Cerebral Infarction Following Bilateral Carotid Artery Ligation in Normotensive and Spontaneously Hypertensive Rats: A Pathological Study

JUN OGATA, M.D., MASATOSHI FUJISHIMA, M.D., YASUYUKI MOROTOMI, M.D., AND TERUO OMAE, M.D.

SUMMARY A pathological examination was performed on normotensive rats (NTR) and spontaneously hypertensive rats (SHR) following bilateral common carotid artery ligation. After ligation, diffuse and extensive cerebral infarcts in the carotid artery territory occurred frequently in SHR, while NTR occasionally had well-circumscribed small infaracts.

The posterior communicating arteries, which are the major anastomotic channels connecting the carotid and vertebrobasilar systems, did not show any anomalies and were well developed in SHR and NTR. Vascular changes secondary to hypertension, such as fibrinoid necrosis or thickening of the wall, were not observed in SHR. Because of the paucity of structural difference of the blood vessels, the more diffuse and extensive cerebral infarcts in SHR after carotid occlusion were attributed to the hemodynamic difference rather than the morphological difference between the two groups.

The results of the present experiment suggest that hypertension per se, i.e., hemodynamic factors, may be operative for the development of cerebral infarction.

Introduction

SPONTANEOUSLY hypertensive rats (SHR) had a marked increase in lactate and lactate-pyruvate ratio of the brain following bilateral common carotid artery ligation and an early mortality, while a slight increase in metabolites and a lower mortality were observed in normotensive rats (NTR). To elucidate the cause of the susceptibility of SHR to brain ischemia, a pathological study of the rat brain was performed.

Methods

The experimental animals used were adult male SHR and normotensive rats (NTR) of the Wistar strain for the controls. The SHR were from the F\textsubscript{10} and F\textsubscript{11} generations raised in our facilities and they corresponded to the F\textsubscript{6} and F\textsubscript{7} generations derived by Okamoto and Aoki. Both groups of rats weighed 250 to 400 gm, with ages of five to nine months. Systolic blood pressure, determined without anesthesia by tail sphygmography, was above 170 mm Hg in SHR, and below 150 mm Hg in NTR.

Wet heart weight, expressed as a ratio to body weight, was measured in 29 NTR and 34 SHR.

In order to study the anatomy of the arteries at the base of the brain, vascular perfusion in vivo was performed in six NTR and six SHR under anesthesia with intraperitoneal amobarbital (100 mg per kilogram). After incision of the jugular veins and thoracotomy, the left ventricle was infused with a suspension of 12.5% barium sulfate in 12.5% citrate, and observed with a JEM T8 electron microscope.

Basilar artery were examined. One side of the arteries was measured when they were paired.

Six NTR and six SHR with intact carotid arteries were killed for histological examination. Three NTR and three SHR were decapitated; the cranium over the cerebrum was opened carefully to prevent compression of the brain by hands or instruments, and more than one-half of the brain surface area was exposed. The brain thus attached on the skull base was fixed by immersion in 10% formalin overnight. The brain then was separated from the skull and kept in the fixative for a week. Three NTR and three SHR were perfused with 3.5% formaldehyde and 7.5% sucrose buffered to a pH of 7.3 with 1/15 M sodium phosphate through the left ventricle for 30 minutes. Then the skull was opened and the brain immersed in the buffered formaldehyde solution. Thus, undesirable cellular changes which might occur during and after fixation were avoided.

The results of the present experiment suggest that hypertension per se, i.e., hemodynamic factors, may be operative for the development of cerebral infarction.

For electron microscopic examination, three NTR and five SHR with intact carotid arteries were perfused with formaldehyde-glutaraldehyde fixative through the left ventricle. Tissue used for electron microscopic study was taken from the cerebrum and the origin of the middle cerebral artery. These were post-fixed for one hour in 2% osmium tetroxide buffered with modified Millonig's phosphate buffer, dehydrated in alcohol, and embedded in Epon 812. Thin sections were stained with uranyl acetate and lead citrate, and observed with a JEM T8 electron microscope.

Under anesthesia, bilateral common carotid arteries of the experimental animals were exposed through a ventral cervical incision, separated from the cervical sympathetic and vagal nerves, and doubly ligated with silk sutures. Bilateral common carotid arteries were ligated in 25 NTR. After ligation, four NTR died spontaneously, and 21 were killed at varying intervals. Ligation of the arteries was performed in 61 SHR. After ligation, 54 SHR died spon-
taneously, and seven were killed. Sixteen out of 28 animals killed were perfused through the left ventricle with a buffered formaldehyde solution. The brains of 12 animals killed and of all the animals which died spontaneously were fixed by immersion, as described for those with intact carotid arteries. A histopathological examination of the brains of these experimental animals was performed.

