Susceptibility to Cerebral Infarction in the Stroke-Prone Spontaneously Hypertensive Rat Is Inherited as a Dominant Trait

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Background and Purpose—Susceptibility to cerebral infarction was compared in stroke-prone spontaneously hypertensive (SHRSP), normotensive Wistar-Kyoto (WKY) rats, and F1 hybrids derived from a SHRSP/WKY cross.

Methods—The proximal left middle cerebral artery (MCA) was occluded under anesthesia and infarct volume assessed 24 hours later by magnetic resonance imaging and confirmed 5 days later by quantitative histopathology. Total hemispheric infarct volume was expressed as a percentage of the total brain volume.

Results—Infarct volumes measured by MRI in adult SHRSP (19.5 ± 2.0%) and F1 hybrid rats (19.4 ± 1.9%) were significantly greater than in WKY (11.1 ± 2.4; CI [6.07, 10.76]) and (5.93, 10.52), respectively, P < .001). Sensitivity to an ischemic insult was unrelated to blood pressure: although systolic blood pressures differed between young versus adult male SHRSP and between female versus male SHRSP and F1 hybrids, infarct volumes were equal. A close correlation was found between infarct volumes measured by MRI and histology (r = 0.92, P < .0001).

Conclusions—Outcome to MCA occlusion (MCAO) measured with MRI provides a reproducible and nonterminal quantitative phenotypic marker of stroke susceptibility in the SHRSP. This is the first study to employ MCAO with MRI to quantify stroke susceptibility in F1 hybrid rats and indicates a dominant mode of inheritance for this phenotype. (Stroke. 1998;29:690-694.)

Key Words: cerebral infarction • genetics • hypertension • magnetic resonance imaging

Permanent MCAO is the definitive model of focal cerebral ischemia. SHR and SHRSP have much larger and less variable infarcts after MCA occlusion than all other rat strains. Furthermore, this increased susceptibility to cerebral ischemia, which we believe is genetically determined, may be unrelated to hypertension because SHR and SHRSP suffer large infarcts at 5 weeks of age before hypertension and vascular hypertrophy are fully established. Studies by Coyle and coworkers suggested that in the SHRSP susceptibility to infarction was inherited as an autosomal recessive trait and that decreased luminal diameters in vascular anastomoses between the MCA and anterior cerebral artery were responsible for the pathophysiology. The importance of vascular anastomoses and genetic predisposition rather than blood pressure alone was stressed further by evidence that rats made hypertensive by deoxycorticosterone acetate and salt administration failed to develop large infarcts after MCA occlusion, whereas adult SHR, and SHRSP, in which hypertension had been treated early, still developed large infarcts.

The analysis of previous data therefore suggests that the large infarcts induced by MCAO were due to inadequate collateral blood flow and that this phenotype is genetically associated with but not directly linked to hypertension. This implies that a gene marker or markers for infarct susceptibility may exist both in animal models and perhaps also in humans. Rubattu and coworkers recently performed a genome-wide screening approach to an SHRSP/SHR cross using an alternative phenotype, latency to stroke after salt loading, as a marker of stroke proneness. They identified three major loci that contributed significantly to the variance of this stroke phenotype in F1 hybrids. Thus, in SHRSP, primary blood pressure-independent genetic factors may play a critical role in both stroke onset and increased susceptibility to infarction.

Previous studies in SHR and SHRSP used histological methods to assess infarct size. These methods are very time-consuming, difficult to perform in the large number of animals required for genetic cosegregation analysis, and involve a terminal end point. In the current study, MRI was used to measure infarct volume after MCAO both in adult (24-week-old) and young (9-week-old) SHRSP, in their normotensive reference strain—the WKY rat, and in adult F1 hybrids obtained by crossing SHRSP and WKY rats. Quantitative...
histology at 5 days postischemia was carried out to confirm the sensitivity and accuracy of the MRI measurements.

