Differential Outcome to Middle Cerebral Artery Occlusion in Spontaneously Hypertensive Stroke-Prone Rats (SHRSP) and Wistar Kyoto (WKY) Rats

PETER COYLE, M.S., PH.D., AND PENTTI T. JOKELAINEN, M.D., PH.D.

SUMMARY  Evidence was found for different outcomes to middle cerebral artery occlusion in the young genetically hypertensive stroke-prone rat (SHRSP) compared to sham operated controls and the Wistar Kyoto rat (WKY). Qualitatively and quantitatively different gross lesions marked by Evans blue-albumin, cortical atrophy, large areas of strikingly altered cortical histology, postoperative survival and motor behavioral deficits differentiate young SHRSP from sham operated controls and the normotensive WKY. We conclude that the limited focal lesion observed in normotensive and sham operated rats is primarily due to surgical trauma of exposing the vessel and passing the ligature deep to it. The grossly larger and qualitatively different lesion in the SHRSP is the result of an inadequate circulation provided by the dorsal cerebral arterial collaterals. Since the 5-6 week old SHRSP were only mildly hypertensive (systolic blood pressure 140 mm Hg), the inadequate collateral circulation appears to be related to either a genetic or acquired problem rather than being secondary to a vascular lesion of chronic hypertension.

Blood Pressure

Tail systolic blood pressure readings were taken

Methods

Blood Pressure

Tail systolic blood pressure readings were taken using a Narco Biosystems model PE-300 Programmed Electro-Sphygmomanometer to automate a constant inflation-deflation cycle. Pressure readings were obtained for ten 35–40 day old WKY and for 10 SHRSP of the same age. Three values for each rat were averaged. Mean value for the WKY was 109 ± 8 mm Hg and for SHRSP 140 ± 10 mm Hg.

Surgery

Ten WKY, 30 SHRSP and 3 NW aged 35–40 postnatal days and representing each sex were anesthetized with ketamine hydrochloride (136 mg/kg body weight, i.m.). One WKY, and 3 SHRSP rats were sham operated such that the right MCA was exposed through a burr hole craniectomy (1–2 mm diameter, 0.785–3.142 mm² in area) performed by a transtemporal route, then the artery was dissected free of the meninges but not ligated. For the ligation procedure the vessel was occluded with a monofilament nylon thread, about 35 μm in diameter and secured with a square knot in the remaining 39 rats. Focal occlusion of the middle segment of the vessel was distal to the striate branches and 700–1000 μm dorsal to the rhinal fissure. Surgery was done with aid of a Bausch and Lomb StereoZoom 7 microscope and Nikon MK II light. Following wound closure with silk suture, 0.5–0.75 ml 2% Evans blue made with physiologic saline was injected intraperitoneally to mark injury to the Evans blue-albumin blood-brain barrier as occurs with severe ischemic injury, cerebral infarction in rat or due to local surgical trauma in exposing or ligating the MCA. Details of the surgery, diet and postoperative procedures and tissue preparations for histology are given elsewhere.

Postsurgical Neurological Tests

On postoperative days 2 and 3, neurological tests were performed to evaluate motor deficits. Each rat was placed on the 25 mm wide or 7 mm narrow surface of a horizontally suspended wooden meter stick. Observations were made on fore- and hind-limb digits,
feet, legs and thighs, their locations and symmetries during station, gait, crawling or running on the meter stick.

Postmortem Determinations

Rats were killed on the third postoperative day to measure Evans blue marks, for histologic analysis, and for injection of media to demonstrate the vessels. Tissue fixation was initiated by injection of 50 ml 10% neutral buffered formalin into the aorta following its clamping at the diaphragm and excision of the right atrium. In 3 NW and 3 SHRSP papaverine hydrochloride (40–50 mg/kg rat in sterile water) was injected intravenously following light ether anesthesia to produce maximal vasodilation. This was to facilitate vessel filling by injection medium. Undiluted liquid photographic emulsion (Rockland BB-201) followed by Vultex, a latex rubber compound, were injected into the aorta after cranial exposure for observation of filling dorsal collaterals. Brains receiving the emulsion were exposed to sunlight while in physiologic saline and were developed in Dektol. The specimens were 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. All were stored in fixative until mounted on glass slides and stained with basic fuchsin or hematoxylin and eosin.

Photography and Computer Assisted Lesion Measurements

Dorsal and lateral views of brains were photographed with blue sensitive film at 1.5 × 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.

