Cerebral Infarction After Middle Cerebral Artery Occlusion in Progenies of Spontaneously Stroke-Prone and Normal Rats

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SUMMARY A differential outcome results from rapid middle cerebral artery (MCA) occlusion in young normotensive Wistar (NW) rats as compared to the spontaneously hypertensive stroke-prone (SHRSP) rat. The SHRSP invariably infarcts; the NW usually does not. To determine if segregation at a single autosomal locus explains the difference between strains, a NW male was crossed with several SHRSP females to produce F1 rats. The segregation of the strain difference was studied in the F2 and backcrosses to the NW and SHRSP parental strains. The relative frequency of infarcting and noninfarcting animals in the segregating progenies supported a single locus recessive model of inheritance for susceptibility to infarction after sudden occlusion of the MCA. Mean infarct size was largest for SHRSP and proportional to the SHRSP gene dosage in the segregating progenies. Variation in the size of the infarct within segregating classes may be attributable to the segregation of polygenes and/or environmental influences during the initial formation of the cerebral anastomoses.

Methods

Breeding Scheme and Environment

Our original source of SHRSP was The National Institutes of Health. Sibmated rats yielding generations/f43–f47 were verified for the infarction trait by the MCA occlusion test prior to this experiment. Four young SHRSP female rats were randomly selected from different litters. To produce the first filial (F1) generation, all females were bred against one NW male rat that was randomly selected from a litter of outbred normal Wistar rats. The Wistar rats were descendants of animals obtained from Albino Farms. One male NW was chosen to minimize the role of background genetic variation that may exist from rat to rat in this heterozygous outbred population. F1 animals were sibmated to generate the second filial (F2) rats. F1 females were backcrossed to the NW male and F1 males were backcrossed to the original SHRSP females to produce progenies denoted BCN and BCS, respectively. Offspring-parental matings were done to exclude the possibility of introducing further genetic variation from the parental strains.

Animals were housed in metal cages in laboratory quarters with a 12 hour light-dark cycle and controlled temperature. Standard tap water was available for drinking ad lib and food was Teklad rat chow. Weaning was at 28 days of age and thereafter no more than 6 rats were housed per cage.

MCA Exposure and Occlusion

Rats were anesthetized with ketamine hydrochloride (132 mg/kg wt, i.m.). Details of the surgery to expose the right MCA for its sudden occlusion are given elsewhere. A 2 mm diameter craniectomy was drilled with a #6 dental burr about 1 mm rostral and 2 mm dorsal to the rostralmost fusion point of the zygoma to the squamosal bone. To prevent the drill from going through the dura mater, the burr hole was not drilled completely through the skull. Bone remaining at the depth of the hole was removed with forceps. Dura mater was carefully pierced with a #11 scalpel blade taking care to avoid branches of the middle meningeal artery. A probe made from an Anchor Brand taper point 1833-2 needle was used to extend the dural opening in order to bluntly dissect the MCA from its surrounding pia-arachnoid.

Monofilament nylon thread, about 35 μm in diameter, was obtained from 3/4 inch nylon rope (K Mart Stores), made visible with a black magic marker, and stiffened at one end by fingernail polish applied the previous day. A square knot with dimensions less than the diameter of the vessel secured occlusion of the MCA about 1700 μm dorsal to the rhinal fissure and 300–500 μm ventral to MCA bifurcations distributing to frontal, parietal and occipital cortical regions. Following cranial wound closure with #4 silk suture, a branch to the right femoral vein was dissected and 2%
Evans blue in physiologic saline was injected at 0.007 ml/gram rat. The vessel was then double ligated and the skin wound sutured.

**Postmortem Determinations**

On the third postoperative day, rats were anesthetized with ether anesthesia. Tissue fixation was initiated by injection of 50 ml 10% neutral buffered formalin into the ascending aorta after excision of the right atrium. Brains were removed from the skull and stored in the fixative until placed in 25% sucrose-formalin several days before sectioning with a microtome. Frozen sections of the forebrain were cut at 25–50 μm in thickness and stored in fixative until mounted on glass slides and stained with hematoxylin and eosin or basic fuchsin.