A preliminary report of these results has been published in abstract form.14

Materials and Methods

Experimental Animals
Inbred colonies of SHRSP and WKY rats have been maintained in the Department of Medicine and Therapeutics at the University of Michigan since 1991.15 The breeding animals were a gift from Dr D.F. Bolin at the University of Michigan where they have been maintained as inbred colonies for more than 15 years. F1 hybrids were produced by mating two SHRSP females with one WKY male. SHRSP, WKY, and F1 hybrids were weaned at 4 weeks, divided by sex, and maintained in family groups (3 to 4 per cage) in constant temperature at 21°C and 12-hour light/dark cycle (7 AM to 7 PM). SBP and heart rate were measured in all animals by plethysmography as previously described.16 To verify these physiological measurements, littermates of SHRSP, WKY, and F1 hybrids underwent direct blood pressure and heart rate recordings using a telemetry system.17 MCAO was performed on SHRSP and WKY at 9 weeks (SHRSP, n=15; WKY, n=10) and 24 weeks (SHRSP, n=10; WKY, n=9) of age; F1 hybrids underwent MCAO at 24 weeks of age (n=11). All experiments were carried out in accordance with institutional and Home Office guidelines.

Surgical Intervention to Produce MCA Occlusion

Rats were anesthetized with isoflurane (1% to 2%) in oxygen–nitrous oxide (1:2) via a face mask. The left MCA was permanently occluded by electrocoagulation using the technique of Tamura et al with minor modifications.17 Anesthesia was given for no longer than 15 minutes.

MRI

A Biospec 47/15 spectrometer (Bruker) with imaging software was used. The radiofrequency probe was a home-built Alderman-Grant type resonator18 with a 40-mm inner diameter and a length of 50 mm. Twelve coronal sections, 1 mm thick, that covered the whole forebrain were taken 24 hours after MCAO using a spin echo (SE) type resonator18 with a 40-mm inner diameter and a length of 50 mm. Further experimental details are described elsewhere.17,19 Infarct area in each section was determined using a semi-automated segmentation procedure based on intensity thresholding. Regional resolution into cortical and striatal infarction involved interactive drawing of a borderline involving blood pressure was statistically significant. Subsequent removal of these terms, beginning with the highest-order interaction or interactions, did not result in lower-order terms involving blood pressure becoming significant.

Consequently, a model was fitted that did not include the main effect of blood pressure, or any interaction involving blood pressure. This reduced model was tested within the full model previously fitted, and was not rejected (F=0.425, df=8, 28, P=.90). It was concluded that blood pressure did not influence infarct volume, on average, in any group of rats.

The reduced model was an ANOVA model in three explanatory variables, namely strain, age (adult or young), and sex. No individual term involving sex was statistically significant and, after checking intermediate models, all of these terms were removed. Again, this reduced model was tested within the previous model and was not rejected (F=0.627, df=4, 36, P=.63). It was concluded that there was no difference, on average, between infarct volumes for male and female rats in}

<table>
<thead>
<tr>
<th>Selected Abbreviations and Acronyms</th>
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<tr>
<td>MCA = middle cerebral artery</td>
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<td>MCAO = MCA occlusion</td>
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<td>SBP = systolic blood pressure</td>
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<tr>
<td>SHR = spontaneously hypertensive</td>
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<tr>
<td>SHRSP = stroke prone SHR substrain</td>
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<td>WKY = Wistar-Kyoto</td>
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Histology

Five days after MCAO, the rats were decapitated, and their brains removed and immediately frozen on a block of dry ice. Coronal cryostatic sections 20 µm thick were cut at 12 equidistant levels (1 mm apart, covering the entire forebrain), mounted on glass slides, and stained with cresyl violet. The area of infarct in each section was determined using a calibrated digitizing tablet from a video-image analyzer. The sum of the infarct areas in the twelve sections, multiplied by the slice thickness, was taken as total infarct volume. All infarct volumes have been expressed as a percentage of the total brain volume.

Statistical Analyses

The effects of systolic blood pressure, sex, strain, and age on infarct volume established by MRI were examined using ANOVA and ANCOVA. The Table displays the sample mean±SEM of SBP and infarct volume for all groups of rats. Since no data were available from young F1 rats, two main analyses were required. In the first, the (population) mean infarct volumes for adult and young SHRSP and WKY rats were compared. In the second, (population) mean infarct volumes of adult SHRSP, WKY, and F1 rats were compared.

Both sets of analyses began by fitting a model containing all main effects and interactions. Nonsignificant effects were then eliminated beginning with the highest-order interaction or interactions. The final model included all statistically significant interactions along with lower-order terms in the same variables. Multiple comparisons were investigated using Tukey’s method with an overall 95% confidence level.

A paired t test was used to compare infarct volumes obtained by MRI with infarct volumes obtained by quantitative histology.