Readily volatile water content of the supratentorial and infratentorial portions of the brain before and one and five hours after carotid occlusion was measured in NTR and SHR according to Feigin’s method.8

Results

The heart and body weight ratio was 0.33 ± 0.04 (mean ± SD) in NTR, and 0.40 ± 0.08 in SHR, showing a statistically significant difference (P < 0.001).

ANATOMY OF THE EXTRACRANIAL AND INTRACRANIAL ARTERIES

The common and internal carotid arteries and vertebral arteries were well developed in both groups of animals. The configuration of the circle of Willis did not differ in either group. There was a definite connection between the anterior cerebral arteries of both sides. The posterior cerebral artery branched off from the internal carotid artery. The posterior communicating artery was well developed in both NTR and SHR, connecting between the posterior cerebral artery and the superior cerebellar artery in most, or the basilar artery in some of these animals (fig. 1).

Table 1 presents the diameter of the arteries in NTR and SHR. Though statistically insignificant, the diameter of the cerebral arteries was somewhat smaller in SHR than in NTR. However, the ratio of the posterior communicating artery to the basilar artery was not different between the two groups.

STRUCTURE OF THE CEREBRAL BLOOD VESSELS AND BRAIN IN INTACT RATS

Extracerebral and intracerebral arteries, neurons, glial elements and white matter of NTR and SHR with intact carotid arteries were histologically normal in all sections examined. There was no fibrinoid necrosis or thickening of the wall of the cerebral arteries in either group. There were no histological artifacts, such as dark neurons, in the brains fixed by immersion or perfusion.*

Ultrastructure of the middle cerebral arteries, intracerebral and pial arterioles, and capillaries was normal in NTR and SHR, and showed no difference between the two groups. There were no abnormalities of the neurons and neuropils of the cerebrum (figs. 2 and 3).

BEHAVIOR OF EXPERIMENTAL ANIMALS

NTR and SHR occasionally had ptosis, and some NTR showed a marked difficulty in feeding themselves following

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**Table 1 Diameter of Cerebral Arteries**

<table>
<thead>
<tr>
<th>Region</th>
<th>NTR (μm)</th>
<th>SHR (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACA</td>
<td>124 ± 23</td>
<td>121 ± 17</td>
</tr>
<tr>
<td>MCA</td>
<td>147 ± 21</td>
<td>134 ± 32</td>
</tr>
<tr>
<td>ICA</td>
<td>216 ± 44</td>
<td>213 ± 54</td>
</tr>
<tr>
<td>PCA</td>
<td>134 ± 30</td>
<td>114 ± 20</td>
</tr>
<tr>
<td>P Com A</td>
<td>61 ± 11</td>
<td>57 ± 11</td>
</tr>
<tr>
<td>BA</td>
<td>203 ± 23</td>
<td>191 ± 31</td>
</tr>
<tr>
<td>Ratio: P Com A/BA</td>
<td>0.30 ± 0.05</td>
<td>0.30 ± 0.05</td>
</tr>
</tbody>
</table>


() = number of animals.

Values represent mean ± SD.

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**Figure 1** Base of the brain of an SHR with postmortem infusion of the arterial system with India ink suspension. The posterior communicating arteries (arrow) are well developed.

**Figure 2** Electron micrograph of the middle cerebral artery at its origin from a seven-month-old SHR with intact carotid arteries. No pathological changes in the endothelial cells (E) surrounding the vessel lumen (VL), internal elastic lamina (EL), smooth muscle cells (SM) in tunica media, and tunica adventitia (A). × 5,500.
FIGURE 3  Electron micrograph of the intracerebral arteriole in the neocortex from an eight-month-old SHR. No pathological changes in the endothelial cells (E), smooth muscle cells (SM), and neuropils. X 12,000.

carotid occlusion. Sixteen percent of the NTR died within the first 24 hours. Upon recovery from anesthesia, difficulty in walking and paucity of movement appeared in SHR following carotid occlusion. In several hours, irritation, circling, aggressiveness, repetitive paw lifting, respiratory distress and disturbance of consciousness occurred progressively. Seizures rarely occurred. Seventy-two percent of the SHR died within the first 24 hours.

