Spatial Relations of Dorsal Anastomoses and Lesion Border After Middle Cerebral Artery Occlusion

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Occlusion of the middle cerebral artery invariably results in infarction of tissue in stroke-prone spontaneously hypertensive rats (SHRSP). To determine if the lesion border extends beyond the territory of the occluded middle cerebral artery or if the lesion enlarges with time after the occlusion, spatial relations of the lesion and the primary anastomosing collateral branches were investigated. Measurements were made 1 day (n = 8) or 21 days (n = 8) after occlusion in 5–8-week-old SHRSP brains marked by triphenyltetrazolium chloride (TTC) or tissue atrophy. After 1 day of occlusion, the border between TTC-marked and unmarked tissue was parallel to, and without spatial displacement from, the medial border of infarcted tissue measured 21 days after the occlusion. Thus, the TTC border accurately localizes the medial border of ischemic tissue that progresses to atrophy. The lesion border was 1.16 ± 0.04 mm downstream from the anastomoses, and the mean distance was not significantly different in frontal, parietal, or rostral occipital regions or between the 2 groups of rats. Without displacement from the anastomoses in 1-Day rats, Large-diameter anastomoses were further from the lesion than small-diameter anastomoses in both groups of rats, thus indicating that protection is greater near large anastomoses than near small ones. After middle cerebral artery occlusion in SHRSP, the limited protection of tissue in the territory of the occluded artery appears to depend on blood supply from pial surface anastomoses. (Stroke 1987;18:1133–1140)

Sudden occlusion of the middle cerebral artery (MCA) just above the rhinal fissure reduces blood flow to the territory of the occluded MCA and invariably results in infarction in stroke-prone spontaneously hypertensive rats (SHRSP). Normotensive rats usually do not have infarction after this occlusion. Following MCA occlusion in normotensive rats, the anastomoses that provide protective collateral supply from the anterior cerebral artery (ACA) enlarge within a month and blood flow to the territory of the occluded MCA returns to virtually normal levels. The mean luminal diameter of the anastomosing ACA-MCA collaterals is appreciably smaller in SHRSP than in normotensive Wistar rats, and after MCA occlusion blood flow to the territory of the occluded vessel is less in SHRSP than in normotensive rats. In SHRSP, tissue infarction and atrophy follow occlusion of the MCA.

One objective was to determine whether after MCA occlusion the lesion border is located within the territory of the occluded MCA or beyond it. If the lesion extends beyond the territory of the occluded MCA, then protective mechanisms other than the collateral circulation may be compromised in SHRSP. Additional goals were to determine whether protection from the lesion is homogeneous or heterogeneous among cortical regions or if the lesion border is located farther from large-diameter anastomoses than small-diameter ones.

Materials and Methods

Animal Preparations

Seven male and 9 female SHRSP were anesthetized with 130 mg/kg i.m. ketamine after tail systolic blood pressure was measured as described earlier. Details of the surgical procedures have been reported previously. In brief, the left MCA was exposed by a transtemporal approach and ligated just above the rhinal fissure with a nylon ligature about 35 μm in diameter. Complete occlusion of the MCA was verified.

Three male and 5 female SHRSP ranging in age from 58 to 60 days were killed 17 ± 4 hours after MCA occlusion and prepared for measurements as described below. This group was designated 1-Day rats. Four male and 4 female SHRSP underwent MCA occlusion at 37–38 days of age and were killed 21–22 days later. This group was designated 21-Day rats. At sacrifice all rats were of similar (59 ± 1 days) age. Blood pressure at 58 ± 1 days was significantly (p<0.05) higher in males (174 ± 4 mm Hg) than in females (156 ± 6 mm Hg). There was no significant difference between blood pressure of the 1-Day and 21-Day groups (p>0.20).
Latex Injection into Vessels

Papaverine hydrochloride (40–50 mg/kg body wt) was injected intravenously into ketamine-anesthetized SHRSP to produce maximal vasodilatation and to kill the rats. Warm Vultex, a white latex-based compound (No. 563, Chicago Latex Products, Schaumburg, Ill.) was injected into the ascending aorta to visualize the lumen size and course of cerebral arterioles. 10 Latex injection pressure at the end of the cannula was 50–100 mm Hg greater than the blood pressure measured within 24 hours of experiment termination. Vessel filling was observed through a closed cranial window to confirm flow of latex through the dorsal anastomoses, to facilitate control of vessel filling, and to reduce the likelihood of vessel bursting. 10

Visualization of the Lesion

Within 5–10 minutes after latex injection, brains of 1-Day rats were placed in 2,3,5-triphenyltetrazolium chloride (TTC) (1% in physiological saline), incubated for 3 hours at room temperature, photographed, and fixed in 10% neutral buffered formalin. TTC reacts with dehydrogenases, accepts electrons, and is reduced to red formazan 11 in normal brain tissue. Tissue lacking grossly observable color was present in the territory of the MCA (Figure 1A).