Results

Lack of Influence of BP on Infarct Volume in Adult and Young Parental Strains

The relationship between infarct volume and blood pressure can be judged from the Table. This shows that infarct volumes are much smaller for the WKY rats (of both sexes and all ages) than for the SHRSP and F1 rats, which also generally have higher blood pressures. There is, however, no evidence of a correlation between infarct volume and blood pressure within any group of rats: (adult SHRSP r=−.360, P= .360, r=.526; young SHRSP r=.262, P=.346; young WKY r=−.039, P=.921; F1 hybrids r=−.275, P=.144).

When the full ANCOVA model was fitted to the data for young and adult SHRSP and WKY rats, no single term involving blood pressure was statistically significant. Subsequent removal of these terms, beginning with the highest-order interaction, did not result in lower-order terms involving blood pressure becoming significant.

Consequently, a model was fitted that did not include the main effect of blood pressure, or any interaction involving blood pressure. This reduced model was tested within the full model previously fitted, and was not rejected (F=0.425, df=8, 28, P=.90). It was concluded that blood pressure did not influence infarct volume, on average, in any group of rats.

The reduced model was an ANOVA model in three explanatory variables, namely strain, age (adult or young), and sex. No individual term involving sex was statistically significant and, after checking intermediate models, all of these terms were removed. Again, this reduced model was tested within the previous model and was not rejected (F=0.627, df=4, 36, P=.63). It was concluded that there was no difference, on average, between infarct volumes for male and female rats in
either age group of either strain. Consequently, data for both sexes were combined for further comparisons of age groups and strains.

**Sensitivity to Ischemic Insult in Adult and Young Parental Strains**

In the reduced ANOVA model, which included only strain and age group, the interaction term was not statistically significant ($P = .214$), but the main effects of both strain and age were statistically significant. Mean infarct volumes were significantly greater in SHRSP than in WKY rats in the same age group (95% confidence interval [7.96 to 10.96], $P < .001$). Also, overall mean infarct volumes were significantly greater in adult rats than in young rats (95% confidence interval [0.25 to 3.25], $P = .023$).

**Lack of Influence of Blood Pressure on Infarct Volume in Adult Parental and F$_1$ Hybrids**

Again, when a full ANCOVA model was fitted to the data from adult rats of all three strains, no term involving blood pressure was statistically significant. Proceeding as before, a reduced model that omitted all the terms involving blood pressure was tested within the full model, and was not rejected ($F = 0.811$, $df = 6$, 18, $P = .58$). We concluded that blood pressure did not influence infarct volume, on average, in any group of adult rats.

In the reduced ANOVA model, which included sex and strain only, neither the interaction term nor the main effect of gender was statistically significant. A reduced (one-way) ANOVA model in strain alone was tested within the previous model and was not rejected ($F = 0.441$, $df = 5$, 24, $P = .82$). We concluded that there was no difference, on average, between infarct volumes for male and female rats in any strain of adult rat. Consequently, data for both sexes were combined for a further analysis of the three strains.

**Sensitivity to Ischemic Insult in Adult Parental and F$_1$ Hybrids**

There was a highly significant effect of strain on the average infarct volume ($P < .001$). Simultaneous confidence intervals for all pairwise comparisons showed that adult SHRSP had a significantly greater mean infarct volume than adult WKY rats (confidence interval 6.07 to 10.76). Adult F$_1$ rats, too, had a significantly higher mean infarct volume than adult WKY rats (confidence interval 5.93 to 10.52). There was no significant difference between the mean infarct volumes of adult SHRSP and F$_1$ rats (confidence interval 2.03 to 2.42); indeed the sample mean infarct volumes of these two groups were virtually identical (see Table).

**MRI Correlated With Histology**

The use of MRI for mapping infarct volume was validated by comparison with measurements made by quantitative histology 4 days after the animals were initially imaged by MRI. Fig 1 illustrates the strong association between the two measurements in adult and young SHRSP and WKY rats, which generated a sample correlation coefficient of $r = .92$ ($P < .0001$).

Although this indicates that the two measures were strongly related, it should be noted that the MRI measurement was greater than the histological measurement in almost every rat because of acute edema of the infarcted area at 24 hours, which would have resolved to a certain extent by 5 days posts ischemia. A 95% confidence interval for the mean paired difference between the MRI and histological measurements on the same rat is 4.35 to 5.72, $P < .0001$.

**Discussion**

Infarct volume after MCA occlusion is a highly relevant phenotype for demonstrating sensitivity to an ischemic insult. MRI is a precise, nonterminal method for quantitation of infarct volume. The current study is the first to demonstrate that combination of this phenotype and technique provides a very powerful means of investigating the genetics of stroke.