Results

Gross Lesion

Gross cortical lesions of SHRSP were ipsilateral to the ligation, were much larger than, and were qualitatively different from those of sham operated rats and the WKY. Evans blue staining was not characterized by isolated focal marks in any case but was massive. For all SHRSP the blue mark extended from the occlusion site towards anastomoses of the MCA with the anterior (ACA) and posterior (PCA) cerebral arteries. In no case did the blue cross the zone of anastomoses between the blue stained tissue and pale surrounding cortex. Variations in the extent of the blue staining was greatest in the region receiving collateral supply from the ACA. In 4 SHRSP rats killed at 10 days, gross cortical atrophy was evident in the blue marked region.

In all WKY and the sham operated SHRSP the blue was localized in cortex adjacent to the burr hole (fig. 1A). Figure 2 indicates the average mark sizes. Evans blue marks for the WKY were less than one-thirtieth the size of the average mark for SHRSP. For the rats with marking limited to the burr hole region there was no white border zone. The MCA field cortex after formalin perfusion was similar in gross appearance to that of the opposite nonoccluded hemisphere. Pial surface branches of the MCA proximal and distal to the occlusion site were frequently lightly stained by Evans blue bilaterally. None of the rats had subarachnoid hemorrhage extending beyond limits of the burr hole.

Figure 1. A. Wistar Kyoto rat (WKY) brain 3 days after occlusion of middle cerebral artery and intraperitoneal injection of Evans blue. Note location of ligature. B. Spontaneously hypertensive stroke-prone rat (SHRSP) brain 10 days after occlusion of the MCA. Note large area of MCA field stained with Evans blue as compared to the WKY rat.
Postoperative Survival

Of the 16 SHRSP not killed on the third postoperative day many died between the second and tenth day. Weight loss was considerable and animals did not drink or feed well on standard rat chow. Brains of animals that died were swollen showing midline shift of the superior longitudinal fissure, cerebellar herniation into the foramen magnum, a flattening of the transverse fissure, and Evans blue marking similar to rats killed on the third day for measurements. All NW and WKY survived 3 days.

Neurological Observations

Observations were made during station and gait while the rat was on the wide or narrow surface of the wooden meter stick. An asymmetric gait was often, but not invariably, observed in SHRSP. This was characterized by the left hindfoot missing its placement on the wide surface of the stick (fig. 3B). After several misses, with or without falling, the rat shifted its hindquarters so a larger surface area of the stick was available for placement of the left hindfoot. This was quickly learned by the rat and no rat was tested on the meter stick prior to MCA occlusion. Similarly, but less frequently observed, the left forefoot utilized a larger surface area than the right one and the digits didn’t grasp the edge of the walking surface (fig. 3B). During station SHRSP sometimes failed to grasp the stick with the left feet and fell. WKY, NW and sham operated SHRSP showed no motor deficit in either station or gait when on the wide or narrow walking surface (fig. 3A). Alternating limb movements were symmetric in action and placement. Foot placements missing the meter stick were rare even during running of these rats.

Histological Findings

Histological sections from brains of SHRSP killed on the third postoperative day invariably have a large cortical lesion (fig. 4B) in the region marked by Evans blue. The tissue was less intensely stained (fig. 4B). Neuronal cell somas were not clearly outlined as in normal tissue. Neurons were shrunken and fragmented. Macrophages, mitotic figures, and spongy neuropil were evident. These features characterize the irreversible brain lesion. In rats having the gross white borderzone there was a sharp transition between the altered tissue and that having well defined neuronal perikarya. Where the white borderzone was lacking, strands or islands of fragmented cells were intermixed with less altered cortex. The lesion extended through all layers of the cortex.

The sham operated rats and all WKY had lesions limited in extent to cortex adjacent to the burr hole (fig. 4A). Shrunken neurons, less intensely stained neuropil, macrophages and mitotic figures were observed. Some hemorrhage was usually present. The lesion was wedge shaped with the apex in a deeper cortical layer (fig. 4A) or cylindrical in shape and extending through all layers. On the average the histologic lesion (mean diameter size in cortical layer I was 1.13 ± 0.21 mm²) was smaller than the mean size of the blue mark. Because the blue was not observable in thin hematoxylin and eosin stained sections the exact location of the blue boundary could not be correlated with the edge of the histologic lesion.

