Risk Area and Infarct Area Relations in the Hypertensive Stroke-Prone Rat

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Background and Purpose: Our purpose was to characterize the surface area of the infarct and the surface area at risk of infarction as defined spatially by arterial anastomoses to determine whether position, size, or shape of the infarct and the area at risk were related in stroke-prone rats or hybrid rats.

Methods: Stroke-prone rats ($n=18$; mean $\pm$ SEM blood pressure, $182 \pm 8$ mm Hg) and hybrid rats ($n=18$; mean $\pm$ SEM blood pressure, $147 \pm 6$ mm Hg; $p<0.05$) were anesthetized and the left middle cerebral artery was occluded with a ligature. The rats were killed 7 days later, arterial anastomoses were made visible with latex, the brains were fixed in formalin, and film recorded the infarct and anastomoses. Anastomoses and infaracts were digitized for measurements of risk area, luminal width, and infarct area.

Results: Mean risk area was similar in size, length, width, and variability in stroke-prone rats (area, $85 \pm 5$ mm$^2$) and hybrid rats (area, $84 \pm 7$ mm$^2$; $p>0.05$), whereas mean infarct area was larger, longer, wider, and less variable in stroke-prone rats (area, $53 \pm 6$ mm$^2$) than in hybrid rats (area, $15 \pm 11$ mm$^2$; $p<0.05$). Infarct length was appreciably greater than infarct width in both groups, indicating that infarct shape was not amorphous. Spatial overlap maps indicated that the infarct area common to all stroke-prone rats was positioned centrally in the risk area and was surrounded by a variable infarct area, which indicated that the likelihood of infarction increased with distance from the anastomoses. Shape factors for both risk area and infaract area were significantly different within each rat group, which indicated that infarct shape did not uniformly parallel the anastomotic sites that determined risk area shape ($p<0.05$). Risk area anastomoses and border zone width were linearly correlated in size and both were significantly wider in hybrid rats than in stroke-prone rats ($p<0.05$), which suggests that the narrower border zone tissue was perfused by narrower anastomoses.

Conclusions: We conclude that the position of the infarct within the risk area relates to luminal widths of conterminous anastomoses that define the risk area, but not to the size or shape of the area at risk of infarction defined spatially by the anastomoses. (Stroke 1993;24:705-710)

KEY WORDS • anastomoses • cerebral arteries • cerebral infarction • rats

Sudden occlusion of the middle cerebral artery (MCA) above the rhinal fissure produces a large ischemic infarct in the spontaneously hypertensive rat$^1$ and the inbred stroke-prone subtype (SHRSP).$^2,3$ A gradient of blood flow, with normal levels near the midline decreasing to abnormally low levels near the site of MCA occlusion,$^4,5$ exists. Thus, inadequate blood flow appears to be primary to lesion initiation after MCA occlusion in spontaneously hypertensive rats.

Distal MCA branches anastomose with distal rami of the anterior cerebral artery (ACA) and posterior cerebral artery (PCA) in rats.$^7$ The dilated anastomoses being of narrower luminal width in SHRSP than in the Wistar-Kyoto rat (WKY)$^8$ and the larger reduction in blood flow in SHRSP than in WKY$^9$ suggest that narrow anastomoses predispose young SHRSP to injury and infarction after MCA occlusion. In contrast, young normotensive WKY with wider anastomoses$^9$ and greater flow reserve are protected from infarction by adequate circulation that is initially less than normal blood flow,$^9$ but within 1 month after MCA occlusion blood flow and flow reserve are restored to normal levels in WKY.$^{10}$ In the study reported here, a map reveals that the infarcted area in SHRSP is circumscribed by anastomoses that define a potential area at risk of infarction. If narrow anastomoses determine lesion initiation and tissue infarction, then placement and size of the anastomoses may determine, in part, infarct location and size. Conversely, if the shape and position of the infarct are haphazardly related to the risk area, then the location and width of the anastomoses would not determine the spatial position of the infarct.