Brains of 21-Day rats were removed from the skull 30–45 minutes after latex injection and placed in the fixative (n = 5) or TTC (n = 3). All 21-Day rat hemispheres had gross cortical atrophy (Figure 1B).

Hemispheres were photographed in the standard dorsal orientation, and prints were made at × 15–70 for measurements.

Measurements and Computations

Anastomoses join distal branches of the 3 major cerebral arteries in rats. 10 An ACA–MCA anastomosis is located at the site of the smallest internal diameter of a collateral or one-half the distance between opposing branch angles (Figure 2B). On the photographs, adjacent anastomoses were connected with a line, and the sum formed a “line of anastomoses” positioned somewhat parasagittal to the midline. At 0.57-mm increments from rostral to caudal, I measured the distance from the midline to the line of anastomoses and to the border between TTC-marked and -unmarked tissue (TTC border) in 1-Day rats or the medial border of the atrophied infarct (atrophy border) in 21-Day rats.

From points spaced at 0.57-mm increments on the line of anastomoses, I measured the shortest distance to the TTC or atrophy border. On coronal tissue sections cut through the septum (Figure 1), I measured the distance from the midline to the TTC border or to the atrophy border.

Statistical Procedures

Profile analysis 12 for independent groups was used to compare points on the TTC border in 1-Day rats with corresponding points on the atrophy border in 21-Day rats. For the 2 groups, the following numbered test points were compared: 3 (frontal), 7 (frontal), 11 (parietal), and 15 (occipital). The objective was to determine whether the TTC and atrophy borders were parallel, and if so, whether displacement from the midline differed for the TTC and atrophy borders. Thus, I determined whether the borders changed form or location between 1 and 21 days of MCA occlusion.

Lines of anastomoses in the 2 groups were also compared by profile analysis to determine if displacement of the anastomoses occurred between 1 and 21 days of MCA occlusion. Student’s t tests with Bonferroni correction for multiple comparisons were computed to evaluate differences between individual points. Paired t tests with Bonferroni correction and analysis of variance for repeated measurements were used to compare the distance between the anastomoses and the lesion (TTC or atrophy) border.

Because resistance is less and flow is greater in large-diameter anastomoses, if large-diameter anastomoses predominate in 1 test region and small-diameter anastomoses characterize other regions, then an analysis of the mean distances from the line of anastomoses to the lesion border could indicate heterogeneous or variable collateral protection among regions. Alternatively, if a normal distribution of large- and small-diameter anastomoses is present in each region, then protection may be homogeneous among regions, and an analysis of means would be expected to obscure local differences in protection provided by large- and small-diameter anastomoses of a region. Thus, the lumen diameter of 1 anastomosis (or 2 averaged) near test point 7 was measured for each (n = 16) rat, and the shortest distance from the anastomosis to the lesion border was measured. Analysis of covariance was used to study diameter–distance relations for the 1-Day and 21-Day rats. All values are expressed as the mean ± SEM, α error < 0.05 (i.e., p < 0.05) is considered significant.

Results

Tissue Marking and Atrophy Patterns in SHRSP

In territories of the ACA and posterior cerebral artery, cortical tissue was intensely red after incubation in TTC. Within 1 day of MCA occlusion, TTC failed to grossly mark all cortical tissue in the MCA territory distal to the occlusion (Figure 1A). However, red cortex was present beneath the anastomoses, and the color extended into the MCA field (Figure 2A). In addition, TTC marked basal nuclei supplied by striate rami that branch from the MCA proximal to the occlusion site (Figure 1A).

Cortical atrophy was evident after 21 days of MCA occlusion (Figure 1B). The atrophy border was located within the territory of the MCA distal to the anastomoses (Figures 1B, 2B). That is, a rim of red tissue was present within the territory of the occluded MCA in 1-Day rats and a border of atrophied tissue was present in the 21-Day rats.