Collateral Filling

Liquid photographic emulsion was used to observe the course of venous vessels, whereas Vultex marked the arterial tree. Collateral channels from the PCA and WCA...
ACA to the MCA field were open to the media in normotensive rats for MCA branches distal to the occlusion contained latex (fig. 5A). MCA field cortical veins were demonstrated with the emulsion (fig. 5A). In contrast for SHRSP, MCA branches distal to the anastomoses were devoid of media and corresponding MCA field cortical veins were not filled (fig. 5B). The area devoid of filling approximated that of the lesion in each strain.

**Discussion**

**Location of the Occlusion and Size of Lesion**

Occlusion of the proximal segment of the MCA was done in adult Sprague-Dawley rats. Ischaemic brain damage occurred in the neocortex and caudate nucleus. A comparison of these findings with ours is difficult to make since details of the histologic lesion and its extent were not given. We assume there was a large lesion. This would not be unexpected since a proximal MCA occlusion requires a greater collateral supply for tissue survival due to inclusion of the caudate nucleus in the field. We occluded the MCA distal to striate branches supplying the caudate nucleus and thus excluded it. Since the tissue volume requiring supply via existing collaterals was less, sparing of the neocortex occurred in young WKY.

Others occluded the MCA dorsal to the rhinal fissure in adult Sprague-Dawley rats. A circular shaped infarct 1-5 mm in diameter developed. The area ranged from 0.79 to 19.63 mm$^2$. Our Evans blue measurements and histological findings for sham operated rats and all WKY were within this size range as were our findings for NW. Earlier studies give evidence that mere exposure of the MCA results in marking of the cerebral cortex by Evans blue and reduced blood flow at the surgical site. Indeed, the sham operated rats and WKY findings reported here support that notion.

Mean lesion size for our SHRSP exceeded the maximum reported for Sprague-Dawley rats by at least 46%. Since the mark was even larger (37 times) in SHRSP compared to WKY, there was a differential outcome to the occlusion in these rat strains.

**Evans Blue Marking and Histology**

Evans blue leaks were in two cortical regions. Leaks...
FIGURE 5. A. Normal Wistar rat (NW) and B. spontaneoushypertensive stroke-prone rat (SHRSP) after occlusion of the right middle cerebral artery and injection of media to demonstrate the location and course of vascular elements (see text).

Cortical cells adjacent to the burr hole were also injured during surgical exposure of the MCA as determined from histologic findings. Heat and trauma due to drilling of the burr hole, incision of the meninges, dissection of the MCA from the pia-arachnoid and passing of the nylon thread deep to the MCA often associated with some hemorrhage were surgical trauma. This trauma likely injured the vasculature and cortical cells at the burr hole site in all rats and accounts for Evans blue leaks and histologic evidence of injury there.

In striking contrast for the SHRSP, Evans blue marks extended far beyond the craniectomy site, a white borderzone existed between the blue and unmarked cortex, and the cortical histology all provide evidence of a massive brain lesion. Such histology characterized ischemic infarcts following MCA occlusion in primates and is indicative of an irreversible brain lesion resulting from an inadequate blood supply. This lesion was irreversible since rats killed after 10 days had gross cortical atrophy indicating tissue removal.

Neurological Deficits
Motor deficits observed in SHRSP were contralateral to the side occluded. Presumably, the basis for the deficits involved cortical neurons that project axons to cells of the spinal cord contralateral to the MCA occlusion. Association, commissural and projection neurons were included in the cortical lesion with axons projecting to the opposite hemisphere, the basal ganglia, thalamus and lower regions. Consequently, the histologic and circulatory response to this large cortical lesion with axons and terminals degenerating outside the blue marked cortical region must be widespread in the nervous system.

Death was common in SHRSP within 10 days following the occlusion. Several variables probably contributed to the demise. Removal of masticatory musculature likely interferes with consumption of standard rat chow. Chow consumption and water intake were depressed for SHRSP. Dehydration, brain swelling and gross hemorrhage were evident in some rats, but not all that died. These alterations, whether due to the large brain lesion or for some other reason, most likely contributed to the demise of SHRSP. None of the sham operated SHRSP or occluded WKY died.

Collateral Filling
The lack of venous and arterial filling in the MCA vascular field of SHRSP suggest interference with the collateral supply, but taken alone, this finding does not indicate brain injury. Whereas the lesion marked by Evans blue and characterized by altered histology crossed under many pial surface veins, interarterial anastomoses of the MCA with the PCA or ACA were not crossed. This is evidence for an arterial supply problem. Since Evans blue was injected following MCA ligation, transport to the lesion was via the collateral supply. Had diffusion of the blue marker occurred from anastomoses near the edge of the lesion inwards to the site of the occlusion, an intensity gradient should have been evident, and the white borderzone should not have been present. Neither was the case. Red blood cells were not usually present in capillaries of the lesioned tissue following formalin perfusion giving evidence of channels for some collateral perfusion and drainage of the MCA tissue field in SHRSP.