Our objective was to characterize the pial surface risk area and core infarct area after MCA occlusion in SHRSP and hybrid rats. Hybrid rats were included because variance in infarct size is greater in hybrid rats$^{11}$ than in SHRSP and variance in infarct size could depend on variance in risk area size. The first goal was to determine if the risk area differs appreciably in size in SHRSP with large infarcts compared to hybrid rats with

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smaller, but more variable, infarcts. Other goals were to learn if the surface of the risk area and the core infarct area were correlated in shape or size if the border zone was related in size to the luminal diameters of conterminous anastomosing arterioles.

Materials and Methods

SHRSP (n=18) and F1 rats (n=18) were investigated. F1 rats were derived from male SHRSP matings with female WKY. Both sexes were sampled. The rats were 2–3 months old when the MCA was occluded. Five to seven tail systolic blood pressure (BP) recordings were obtained for each animal 1–3 days before MCA occlusion. BP recordings followed procedures detailed previously.\(^2\) The last three recordings were averaged to yield one BP value per rat. Average tail systolic BP before MCA occlusion was greater in SHRSP (182±8 mm Hg) than in F1 rats (147±6 mm Hg, p<0.05).

All rats were anesthetized with 132 mg/kg wt i.m. ketamine hydrochloride. The left MCA was exposed above the rhinal fissure beyond the origins of lenticulostriate arteries supplying subcortical structures.\(^12\) A 2-mm-diameter craniectomy was drilled with a #6 dental burr approximately 1 mm rostral and 2 mm dorsal to the rostral fusion point of the zygoma to the squamosal bone. Monofilament nylon thread, about 35 μm in diameter, was used to ligate the MCA with a square knot 1.56±0.04 mm (n=36) above the rhinal fissure. Flow interruption could be observed in the MCA immediately distal to the knot and confirmed occlusion.\(^12\) The rats were not paralyzed, no mechanical ventilation was used, and no respiratory gases were administered. After wound closure with sutures, postsurgical care followed procedures previously outlined.\(^12\)

On the seventh day after MCA occlusion all rats were anesthetized again. Papaverine (40–50 mg/kg body wt) was administered intravenously to produce maximal vasodilation and death. Under controlled conditions, latex (Chicago Latex Products, Schaumberg, Ill.) was injected into the arterial tree to visualize for measurement the luminal diameters of anastomosing rami.\(^7\) The brains were immersion-fixed in 10% neutral buffered formalin for 9 months before photography. Our impression is that photographic contrast is increased with fixation time.

The brains were placed in a custom-made holder to photograph the hemisphere at an angle of 65° to the midsagittal plane. This angle of orientation permitted observation of the cortical surface of the infarct in one image (Figure 1). Fine grain release film was used for photography because this film is sensitive to the spectral properties of infarcted tissue. Infarct borders were drawn from negative projections. Digital coordinates were obtained with a SummaSketch digitizing pad.
(Summagraphics Corp., Fairfield, Conn.). Infarct area and perimeter were calculated using Sigma-Scan software (Jandel Scientific, San Rafael, Calif.) running on a microcomputer.

The location of an anastomosis was determined on the basis of vessel branch angle reversal patterns. The site of an anastomosis was halfway between vessel branch angle reversal points or where the luminal diameter was minimal. Sites of anastomosing vessels were used to identify spatial limits of the area at risk of infarction on the surface of the brain and to measure vessel luminal diameter. Luminal diameters of the 10 widest anastomoses were measured on photographic prints at ×75 magnification using the digitizing pad.

Maps of infarct area and risk area were constructed from film negatives. For spatial overlap maps, the site of MCA occlusion was used as the registration (fiducial) alignment point. The common area was that which overlapped spatially in the 18 SHRSP or in the 18 F1 rats. The variable area was located spatially outside of the area common to a group of 18 rats. The arithmetic mean of the variable area was calculated by subtracting the common area obtained for the group from the area for an individual rat. Shape was evaluated using a shape factor defined as the square root of the area divided by the perimeter of the area.