Relation of TTC Border to Atrophy Border

Spatial features of the 2 borders were compared using the midline as reference in measurements made.
FIGURE 1. Coronal section through brain of stroke-prone spontaneously hypertensive rats (A) 1 day and (B) 21 days after occlusion of left middle cerebral artery (MCA). Brain (A) reacted with 2,3,5-triphenyltetrazolium chloride (TTC). Branch of anterior cerebral artery anastomoses (arrow) with branch of MCA distal to the lesion (arrowhead).
FIGURE 2. Dorsal view, left hemisphere (A) 1 and (B) 21 days after middle cerebral artery (MCA) occlusion and incubation in 2,3,5-triphenyltetrazolium (A). Dashed line, border of lesion downstream from anterior cerebral artery (ACA)–MCA anastomoses (arrows). PCA, territory of posterior cerebral artery.
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on coronal sections (Figure 1) and the dorsal perspective (Figure 2). The TTC and atrophy borders were parallel to \( p = 0.57 \), and without displacement from \( p = 0.53 \), each other (Figure 3). The lesion border was not parallel to the midline \( p < 0.01 \). Thus, distance of the lesion border from the midline depended on the level of section along the rostral-caudal extent of the midline.

Location of ACA–MCA Anastomoses After MCA Occlusion

On the side of MCA occlusion, the lines of anastomoses were parallel in 1-Day and 21-Day rats \( p = 0.13 \). Mean distance from the midline to the anastomoses was not significantly different in 1-Day and 21-Day rats (Figure 4). After 21 days of MCA occlusion, the anastomoses were symmetric in distance from the midline on occluded and unoccluded sides. Thus, after 21 days of MCA occlusion, the anastomoses were in virtually the same position as anastomoses in 1-Day rats and in the same position as anastomoses on the contralateral unoccluded side.

Distance Relation of Anastomoses to Lesion Border

The lesion border was further from the midline than the anastomoses in each group of rats \( p < 0.05 \); Figure 4, Table 1). The null hypothesis, that the distance from the anastomoses to the lesion border is not \( > 0 \) in each test group, was rejected \( p < 0.05 \); Hotelling's \( T^2 \) test). Thus, there was a significant amount of tissue between the line of anastomoses and the lesion border.

Figure 5 illustrates distance from the anastomoses to the lesion border. Mean distance was 1.14 ± 0.05 mm for 1-Day rats and 1.18 ± 0.06 mm for 21-Day rats. The lines are parallel \( p = 0.78 \), of uniform width \( p = 0.25 \), and without displacement \( p = 0.88 \) from each other. Thus, the tissue between the anastomoses and the lesion border was homogeneous in width \( p > 0.05 \) from frontal to parietal and occipital regions after 1 or 21 days of MCA occlusion. Anastomosis-diameter lesion–distance relations for individual anastomoses were then studied.

Large-Diameter Anastomoses Protect More Tissue Than Small-Diameter Ones

A linear regression relates the lumen diameter of an anastomosis with its distance from the lesion border. The slope of the regression line (Figure 6) was positive and \( > 0 \) for 1-Day rats \( p < 0.001; r^2 = 0.92 \) and 21-Day rats \( p < 0.03; r^2 = 0.74 \). Regression lines for the 2 groups were parallel \( p = 0.91 \), but there was
Table 1. Regional Differences and Effects of Duration of Middle Cerebral Artery Occlusion on Distances Between Midline and Anastomoses, Midline and Lesion Border, and Anastomoses and Lesion Border in Stroke-Prone Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Test point</th>
<th>Distance from midline</th>
<th>21-Day rats</th>
<th>Distance to lesion border (anastomoses to lesion border)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anastomoses</td>
<td>Lesion border</td>
<td>Anastomoses</td>
</tr>
<tr>
<td>3</td>
<td>1.39 ± 0.24</td>
<td>2.63 ± 0.38*</td>
<td>2.04 ± 0.16</td>
</tr>
<tr>
<td>7</td>
<td>1.13 ± 0.15</td>
<td>2.23 ± 0.25*</td>
<td>1.73 ± 0.16</td>
</tr>
<tr>
<td>11</td>
<td>1.56 ± 0.13</td>
<td>2.74 ± 0.25*</td>
<td>1.78 ± 0.16</td>
</tr>
<tr>
<td>15</td>
<td>1.89 ± 0.09</td>
<td>3.29 ± 0.25*</td>
<td>1.96 ± 0.11</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *p<0.05 with Bonferroni correction.

significant displacement ($p<0.01$; Figure 6). The lesion border was further from large- than small-diameter anastomoses in both groups of rats. The mean lumen diameter of the anastomoses was greater for 21-Day rats (72 ± 7 µm) than for 1-Day rats (51 ± 5 µm; $p<0.05$). Thus, for a fixed distance to the lesion border, lumen diameters of the anastomoses were larger in 21-Day rats than 1-Day rats (Figures 2A, 2B, and 6).

