slice as that used for PWI was used to calculate the diffusion-perfusion mismatch area. Figure 4 illustrates the evolution of the ADC-derived lesion on the single slice used for calculating the diffusion-perfusion mismatch. The ADC-derived lesion was significantly larger in SHRSP from as early as 1 hour after stroke.

**Figure 1.** Representative thresholded CBF map with ADC abnormality (black) overlaid highlighting the DWI-PWI mismatch (shown in white) at 1 and 6 hours after stroke in a WKY (top) and SHRSP (bottom).

**Figure 2.** Temporal evolution of the ADC-derived lesion volume after permanent MCAO in SHRSP and WKY. Data are presented as mean ± SD. *Significant statistical difference in ADC lesion volume between strains (2-way ANOVA with Bonferroni post test, P < 0.05, n = 7 or 8).

**Figure 3.** Temporal evolution of the PWI-derived perfusion deficit after permanent MCAO in SHRSP and WKY. Data are presented as mean ± SD. *Statistically significant increase in PWI lesion area from 1- to 6-hour time points (paired t test, P < 0.05, n = 7 or 8).

**Figure 4.** Temporal evolution of the ADC-derived lesion area after permanent MCAO in SHRSP and WKY. Data are presented as mean ± SD. *Statistically significant difference in ADC lesion area between strains (2-way ANOVA with Bonferroni post test, P < 0.05, n = 7 or 8).
Figure 5. Temporal evolution of DWI-PWI mismatch area after permanent MCAO in SHRSP and WKY. Data are presented as mean±SD. *Statistically significant difference in mismatch area between WKY and SHRSP (2-way ANOVA with Bonferroni post test, *P<0.05). #Statistically significant decrease from 1- to 6-hour time points in WKY (P<0.05, paired t test, n=7 or 8).

(40.6±5.8 vs 29.3±4 mm², respectively, at 1 hour after MCAO; *P<0.001) and throughout the 6-hour time course (P<0.001 for all time points). There was no significant difference in the evolution of the ADC lesion area between strains (increase of 7 and 6 mm² in WKY and SHRSP between 1 and 6 hours after MCAO, respectively; *P>0.05, unpaired t test).

Mismatch was determined as the difference between the ADC-derived lesion area and the perfusion deficit area from the same slice. At 1 hour after MCAO, the diffusion-perfusion mismatch area was significantly smaller in SHRSP (3.4±5.8 vs 9.7±3.8 mm² in WKY, *P=0.03; Figure 5), even though the perfusion deficit area was not significantly different between strains at this time point. This resulted in 65% less penumbral tissue in SHRSP compared with WKY at 1 hour after stroke. Over time, there was a significant decrease in mismatch in WKY (from 9.7±3.8 mm² at 1 hour to 3.2±2.7 mm² at 6 hours after MCAO; *P=0.01, paired t test), resulting in a 66% reduction in the penumbra at 6 hours. In SHRSP, there was a small but nonsignificant decrease in the already-small mismatch area during the same time course (from 3.4±5.8 mm² at 1 hour to 1.7±4.2 mm² at 6 hours after MCAO; *P=0.23, paired t test). This lack of change in mismatch area over time in SHRSP was due to the increase in perfusion deficit area alongside the increase in ADC lesion area.