GROSS APPEARANCE OF BRAIN AFTER CAROTID OCCLUSION

No macroscopic lesions appeared in NTR after carotid occlusion. Downward herniation of the inferior cerebellar vermis was seen in approximately one-half of the SHR which had severe neurological symptoms and died between five and 24 hours after carotid occlusion (fig. 4). At the time sectioning was done, such a brain occasionally contained focal and confluent petechial hemorrhages in the thalamus (fig. 5).

FIGURE 4  Herniation of the inferior cerebellar vermis in an SHR dying 12 hours after carotid occlusion (BCAL). The brain on the right is from an SHR with intact carotid arteries (CONTROL).

MICROSCOPIC APPEARANCE OF BRAIN AFTER CAROTID OCCLUSION

No cerebral lesions were detected in four NTR killed and three NTR which died less than five hours after carotid occlusion. Of three NTR killed between five and 24 hours, one had a large well-circumscribed area of pallor in the neocortex and the subjacent white matter of the bilateral middle and anterior cerebral artery territories, and another had small pale areas in the neocortex of the middle cerebral artery territory. These lesions consisted of neurons showing shrinkage and eosinophilia of the cytoplasm and smudgy deep purplish staining of the nuclei, which were surrounded by empty perineuronal spaces. Some neurons were pale and swollen. There were no detectable lesions in the remaining NTR killed.

There were no detectable lesions in one NTR which died during this period.

Of 11 NTR killed between 24 hours and five days, one had a large well-circumscribed area of pallor in the neocortex and the subjacent white matter of the unilateral middle cerebral artery territory (fig. 6), four had multiple small lesions in the neocortex and subcortical white matter (fig. 7), and six had no detectable lesions. The lesions showed more advanced neuronal changes and vacuolation of neuropils than in those animals killed earlier. Polymorphonuclear leukocytic infiltration and macrophages were not seen. The margin of the lesions occasionally showed prominentvacuolation of neuropils and dark shrunken or pale swollen neurons. The small lesions appeared frequently in the middle cerebral artery territory, and occasionally in the anterior cerebral artery territory. The paramedian region, which is the watershed zone between the anterior and middle cerebral arteries, was a site of predilection of these small lesions.

Three NTR killed seven days after carotid occlusion showed no detectable lesions. Lesions never appeared in an area other than the neocortex and subcortical white matter in NTR following carotid occlusion.

Of 14 SHR dying during the five hours after carotid occlusion, four showed some sponginess at the center of the
thalamus, while no lesions were detectable in ten. There were no detectable lesions in two SHR killed during this period.

Of 37 SHR dying between five and 24 hours, 12 showed diffuse and extensive pallor and sponginess of the neocortex. The lesions consisted of neurons showing shrinkage and eosinophilia of the cytoplasm and smudgy deep purplish staining of the nuclei, and empty perineuronal spaces. Pale swollen neurons were seen occasionally. The cortical lesions were bilateral and diffuse throughout the cerebrum, involving the frontal, medial and occipital regions. These changes showed a tendency to appear more advanced in the paramedian region, but rarely appeared in the basal aspect of the cerebrum. Of these 37 SHR, a large well-circumscribed area of sponginess of the tissue appeared in the thalamus in 29, among which four also had multiple small hemorrhages (fig. 8). The sponginess of the tissue in the thalamus was large and round, usually symmetrical, at the center of the thalamus on coronal section through the optic chiasm. Parasagittal section of these SHR showed a margin of the sponginess which was perpendicular from the base of the hypothalamus and curved caudally in the thalamus (fig. 9). The sponginess of the tissue was more prominent toward the periphery of the lesion, where dark shrunken and pale swollen neurons were seen. Beyond the lesion, the tissue abruptly returned to an almost normal appearance (fig. 10). Ten of these 37 SHR demonstrated an association of the lesions in both the neocortex and thalamus. The remaining six among these 37 SHR showed no detectable lesions.

Of five SHR killed between five and 24 hours, two had diffuse and extensive changes of the neurons and neuropils in the neocortex, and three had sponginess of the tissue in the thalamus. There was an association of the lesions in the neocortex and thalamus in one animal, and the remaining animal had no detectable lesions.

Of three SHR dying between 24 hours and five days, two had diffuse changes of the neurons and neuropils in the neocortex. There were neuronal changes in the unilateral Sommer sector in one. The lesions of the neocortex in this period were more advanced than in those animals killed earlier (figs. 11 to 13). No macrophages were seen in the tissue. Sponginess of the tissue in the thalamus was seen in one animal. The remaining one had no detectable lesions.