**Photography and Computer Assisted Lesion Measurements**

Dorsal and lateral views of brains were photographed with fine grain release blue sensitive film at 1.5 x magnification. Negative images of the lesions were projected onto paper with a Leitz Prado projector. Lesion boundaries were traced, then digitized with a Summagraphics Digitizer interfaced to a Commodore Microcomputer. The loci intersecting the lesion in lateral and dorsal projections were determined by line projections. Surface area of the lesion fraction obtained from the lateral projection was computed without correction for curvature. For the dorsal projection, correction for curvature in the lesion surface was obtained by an algorithm designed specifically for rat, based upon curvatures measured from a brain atlas, and run on the microcomputer.

**Statistical Analysis**

The Fl, F2 and backcross progenies were compared for the relative frequency of infarction and the difference in average infarct size among animals with infarcts. The analysis of infarction frequency was based on classifying rats with lesions less than 13 mm² as noninfarcting animals whereas those rats with lesions greater than 13 mm² in size were classified as rats with infarcts. The rationale justifying two lesion groups is given in the discussion section. A Chi-square goodness of fit analysis was used to compare the observed frequencies of infarcts with those expected assuming that the difference in infarction frequency between the parental strains was due to allelic differences at a single gene locus.

Lesion size for those rats with infarcts was analyzed as a continuous variable to determine whether the average infarct size varied significantly among the various classes of rats. To adjust for differences in lesion size among progenies that were attributable to factors other than the MCA occlusion that induced infarction (e.g. surgical trauma), infarct size was evaluated as the lesion size minus the within class mean noninfarct lesion size. For the analyses that assume a normal distribution, a fourth root transformation was found to minimize the skewness and kurtosis of the distribution of lesion size within the noninfarction and infarction classes. Histograms of the untransformed (fig. 1A) and transformed (fig. 1B) data are presented in figure 1. Prior to transformation the skewness and kurtosis were 1.503 and 1.051, respectively, in noninfarcting rats and 0.266 and 0.084 in rats with infarcts. Following transformation, the skewness and kurtosis were reduced to 0.429 and -0.734, respectively, in noninfarcting animals and 0.167 and -0.733 in rats with infarcts.

An analysis of variance was used to evaluate the distribution of infarct sizes among the NW, SHRSP, F1, F2, BCN, and BCS classes of rats. Two-way analyses of variance of infarct size and weight among the sex by rat class strata and weight among infarcting/noninfarcting rats by the rat class strata were performed. The analysis of covariance was used to evaluate the homogeneity of the relationship between infarct size and weight in the different classes of rats. Since the linear relationship was not homogeneous among
different classes, a separate regression was used for each class to adjust infarct sizes for differences in weights among rats.

Results

Tissue Histology

Infarcting Rats

Evans blue grossly marked lesioned cortical tissue. In histological sections, the lesion was less intensely stained with hematoxylin and eosin than normal tissue. The lesion was characterized by the following features. Fragmented and pyknotic nuclei of cells were evident. Neuronal cell somas were not clearly outlined. Neuronal cells were present as were mitotic figures. Polymorphonuclear leucocytes and phagocytes were common. As observed in coronal sections this irreversible lesion included all layers of the cortex and was large. The lesion extended from the occlusion site to far beyond the territory exposed by the burr hole. However, the infarct did not cross under anastomoses of the MCA with the anterior and posterior cerebral arteries to enter cortical fields normally supplied by those vessels.

Noninfarcting and Sham Operated Rats

Noninfarcting and sham operated rats had small (table 1) lesions in the cortex next to the 2 mm diameter (area = 6.28 mm²) burr hole required for dissection and ligation of the MCA. The lesion was often, although not invariably more intensely marked with Evans blue than the large infarct resulting from MCA occlusion. A small amount of hemorrhage due to dissection of the MCA and passage of the ligature deep to the vessel was usually present. Phagocytes containing vacuoles (possibly mineral oil or bone wax) were seen near the cortical surface. Less intense tissue staining, pyknotic cells, and mitotic figures were present. Often this lesion did not extend through all layers of the cortex but only involved the superficial layers.