Speculations Concerning the Lesion in SHRSP
The histologic features characterize an ischemic lesion. Its location within the MCA vascular field distal to the ligation is evidence of an inadequate blood supply through existing pial surface collaterals, since these are the only source distal to the ligation for collateral supply to the cortex. The inadequate supply may be due to increased cerebral metabolism, a reduced oxygen transport system, reduced blood flow due to inadequate or insufficient regulation of collaterals, fewer or smaller diameter existing collaterals in
SHRSP. Or there may be some other single factor or a multifactorial combination involving metabolic, vascular structural or hemodynamic alterations.

Relationship of Hypertension and Lesion-Proneness

The proneness is not secondary to chronic hypertension since it does not exist yet in young rats. Vascular structural change including hyaline degeneration,\textsuperscript{13} fibrinoid necrosis,\textsuperscript{27} thrombotic vascular occlusions\textsuperscript{28} and subintimal deposition of fibrinoid materials and fibrous thickenings of the wall involving only distal ramifications and small muscular arteries\textsuperscript{27} reduce cerebral blood flow. These features characterize vascular lesions secondary to chronic or fully established hypertension when systolic blood pressure is often over 200 mm Hg.\textsuperscript{28, 29} Since even mild medial hyperplasia in cerebral vessels was not evident in 16–34 week old SHR, but present at 40–82 weeks,\textsuperscript{29} neither it nor other vascular lesions secondary in appearance to chronic high blood pressure can be responsible for the infarct in young rats never having lived in this phase of hypertension nor having the vascular structural changes resulting from it. That proneness for the lesion following MCA occlusion codevelops in time with initiation of blood pressure elevation for whatever reason, be it structural or functional, is not ruled out. In fact, blood pressure in SHRSP is elevated compared to WKY at the 5–6 week test time. Even cerebral vascular differences exist between SHR and WKY at 15 days of age,\textsuperscript{30} but strain differences preclude meaningful interpretations that remain for experimental testing. We can only conclude the infarct must relate to either a genetic or acquired problem, since adulthood and its pathologic vascular changes do not exist yet in young SHRSP.

The outcome of this specific cerebral vascular occlusion done in young spontaneously hypertensive stroke-prone rats differs from responses of sham-operated ones and age matched WKY. WKY develop an appropriate collateral circulation to maintain the tissue field distal to the occlusion. Injury of the blood-brain barrier, gross cortical atrophy, histology indicating an irreversible ischemic neuronal lesion, and motor behavioral deficits differentiate SHRSP from WKY and NW rats. The type of lesion observed, its consistent staining in vivo by Evans blue and the pattern of vascular filling deficits provide evidence that a collateral circulation existed in SHRSP. The arterial component of the collateral circulation in SHRSP was inadequate to maintain the viable MCA tissue field distal to the occlusion, and the large irreversible brain lesion was accompanied by motor deficits and death. Since proneness to infarction exists for young SHRSP prior to vascular lesions appearing secondary to chronic hypertension, outcome of the ligation can not depend upon them, yet the proneness and elevated blood pressure exist at test time. Whether or not one precedes the other in time remains to be determined.

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References

The canine cerebrum. In general, their findings indicate that the pial microvessels are more metabolically active than arterioles from either the gray or white matter. To our knowledge, the present study is the first to apply histochemical methodology to describe the metabolic profiles of normal mammalian spinal cord arteries and arterioles.

Methods

Eight normal adult Long-Evans female rats were anesthetized with intraperitoneal injections of sodium pentobarbital (200 mg/kg body weight). In four of these animals, the thorax was opened and each animal was perfused through the left ventricle with 10% buffered neutral formalin. Then, segments of spinal cord were surgically removed from the mid-thoracic region from both the perfused and non-perfused animals. The segments of spinal cord from the non-perfused animals were quickly frozen in isopentane, cooled in an acetone-dry ice mixture at \(-45°C\), then mounted on cryostat chucks and sectioned at 6 microns on a cryostat (OCT compound). Tissue samples from the perfused animals were transferred to absolute alcohol, routinely processed for paraffin embedding, and sections cut at 4 microns.

Selected enzymes or components of key metabolic pathways in the arterial and arteriolar walls of the rat
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