Except for the sponginess of the tissue in the thalamus, lesions were not detectable in the striatum and thalamus in SHR after carotid occlusion. Thrombosis or fibrinoid necrosis of the blood vessels was not observed in the brain after carotid occlusion. There were no histological abnormalities in the infratentorial structures of the brain in both NTR and SHR.

WATER ANALYSIS OF BRAIN AFTER CAROTID OCCLUSION

SHR showed a significant increase of water content of the supratentorial portion of the brain five hours after carotid occlusion, while there was no change of the value after one hour. NTR showed no change of the values in one and five hours after carotid occlusion. There was no change of the water content of the infratentorial portion of the brain in either group (table 2).
Table 2  Water Content of Supratentorial and Infratentorial Portions of the Brain Following Carotid Artery Ligation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>1 Hour</th>
<th>5 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 7)</td>
<td>(n = 7)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>NTR Supratentorial</td>
<td>78.3 ± 0.7</td>
<td>78.5 ± 0.6</td>
<td>78.4 ± 0.4</td>
</tr>
<tr>
<td>NTR Infratentorial</td>
<td>75.5 ± 1.1</td>
<td>75.5 ± 1.0</td>
<td>75.6 ± 0.7</td>
</tr>
<tr>
<td>SHR Supratentorial</td>
<td>78.4 ± 1.0</td>
<td>78.4 ± 0.9</td>
<td>79.6 ± 1.1*</td>
</tr>
<tr>
<td>SHR Infratentorial</td>
<td>75.9 ± 0.4</td>
<td>75.7 ± 0.4</td>
<td>75.7 ± 0.4</td>
</tr>
</tbody>
</table>

*P < 0.05.
Values are percent of wet weight ± SD.

were slight. The cerebral lesions were mostly small and they appeared only in 39% of NTR killed more than five hours after carotid occlusion. There were no herniation of the vermis and no increase of water content of the brain. On the other hand, after carotid occlusion in SHR, severe neurological symptoms were present and the cerebral lesions were diffuse and extensive. Herniation of the vermis appeared often and water analysis revealed a significant increase in the supratentorial portion of the brain. Cerebral lesions were seen in 82% of SHR killed more than five hours after carotid occlusion.

Of 14 SHR dying five hours after carotid occlusion, there were no histological abnormalities in the brains of ten. The significant increase of water content in the supratentorial portion of the brain in SHR during the five hours after carotid occlusion (not apparent in one hour) suggests that ischemic brain injury had evolved in this period, and later became histologically manifest.

These results indicate that the high mortality following carotid occlusion in SHR2,3 may be closely related to the severe cerebral infarcts. Surgical intervention and circulatory disturbance in the external carotid artery distribution, of course, might play some role in this mortality.

The circumscribed lesions in NTR frequently had a marginal zone12 consisting of prominent vacuolation of neuropils and dark shrunken or pale swollen neurons. In SHR the lesions of the neocortex were very diffuse and rarely showed a marginal zone. The sponginess of the tissue in the thalamus of SHR could be interpreted to be the...
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FIGURE 12  Neocortex of the SHR shown in figure 11. There are irregular and diffuse loss of neurons and sponginess of the tissue. Hematoxylin and eosin, × 55.

FIGURE 13  Higher magnification of figure 12, demonstrating dark and shrunken neurons and empty perineuronal spaces. Hematoxylin and eosin, × 280.

Marginal zone, which corresponds to the watershed zone between the carotid and vertebrobasilar systems. These might indicate that reduction of cerebral blood flow was significant in the whole internal carotid artery distribution in SHR, while that in NTR developed in a more peripheral portion of the arterial tree.

The present experiment on SHR is reminiscent of a study using gerbils 13 which had cerebral infarction following unilateral carotid artery ligation. This is attributed to the lack of connecting arteries between the carotid and vertebrobasilar systems, 14 and to the inadequacy of communication between the anterior cerebral arteries. 15'16 However, this is not the case in SHR.