Student's *t* test was used to compare the two groups. Within-group comparisons used a two-tailed paired *t* test. An *α* error below 0.05 (i.e., *p* < 0.05) was considered significant. All values were expressed as mean ± SEM unless otherwise noted.

### Results

Collateral supply branches from the ACA, PCA, and proximal MCA anastomose with MCA branches in a configuration that is distal to and that circumscribes the occlusion site (Figure 1, left). This circumscribed region outlined by anastomoses is at risk for reduced blood flow and infarction after MCA occlusion because there is no source beneath the cortex of collateral blood to the cortex in rats. Within the risk area, the infarct is surrounded by a border zone of tissue that extends to the anastomoses (Figure 1, right). Adjacent anastomoses are not displaced in a sharp zigzag pattern, but rather the anastomoses align along a broad curve (Figure 1, right). There was no evidence of nonanastomosing interdigitating arteries. Thus, the outer edge of the border zone is not highly serpiginous (serrated) as might occur if nonanastomotic vessels were inserted.

Mean surface area at risk of infarction was similar in size and variability in SHRSP and F1 rats (*p* > 0.05, Table 1). Mean length of the risk area was greater than mean width of the risk area in both SHRSP and F1 rats (*p* < 0.05, Table 1). There was no significant difference in mean length or mean width of the risk area between SHRSP and F1 rats (*p* > 0.05, Table 1). For SHRSP and F1 rats, risk area overlap maps provided size and position information relative to the MCA occlusion site. Overlap maps indicate that the common risk area size was similar for SHRSP and F1 rats (Table 1). The variable risk area completely circumscribed (Figure 2) and was nearly as large as (Table 1) the common risk area in SHRSP and F1 rats. Mean variable risk area was not significantly different between the two groups (*p* > 0.05, Table 1).

Mean pial surface infarct area was greater in SHRSP than in F1 rats (*p* < 0.05, Table 1), although variability in infarct area was appreciably greater in F1 rats than in SHRSP (SD values significantly different, *F* = 3.45,

### Table 1. Parameters of Risk Area and Infarct Area in Brains of Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>SHRSP (n=18)</th>
<th>F1 (n=18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk area (mm²)</td>
<td>85±5</td>
<td>84±7</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>10.1±0.1†</td>
<td>10.0±0.1*</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>6.2±0.1</td>
<td>6.1±0.1</td>
</tr>
<tr>
<td>Width/length</td>
<td>0.62±0.01†</td>
<td>0.61±0.01</td>
</tr>
<tr>
<td>Overlap area (mm²)</td>
<td>109</td>
<td>108</td>
</tr>
<tr>
<td>Common (mm²)</td>
<td>59</td>
<td>57</td>
</tr>
<tr>
<td>Variable (mm²)</td>
<td>50</td>
<td>52</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>26±1</td>
<td>27±2</td>
</tr>
<tr>
<td>Infarct area (mm²)</td>
<td>53±6</td>
<td>15±11†</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>7.6±0.2*†</td>
<td>3.6±0.4†</td>
</tr>
<tr>
<td>Width (mm)</td>
<td>4.5±0.1</td>
<td>2.3±0.2†</td>
</tr>
<tr>
<td>Width/length</td>
<td>0.60±0.06</td>
<td>0.66±0.04</td>
</tr>
<tr>
<td>Overlap area (mm²)</td>
<td>81</td>
<td>49</td>
</tr>
<tr>
<td>Common (mm²)</td>
<td>28</td>
<td>0.3</td>
</tr>
<tr>
<td>Variable (mm²)</td>
<td>53</td>
<td>49</td>
</tr>
<tr>
<td>Mean±SEM</td>
<td>25±1</td>
<td>15±3†</td>
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<tr>
<td>Shape factor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk area</td>
<td>0.26±0.00*</td>
<td>0.25±0.00</td>
</tr>
<tr>
<td>Infarct area</td>
<td>0.24±0.00‡</td>
<td>0.23±0.01‡</td>
</tr>
</tbody>
</table>

SHRSP, stroke-prone spontaneously hypertensive rats; F1, first filial generation rats. Values are mean±SEM, except mean±SD for infarct area and risk area.