Characteristics of Experimental Animals

Table 1 is a list of the animal types, number of rats tested by sex, mean ages and weights on operation day, and mean infarct size. One hundred thirteen rats (55 males, 58 females) representing parental and progeny classes were sampled. The right MCA was rapidly occluded in 104 rats. In addition 9 rats were sham operated such that the MCA was exposed and dissected through surgery but the vessel was not occluded. This was done to obtain a measure of lesion size due to the surgery alone. No sham operated rat had an infarct; no lesion was greater than 9 mm². Mean lesion size for shams was not significantly different from the noninfarcting group mean for rats (p > 0.71) with MCA occlusion.

For parental types, 16 SHRSP and 6 NW rats of 5–6 weeks age and 5 older (>14 weeks) NW animals were tested. All MCA occluded SHRSP had infarcts. Only 1 of the 11 MCA occluded NW rats had an infarct.

All seventy-seven progeny rats underwent the MCA occlusion as young rats (37.0 ±1.2 SD days old). Thirty-three animals had infarcts; the remaining 44 rats did not have infarcts. Infarction frequency among the progeny was not significantly different in males as compared to females (x² = 2.47, p > 0.10). No progeny rat died as a result of the MCA occlusion.

Mean body weights from lowest to highest were SHRSP<BCS<BCN<F1<F2<NW. Mean weights of the SHRSP and BCS were significantly lower than those of the other classes of rats (p < 0.0001) whereas the mean weights of young BCN, F1, F2 and NW animals were not significantly different from one another. In each class of rats, females weighed less than

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Summary of Experimental Results</th>
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<tr>
<td>Rats</td>
<td>N</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
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<tr>
<td><strong>Parental classes</strong></td>
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</tr>
<tr>
<td>SHRSP</td>
<td>16</td>
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<tr>
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</tr>
<tr>
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<td>5</td>
</tr>
<tr>
<td><strong>Progeny classes</strong></td>
<td></td>
</tr>
<tr>
<td>F1</td>
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</tr>
<tr>
<td>F2</td>
<td>10</td>
</tr>
<tr>
<td>BCS</td>
<td>29</td>
</tr>
<tr>
<td>BCS</td>
<td>23</td>
</tr>
<tr>
<td><strong>Shams</strong></td>
<td></td>
</tr>
<tr>
<td>SHRSP</td>
<td>4</td>
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<tr>
<td>NW</td>
<td>2</td>
</tr>
<tr>
<td>BCN</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>113</td>
</tr>
</tbody>
</table>

Numbers in parentheses (male,female).
males. There was evidence for a significant \( p < 0.05 \) class of rat by sex interaction effect on weight. The difference in weight between males and females was greater in NW, BCN and BCS than for the other classes of rats.

The mean weights of rats with infarcts were not significantly different from the mean weights of rats without infarcts. Among rats with infarcts, however, there was a significant linear relationship between size of the lesion and weight. This relationship was not consistent among different classes of rats \( p < 0.05 \). Within the SHRSP class, there was a statistically significant negative relationship between infarct size and weight, but within the BCN class there was a significant positive relationship. For the other progenies; BCS (negative), F2 (positive), and F1 (negative), the regressions were not significantly different from zero. The infarct size variable used in the following analyses was adjusted for weight separately in each class of rats.

Lesion Frequency

Figure 2A is a composite frequency histogram of lesion sizes for the 104 MCA occluded rats. Rats with infarcts comprise one distribution. The other distribution is of noninfarcting rats. Mean lesion size was 3.1 ± 3.6 SD mm\(^2\) for noninfarcting rats and 50.1 ± 20.4 SD mm\(^2\) for the infarct group. An 8.9 mm\(^2\) gap in continuity exists between the two groups (fig. 2A). All SHRSP had infarcts (fig. 2B); the mean lesion size was 61.6 ± 11.2 SD mm\(^2\). Except for one rat (fig. 2C), all NW were noninfarcting animals. Figures 2D-G display the lesion size of each rat for F1, BCS, BCN, and F2 classes of rats. Some, but not all rats of each class had infarcts.