Discussion
This is the first study to investigate the spatiotemporal evolution of ischemic damage during the acute phase in SHRSP and WKY after MCAO. Previous studies have demonstrated that SHRSP display increased sensitivity to stroke and have increased infarct volume after MCAO when compared with their normotensive WKY counterparts.6,9,15–17 However, none of those studies investigated the presence of penumbral tissue nor its fate in SHRSP compared with WKY. The present study demonstrates that the ADC-derived lesion was significantly larger in SHRSP from 1 hour after MCAO and remained so throughout the 6-hour time course. However, there were no significant differences in the rate of lesion evolution between the strains. Parameters such as blood pressure and blood gases, which can influence infarct size and development, were maintained within normal levels throughout anesthesia. Therefore, it is unlikely that the increased lesion volume can be attributed to physiologic variables in the SHRSP. It has been previously shown that when experimental stroke is induced in young SHRSP before the onset of hypertension, infarct volume is still significantly larger than in age-matched WKY, indicating that factors other than hypertension contribute to increased stroke sensitivity in the SHRSP.8,18 Increased release of glutamate has previously been demonstrated after stroke in the SHRSP,19 suggesting neuronal damage through glutamate-mediated excitotoxicity as a potential mechanism that could contribute to increased lesion volume in SHRSP. Another proposed mechanism is reduced blood flow through collateral vessels linking the MCA with anterior and posterior cerebral arteries. Collateral blood vessels, investigated 1 month after MCAO in SHRSP and WKY, revealed that anastomoses arising from the MCA were significantly narrower and blood flow less than in WKY.20 A recent study demonstrated that the potent cerebral vasoconstrictor, 20-hydroxyeicosatetraenoic acid, is increased in the cerebral vasculature of SHRSP, and inhibition with HET-0016, administered before transient MCAO, resulted in a marked reduction in infarct volume, associated with reduced production of cerebrovascular reactive oxygen species and reduced endothelial dysfunction.21 In the present study, the perfusion deficit area increased during 6 hours in SHRSP but remained unchanged in WKY. This increase in size of the perfusion abnormality may be attributed to failure of collateral supply, possibly mediated via increased 20-hydroxyeicosatetraenoic acid, and demonstrates the potential for the perfusion deficit to gradually worsen over time. Additionally, this increase in perfusion deficit in SHRSP could further exacerbate ischemic damage, resulting in greater incorporation of penumbral tissue into the infarct core.

One limitation of the present study was the fact that the perfusion abnormality was assessed on a single coronal slice within the MCA territory, owing to technical limitations of the current protocol. It is possible that more anterior or posterior perfusion scans within the MCA territory would have shown a greater difference in perfusion deficit between strains.

The diffusion-perfusion mismatch method22 has been used to provide an index of the penumbra and therefore, the amount of potentially salvageable tissue remaining over time. The diffusion abnormality detects the development of cytotoxic edema due to ischemic damage,23 whereas the perfusion abnormality shows the region of reduced CBF. Because the DWI-PWI mismatch method is characterized as a region of tissue with a CBF abnormality but no evidence of cytotoxic edema, it is assumed to be potentially viable. In the present study, SHRSP had significantly less mismatch tissue available at 1 and 2 hours after stroke when compared with WKY. This was due to the fact that the diffusion abnormality on the same slice was significantly larger in the SHRSP from 1 hour after stroke and thereafter throughout the 6-hour time course. The mismatch area gradually became incorporated into the ADC.
lesion in both strains over time, indicating a reduction in the amount of potentially salvageable tissue remaining. The insignificant amount of mismatch tissue in SHRSPr from as early as 1 hour after stroke indicates that the ischemic injury occurs very rapidly in this strain and suggests that there is less potentially salvageable tissue available for acute stroke therapies. In fact, it has previously been shown that the DWI lesion is maximal at 1 hour after distal MCAO in the related spontaneously hypertensive rat and that this is well correlated with the final infarct at 24 hours.24,25 Legos and colleagues24 have also demonstrated that the spontaneously hypertensive rat has little mismatch tissue at 1 hour after distal MCAO, which has been attributed to poorer collateral flow in these animals.

The increase in perfusion deficit observed in SHRSPr with the resulting increase in ADC lesion resulted in little change in the already-small mismatch area over time. The expanding perfusion deficit would partly explain why the ADC lesion is larger in SHRSPr at later time points. However, at earlier time points, there was no significant difference in the perfusion deficit between strains.

In conclusion, the present study demonstrates that genetic hypertension can influence the amount and lifespan of penumbral tissue. SHRSPr had significantly more ischemic damage and less penumbral tissue than did WKY within 1 hour of stroke onset. In addition, the expanding perfusion deficit in SHRSPr could indicate that these rats have a greater volume of tissue at risk of infarction compared with WKY. These results could have important implications for the management of stroke patients with preexisting risk factors and suggest that ischemic damage could progress at a faster rate and over a longer time frame in the presence of hypertension.

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Disclosures
None.

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