†*p* < 0.05 versus width.

‡*p* < 0.05 versus SHRSP.

§*p* < 0.05 versus risk area.
Both mean infarct length and mean infarct width in SHRSP were significantly greater than the corresponding parameters in F1 rats ($p<0.05$, Table 1). Mean infarct length was appreciably greater than mean infarct width in both groups of rats ($p<0.05$, Table 1). Neither the infarct shape factor nor the quotient of infarct width divided by infarct length was significantly different between the two groups ($p>0.05$, Table 1). However, the angle of the length axis to the midline was appreciably greater in F1 rats with small infarcts ($48.4\pm5.0^\circ$, $p<0.05$) than in SHRSP with large infarcts ($4.1\pm1.0^\circ$), which indicates that infarct bearings change with infarct size. Furthermore, the risk area shape factor was significantly greater than the infarct area shape factor in both groups of rats ($p<0.05$, Table 1), thus indicating different shapes. The infarct area:risks area ratio was significantly greater in SHRSP ($0.62\pm0.02$, $p<0.05$) than in F1 rats ($0.18\pm0.03$), but there was no significant difference between the sexes among SHRSP (10 females, $0.61\pm0.02$ versus eight males, $0.64\pm0.02$) or F1 rats (11 females, $0.18\pm0.03$ versus seven males, $0.18\pm0.06$, $p>0.05$).

There was no significant linear correlation of infarct area with risk area, or infarct length with infarct width, or risk area length with risk area width in either group of rats ($p>0.05$). Thus, spatial determinants of the surface risk area do not determine infarct size.

Infarct area overlap maps indicate that infarct frequency for each group was greatest (100%) at the site of MCA occlusion and least peripherally, nearest the anastomoses (Figure 3). Mean distance of the infarct from the midline was $2.99\pm0.17$ mm at $2.50$ mm from the rostral pole of the brain and $2.81\pm0.15$ mm (not significantly different) from the midline at $10.50$ mm further caudal in SHRSP. Infarct area common to all SHRSP was $28$ mm$^2$, less ($0.3$ mm$^2$) in F1 rats. The variable area of infarction for the group was $53$ mm$^2$ for SHRSP and $49$ mm$^2$ for F1 rats. Mean variable area of infarction was $25\pm6$ mm$^2$ for SHRSP, less ($15\pm11$ mm$^2$, $p<0.05$) in F1 rats.

Mean luminal width of the 10 widest anastomoses in each rat was less in SHRSP ($35\pm0.8$ $\mu$m, $p<0.05$) than in F1 rats ($44\pm1.3$ $\mu$m). Also, mean border zone surface area was less in temporal region$^{13}$ 1-3 of SHRSP ($14\pm1$ mm$^2$, $p<0.05$) than of F1 rats ($25\pm1$ mm$^2$). Conterminous anastomoses to the temporal border zone were narrower in SHRSP ($52\pm3$ $\mu$m, $p<0.05$) than in F1 rats ($64\pm2$ $\mu$m). Furthermore, the temporal border zone area and mean luminal diameter of corresponding anastomoses were related by significant linear correlation in each group ($r=0.6091$, $p<0.05$ for SHRSP; $r=0.6623$, $p<0.05$ for F1 rats).