To determine if the difference in the relative frequency of infarction between the parental types was due to the segregation of a single gene, observed infarction frequencies were compared to those predicted by a single dominant gene model and a single recessive gene model. The inbred SHRSP were assumed to be homozygous; the single outbred NW was first evaluated as a homozygote and then as a heterozygote for the hypothesized single gene models.

The results of the analyses of genetic models are presented in table 2. A single locus model of inheritance, where the susceptibility to infarction is dominantly inherited was strongly rejected. If the difference in infarction frequencies between the NW and SHRSP was attributed to a single dominant gene, all F1 and all BCS animals would be expected to have infarcts. This clearly was not the case.

If the NW male was assumed to be homozygous AA, a single locus recessive model was also rejected. Under this assumption, one would expect none of the F1 and none of the BCN animals to infarct. In actual-
were in close agreement with those expected if a single gene recessive model accounted for the difference in infarction frequency between the NW and SHRSP (table 2).

Lesion Size

The mean infarct size in SHRSP was significantly larger than the lesion size estimated from the aggregate of all progeny types (p < 0.001). Infarct size was not (p > 0.05) significantly different among the progeny types; nor did the variance of infarct size among animals with an infarct differ significantly among these groups. One F1 and 4 BCS rats had markedly larger infarcts than those of other progeny type animals (fig. 2). Neither the mean nor the variance of infarct size was significantly different in male progeny as compared to female progeny.

Discussion

Two Lesion Groups

The rationale for grouping rats into two lesion groups follows. First, mere exposure and dissection of the MCA by surgery causes tissue trauma that results in Evans blue marks indicating injury to the Evans blue-albumin blood-brain barrier. Our data confirm the earlier reports and histologic lesions occurred in sham operated rats without MCA occlusion. Lesions that were <13 mm² in rats with MCA occlusion had histologic features similar to sham operated animals. There was neither a mean size nor histologic difference separating these MCA occluded rats from sham operated ones undergoing the same surgical procedure including passage of the ligature deep to the MCA but without occlusion. Second, in rats with lesions greater than 13 mm² in size, the histologic findings characterized an infarct. An 8.9 mm² gap in lesion size separated the infarcting group from the group with lesions due to the surgery alone. Thus, there was a clearly defined bimodality that differentiated the animals into two groups; rats with small lesions due to the trauma of surgery and rats with large infarcts resulting from MCA occlusion.

Pathophysiologic Basis for the Infarct

Earlier studies noted large infarcts in young SHRSP after sudden occlusion of the middle cerebral artery. Since infarcts in SHRSP did not cross under anastomoses into cortical fields supplied by the posterior or anterior cerebral artery, rats with infarcts did not receive adequate collateral blood supply. This evidence suggests the problem is related to the dorsal arterial anastomoses linking branches of the anterior and posterior cerebral arteries to rami of the MCA. Additional studies are needed to determine if infarcts occur in SHRSP due to fewer anastomotic junctions, structurally altered ones or inappropriate regulation of existing anastomoses that may lack nerves and sphincters, or because of increased metabolic demands in SHRSP requiring greater blood supply than for normotensive rats or for some other single or multifactorial reason.

Comparisons to Other Studies

Lesion size data for both young and adult NW was like that obtained by Robinson et al. for adult Sprague-Dawley rats 5, 20, or 40 days after occlusion of the MCA. Cortical lesion sizes in their study varied from 0.8 to 19.6 mm². In ours the range was 1.5—7.2 mm² except one young NW had a lesion of 46 mm². Adult NW had small lesions providing evidence age is not a major discriminating factor in NW. Neither mean lesion size nor individual lesion size values was reported in their investigations making comparisons to this study impossible. Tamura and Coworker investigated the adult Sprague-Dawley rat but occluded the MCA proximal to branches supplying the caudate nucleus. Whereas blood flow to cortex was reduced to 13 percent of control levels, the tissue field requiring collateral supply was much larger than for this study that excluded the caudate nucleus from the collateral field. The middle cerebral artery was occluded 30 minutes prior to measurement of cerebral blood flow and this precluded a comparison with our third postocclusion day data characterizing irreversible structural change.