The present results confirm the bimodal distribution of the MCAO phenotype in parental SHRSP and WKY strains (Fig 2), which has been reported previously. Two further important findings are presented. First, a number of results in this study support the hypothesis that sensitivity to an ischemic insult in the SHRSP is independent of blood pressure: (1) SBP...
is significantly lower in young versus adult male SHRSP while there is no difference in infarct volume; (2) in young animals, before hypertension is established, there is no significant difference in SBP in male SHRSP and WKY but infarct volume is significantly greater in the SHRSP; and (3) in adult SHRSP and F1 hybrids, SBP is significantly higher in males than females but there is no sex-related difference in infarct volume.

The second and most interesting finding is that the distribution of infarct volumes in F1 rats was virtually identical to the distribution in the SHRSP, strongly suggesting a dominant mode of inheritance for this phenotype. The significance of this finding has prompted a genome-wide screen in F2 hybrids (F1 × F1 cross) to investigate genetic markers for stroke severity. Previous studies that examined the genetics of stroke in the SHRSP include an earlier cosegregation study by Coyle et al in which focal ischemia was used to characterize the stroke-prone phenotype and two studies in which an alternative phenotype was used, latency to stroke on a high salt diet. Coyle's studies suggested a single recessive gene was responsible for the pathogenesis of stroke in the SHRSP. Possible reasons for the different conclusion from the current study may include their use of outbred normal Wistar rats instead of inbred WKY rats and the possibility that there was a less severe, more distal occlusion in much younger animals (F1 hybrids 8 to 12 weeks old). When they used latency to stroke, Nagaoka et al reported this phenotype to be characterized by a polygenic inheritance and more recently, Rubattu et al performing a genome-wide screen on an F2 cross (SHRSP × SHR) identified three major quantitative trait loci that together accounted for 28% of the overall phenotypic variance. However, it should be stated that the genes responsible for the latency to stroke with a high salt diet may be quite independent of those that determine the size of infarct after cerebral vessel occlusion (ischemic sensitivity genes).

A number of hypotheses have been put forward to explain the increased ischemic sensitivity in the SHRSP. The most commonly cited hypotheses propose that the SHRSP exhibit arterial hypertrophy in cerebral arteries, resulting in decreased functional compliance with limited dilatation to ischemia. This may be particularly important in collateral vessels where a reduction in anastomotic diameter would result in collateral flow impairment during ischemia. In support of this, MCAO in normotensive animals is associated with a marked increase in nitric oxide release in the ischemic region. Since central nervous system nitric oxide synthase activity is reduced in SHRSP compared with WKY, defective nitric oxide release may be a contributory factor in the impaired collateral perfusion of the ischemic area in SHRSP. In addition, SHRSP may also demonstrate an intensified inflammatory response to the ischemic insult that could further compromise flow. Indirect evidence for an impaired collateral supply comes from neuroprotection studies in hypertensive strains in which drugs with flow-enhancing properties (eg, L-type calcium channel blockers) are found to be more effective than NMDA glutamate antagonists with proven efficacy in normotensive strains.

The present findings are consistent with a dominant mode of inheritance for ischemic sensitivity in the SHRSP. Recent studies from our laboratory and from Rubattu and cowork-
en13 provide strong evidence for the existence of primary blood pressure independent genetic factors which influence both latency to stroke13 and sensitivity to an ischemic insult20 in the SHRSP. Whether outcome to stroke has a genetic basis in man needs to be examined further but such research should be strongly encouraged in view of these results.

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References

The most widely used experimental model of cerebral infarction results from MCA occlusion in the rat. The severity of this infarct is quantified by its volume, and in the current decade MRI has been established as a precise tool for monitoring this volume. Using these techniques, the Glasgow investigators observed much larger infarcts in the hypertensive SHRSP rats than in the normotensive WKY, yet they firmly established that infarct size was independent of blood pressure. They do consider alternative hypotheses that could explain a decrease in collateral blood flow and thereby be responsible for the large infarcts in SHRSP. Most attractive among these hypotheses are arterial hypertrophy and a deficit in nitric oxide release.1 Following cross-breeding between SHRSP and WKY rats, they found that the infarct size in the F1 generation rat was as large as that in the SHRSP parental strain. This clear evidence for dominance of the genetic trait of the large infarct size adds to the spectacular identification by these investigators of the quantitative trait locus on chromosome 5 as responsible for stroke size in SHRSP.2 It is of special interest to see Glasgow University regaining its leadership role in hypertension-related research.

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References
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