**Discussion**

This study provides three new findings. First, the core area of infarction is located within an area circumscribed by anastomoses, which suggests that the area at risk of infarction after MCA occlusion is defined by sites of anastomoses on the pial surface of the brain. Second, the nearly equal areas at risk of infarction in SHRSP and F1 rats but the significantly larger infarct in SHRSP than F1 rats are evidence that risk area is not a major determinant of infarct size. Third, infarct area overlap maps, border zone size data, and anastomosis width information indicate that infarct likelihood increases as a function of anastomosis width and distance from the anastomoses that define the risk area.

This study is the first known attempt to identify an area at risk of infarction based on the location of narrow anastomoses that may restrict blood flow and thus predispose SHRSP to infarction after an arterial occlusion. Whether hypertension-related differences elsewhere in the vascular tree also contribute to predisposing this risk area to large infarcts after MCA occlusion is not ruled out. The risk area was identified in two dimensions because the methods precluded an analysis of anastomoses that may exist within the cortex. This limitation was not considered exclusionary to the analysis undertaken for the following reasons. First, the neocortex receives no blood supply from the underside,$^{14-17}$ thus the possibility is eliminated that anastomoses exist at the interface with white matter.$^{16}$ Second, speculation that anastomoses join adjacent, radially
directed, cortical penetrating arteries within the cortex is contrary to functional evidence\textsuperscript{16,18} and without quantitative anatomical support.\textsuperscript{14} The concept that penetrating cortical arteries are functional end arteries\textsuperscript{19,20} (without anastomoses to contiguous arteries) was never excluded since collateral supply is considered minimal through precapillary arterioles and capillary anastomoses. Thus, the possibility that collateral blood flowed laterally within the risk area was not excluded, but current information suggests that the distance was minimal, if any, beyond the field of a radial artery.

If the MCA anastomoses provided no blood flow and the upstream (preanastomotic) territory was unimpaird, then infarct size and shape would theoretically equal risk area size and shape as defined by sites of anastomoses. Neither size nor shape of the infarct was correlated with size or shape of the risk area, which is evidence that tissue was protected from infarction downstream of the anastomoses in both SHRSP and F1 rats. Because the risk area was nearly the same size in SHRSP with large infarcts and F1 rats with small infarcts, risk area size per se was not the major determinant differentiating infarct size in SHRSP and F1 rats.

Infarct overlap maps indicate that the likelihood of infarction was not uniform within the area at risk of infarction in either SHRSP or F1 rats. Likelihood of infarction being greatest at the site of MCA occlusion and least closest to the anastomoses suggests a graded protection having greater effect near the anastomoses than at the site of occlusion. Others\textsuperscript{4-6} have demonstrated a gradient of blood flow that increases with distance from the site of MCA occlusion, which suggests that more tissue protection was present near the anastomoses (where blood flow was higher) than near the MCA occlusion site (where blood flow was lowest).

The common and variable areas of infarction being nearly equal for SHRSP and the variable area being 50 times greater than the common area in F1 rats suggest the presence of a determinant of infarction that varies in cortical spatial position. That is, the variation in spatial location of the infarct among rats suggests that the determinant of the variation is not consistent in spatial location in all rats. Thus narrow, flow-restricting anastomoses that are inconsistent in spatial location and width may account for the large variable area of infarcted tissue observed for SHRSP and F1 rats.

The correlation of anastomosis luminal width with border zone surface area is evidence that narrow anastomoses are spatially associated with less protected tissue. The mechanism that relates narrow anastomoses with narrow border zones and wide anastomoses with wide border zones is not clear. Others have demonstrated a gradient of blood flow that increases with distance from the site of MCA occlusion,\textsuperscript{4-6} which could suggest pressure-dependent blood flow. We speculate that narrow anastomoses reduce BP within the risk area. With pressure-dependent blood flow\textsuperscript{21} decreasing proportional to the distance from anastomoses defining the risk area boundary, less than adequate blood flow\textsuperscript{22} in the center of the risk area resulted in infarction. Thus, a greater drop in pressure due to increased vascular resistance of narrower anastomoses would result in less blood flow, a narrower border zone, and a larger infarct in SHRSP than in F1 rats.

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