Genetic Models

Our findings were consistent with a single recessive gene accounting for the difference in infarction frequency between the NW and SHRSP when the NW was assumed to be heterozygous, but not when the NW was assumed to be homozygous normal. We estimate that approximately 9% (1/11) of randomly selected NW rats infarct in the occlusion test. If the assumptions are made that 1) all rats with infarcts were aa, 2) the NW strain was in Hardy-Weinberg equilibrium, and 3) that the increased susceptibility to infarction was inherited as a single gene recessive trait, the fre-

<table>
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<th>Genotype</th>
<th>Model</th>
<th>F1</th>
<th>F2</th>
<th>BCS</th>
<th>BCN</th>
<th>Goodness of Fit</th>
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<tbody>
<tr>
<td></td>
<td>total sample</td>
<td>n = 15</td>
<td>n = 10</td>
<td>n = 23</td>
<td>n = 29</td>
<td>Chi-sq 5% CV</td>
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<tr>
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<td>SHRSP</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>aa</td>
<td>AA</td>
<td>Dominant</td>
<td>4 15</td>
<td>4 7.5</td>
<td>14 23</td>
<td>11 14.5</td>
</tr>
<tr>
<td></td>
<td>aa</td>
<td>Recessive</td>
<td>4 0</td>
<td>4 2.5</td>
<td>14 11.5</td>
<td>11 0</td>
</tr>
<tr>
<td>Aa</td>
<td>aa</td>
<td>Recessive</td>
<td>4 7.5</td>
<td>4 5.63</td>
<td>14 17.25</td>
<td>11 10.88</td>
</tr>
</tbody>
</table>
Frequency of the a allele in the NW strain is estimated as the square root of 0.09 = 0.30 and the frequency of heterozygotes is estimated as 2(1 – 0.30) (0.30) = 0.42. Thus, the probability of randomly selecting a heterozygous NW from the population of NW rats was approximately 42%.

Material Effect

Since the BCS and F1 were born and reared from the same SHRSP dams, but had significantly different infarction frequencies as compared to the SHRSP, the maternal environment would not appear to be a major determinant of the infarction frequency. More likely, susceptibility to infarction in the progeny is strongly influenced by inherited autosomal factors.

Infarct Size

In the progeny lines, the BCS, the F1 and F2 combined, and the BCN inherited ⅓, ⅔ and ⅔ of their genes respectively, from the SHRSP dams. The mean infarct sizes in mm² for these groups were 40.6 in the BCN, 41.6 in the F1 and F2 combined, 49.8 in the BCS, and 61.6 in the SHRSP. Thus, although infarct size was not significantly different among progeny types, there was a trend of increasing infarct size as the SHRSP gene dosage increased. These data are consistent with background polygenes contributing to the lesion size differences among the progeny types. In addition there is some evidence of bimodality of infarction size in the progeny types; especially in the BCS (fig. 2). This finding suggests that there may be other segregating genes that influence the size of infarcts in progeny issued from the matings.

Closing Comments

Rapid occlusion of the middle cerebral artery in young SHRSP invariably results in a large infarct. In contrast young and adult normal Wistar (NW) rats are protected from this lesion by adequate collateral circulation. Outcome of the MCA occlusion test is different in progeny of one NW male mated to SHRSP females or their F1 offspring; some progeny rats infarct, others do not. The maternal environment was probably not a major determinant of infarction frequency. Genetic factors were implicated. A dominant model for inheritance of the infarction trait was rejected. The data support a concept that the trait of susceptibility of infarction after sudden MCA occlusion is consistent with that expected from a single locus recessive model of inheritance and that polygenes contribute to infarct size differences among the progeny.

Acknowledgments

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Cerebral infarction after middle cerebral artery occlusion in progenies of spontaneously stroke-prone and normal rats.
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