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
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 Glasgow since 1991.13 The breeding animals were a gift from Dr D.F. Bohr 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 and humidity. The radiofrequency probe was a home-built Alderman–Grant type resonator16 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) sequence with an echo delay of 60 ms and a repetition delay of 2000 ms (SE 2000/60). The spatial resolution in the imaging plane (pixel dimension) was 0.16 × 0.16 mm² (field of view = 40 mm). Further experimental details are described elsewhere.17–19 Infarct area in each section was determined using a semiautomated segmentation procedure based on intensity thresholding. Regional resolution into cortical and striatal infarction involved interactive drawing of a borderline between the respective structures prior to intensity thresholding. The infract size determined either by thresholding alone or by adding up cortical and striatal values yielded identical numbers within error limits. The total infract volume was calculated by summation of the number of pixels in each slice and multiplication by the pixel size and slice thickness. Infract volumes generated by MRI and histology were expressed as a percentage of total brain volume to account for brain swelling and differences in brain size between sexes and strains. Image analysis was carried out by a person unaware of strain, age, or sex of the animal.

MCA = middle cerebral artery
MCAO = MCA occlusion
SBP = systolic blood pressure
SHR = spontaneously hypertensive rat
SHRSP = stroke prone SHR strain
WKY = Wistar-Kyoto

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 infract 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 infract volume established by MRI were examined using ANOVA and ANCOVA. The Table displays the sample mean ± SEM of SBP and infract 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 infract volumes for adult and young SHRSP and WKY rats were compared. In the second, (population) mean infract 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 infract volumes obtained by MRI with infract volumes obtained by quantitative histology.

Results

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

The relationship between infract volume and blood pressure can be judged from the Table. This shows that infract 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 infract volume and blood pressure within any group of rats: (adult SHRSP r = .360, P = .307; adult WKY r = −.245, P = .526; young SHRSP r = .262, P = .346; young WKY r = .039, P = .921; F1 hybrids r = −.275, P = .14). 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 infract 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 infract volumes for male and female rats in

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Selected Abbreviations and Acronyms

MCA = middle cerebral artery
MCAO = MCA occlusion
SBP = systolic blood pressure
SHR = spontaneously hypertensive rat
SHRSP = stroke prone SHR strain
WKY = Wistar-Kyoto
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 F1 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 F1 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 F1 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 F1 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.5,6 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 coworkers...
provide strong evidence for the existence of primary blood pressure independent genetic factors which influence both latency to stroke and sensitivity to an ischemic insult 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 discuss 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. 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. 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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