Discussion

Major Findings for SHRSP

First, following occlusion of the MCA, tissue is infarcted downstream from, but not at, the ACA-MCA anastomoses. Protection from infarction was maintained upstream from the anastomoses secondary to lesion development downstream from the anastomoses. Infarction downstream from the anastomoses is evidence of inadequate collateral protection in SHRSP. Thus, the anastomoses limit the amount of collateral protection received. Second, the position of the lesion border was virtually the same after 1 or 21 days of MCA occlusion. This finding suggests that tissue infarction did not progress medially after 1 day of MCA occlusion. Third, large-diameter anastomoses were further from the lesion border than small-diameter anastomoses. Thus, local protection against the lesion was greater near large- than small-diameter anastomoses. Fourth, the protected tissue downstream from the anastomoses was, on the average, of uniform width in frontal, parietal, and rostral occipital regions. The uniform width of protected tissue among regions suggests that neither large- nor small-diameter anastomoses are unique to any one region in SHRSP.

Different Consequences of MCA Occlusion

In normotensive rats, MCA occlusion above the rhinal fissure initially reduces blood flow to the territory of the occluded MCA.1 After 1 month of MCA occlusion in normotensive rats, blood flow and collateral reserve are virtually normal, tissue infarction is usually absent, and the ACA–MCA anastomoses are...
of larger diameter than in controls. Thus, unlike SHRSP normotensive rats are protected from infarction in the territory of the occluded MCA.

In SHRSP, occlusion of the MCA above the rhinal fissure reduces blood flow to the territory of the occluded MCA, and tissue infarction occurs. Tissue sections from brains of SHRSP killed on the third postoperative day reveal shrunken and fragmented neurons, macrophages, mitotic figures, and spongiform neuropil in the territory of the occluded MCA. As shown here, the lesion does not extend into the field supplied by the ACA. Thus, tissue infarction is limited to the field receiving collateral blood supply.

Infarction after MCA occlusion is primarily a result of inadequate blood supply. Some of the basis for inadequate blood supply in SHRSP seems clear. The anastomosing ACA-MCA branches that exist before MCA occlusion are significantly smaller in luminal diameter, not fewer in number, in 5-week-old SHRSP than in normotensive controls. The smaller ACA-MCA anastomoses that exist in young SHRSP are defective prior to the established form of hypertension. Because young SHRSP never lived in that phase of the disease, the defect cannot be secondary to established hypertension. As a consequence of the vascular abnormality in SHRSP, occlusion of the MCA produces tissue infarction resulting from inadequate blood flow through cerebral anastomoses with small lumens.

**Borderzone Protection**

After occlusion of the MCA, several mechanisms may contribute more or less to the protection of tissue downstream from the pial surface anastomoses. First, protection resulting from gases and metabolites diffusing from microvascular beds located at, or upstream from, the anastomoses > 1 mm away does not seem likely because cortical intercortical distances are < 1/20 of that distance in rats. Second, blood flow through intracortical collateral channels to microvascular fields at the lesion border was not ruled out, but cortical penetrating arteries are small in diameter and intracortical arteries are end arteries. Alternatively, after MCA occlusion in normotensive rats protected from infarction, cellular mitotic activity occurs in the vascularized pia mater containing the anastomosing vessels, but not in the cortex. This finding of mitotic activity and the presence of longer and larger-diameter collaterals in rats with collateral protection against infarction are evidence that vascular adaptation occurs, increasing blood flow after MCA occlusion, and that the changes occur primarily in the pial surface anastomoses not in the intracortical vessels. Thus, protection of the borderzone tissue appears to depend primarily on blood flow through the pial surface anastomosing vessels.

**Implications of Findings**

Large-diameter pial surface anastomoses are located farther from the lesion than small-diameter anastomoses. Thus, more tissue was protected near large-diameter anastomoses than near small ones, probably as a result of more blood flowing through large-diameter anastomoses than through small ones. Furthermore, for a given amount of protection, the anastomoses are larger in rats with MCA occlusion for 21 days than for 1 day, which suggests that the anastomoses undergo enlargement after the occlusion and that more blood flows through the surface anastomoses in 21-Day rats than in 1-Day rats.

In summary, after MCA occlusion in SHRSP, tissue beneath the distal anastomosing branches is protected from infarction. The borderzone of protected tissue is, on the average, of uniform width in frontal, parietal, and rostral occipital regions. However, more tissue is protected near large-diameter anastomoses than near small ones, probably as a result of more blood flowing through large-diameter anastomoses than small ones. For a given amount of protection, the anastomoses after 21 days of MCA occlusion are of larger diameter than after 1 day of occlusion, thus suggesting that blood flow through the anastomoses may increase with time after MCA occlusion in SHRSP.

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**KEY WORDS**

- stroke-prone spontaneously hypertensive rats (SHRSP)
- infarction
- borderzone width
- anastomoses
- collaterals
- middle cerebral artery
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