Due to the bilateral ligation of the common carotid arteries, the posterior communicating arteries are considered to be the major anastomotic channels for blood circulating toward the area irrigated by the internal carotid arteries. The anatomical study of the cerebral arteries revealed no anomaly of the posterior communicating arteries in either NTR or SHR. Although the diameter of the cerebral arteries of SHR was somewhat smaller than that of NTR, the ratio of the posterior communicating artery to basilar artery was not different between NTR and SHR, thereby demonstrating that the posterior communicating artery was not unproportionally small in comparison with the other cerebral arteries in SHR. It might be concluded that there is no difference of the posterior communicating artery between NTR and SHR to support the difference of the brain pathology after carotid occlusion between the two groups. Morphological changes of the blood vessels of the brain secondary to hypertension were not observed in SHR. The normalcy of the blood vessels makes it logical to assume that the cerebral lesions in SHR can be attributed to the hemodynamic rather than the morphological difference between the two groups.

Fujishima et al. 2 have shown that the lower blood pressure limit of cerebral autoregulation was 75 mm Hg in SHR and 50 mm Hg in NTR. They also have shown that, after carotid occlusion, the carotid back flow pressure was markedly lowered and remained unchanged in response to changes in systemic blood pressure in SHR. In NTR the relationship between carotid back flow pressure and systemic blood pressure was essentially the same as that before the occlusion. Eklöf and Siesjö 17 reported that bilateral ligation of the carotid arteries in NTR reduced the cerebral blood flow to almost 50% of the normal value. Cerebral blood flow in SHR might fall far below those reported in NTR. Therefore, the difference of the cerebral autoregulation may explain the diffuse and extensive cerebral infarction observed in SHR after carotid occlusion.

The diffuse and extensive cerebral infarcts in SHR correspond well to the marked increase in brain lactate and the lactate-pyruvate ratio. 18'19 On the other hand, in NTR these metabolites did not increase as in SHR, compatible to the pathological findings consisting of occasional small cerebral infarcts.

Cerebral infarction develops more frequently in individuals with hypertension than in those with normal blood pressure. 18'20 This usually has been attributed to the occurrence of more severe vascular changes in hypertensive humans. Considering the upward shift of the lower blood pressure limit of cerebral autoregulation in severely hypertensive patients, 21 the present experiment suggests that hypertension per se, i.e., hemodynamic factors, might be operative for the development of cerebral infarction, in addition to the associated vascular changes.

References
5. Holt SJ, Hicks KM: Studies on formalin fixation for electron microscopy
Platelet Survival Studies in Stroke-Prone Spontaneously Hypertensive Rats (SHRSP)

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SUMMARY Platelet survival was studied by using "Cr-labeled platelets in stroke-prone spontaneously hypertensive rats (SHRSP), stroke-resistant SHR (SHRSR), and normotensive control rats of the Wistar-Kyoto (WK) strain. Relatively young animals of the same age prior to the development of cerebrovascular lesions (cerebral infarction and/or hemorrhage) were used. Platelet half-life time in SHRSP was slightly but significantly shorter than in any other groups of rats, irrespective of the type of platelet donors. Mean platelet consumption was also significantly increased in SHRSP only. Platelets of SHRSP injected into SHRSR showed normal survival. These data support the concept that the shortened platelet survival in SHRSP is brought about by some extracorporeal abnormalities. Although the vascular changes in SHRSP could be the most likely explanation for the shortened platelet survival, its mechanism remains to be solved. This investigation suggests that studies of the platelet survival in hypertension may be useful in predicting the development of stroke before its clinical recognition.

Introduction

THE STROKE-PRONE spontaneously hypertensive rats (SHRSP) in which cerebrovascular lesions (cerebral infarction and/or hemorrhage, or stroke) would spontaneously develop were established by a proper successive selective breeding of the SHR in which stroke developed spontaneously.1 The involvement of genetic factors in the development of stroke in the rat was postulated from observations on its familial occurrence.2 SHRSP thus selected at the Department of Pathology, Faculty of Medicine, Kyoto University, were the offspring of one or both parents who had died from stroke for the past eight or ten generations, and their spontaneous incidence of stroke was more than 80%. Studies on the mechanisms of stroke up to the present indicated that hypertension was an important systemic factor for stroke,1,3 while local factors such as recurrent branching and boundary zone were similar to those of stroke in man.4 Therefore, SHRSP were expected to be useful as the best animal model for human stroke.

Attempts to develop hematological methods which can anticipate the development of thrombosis or detect occult thrombotic disease before it is recognized clinically have been mostly fruitless. Tests for detecting alterations in platelet function and blood coagulation cannot reliably indicate a prethrombotic state and may reveal abnormalities following the development of thrombosis.5 It has been well known that the platelet is not only related to blood coagulation but plays an important role in the maintenance of vascular integrity and in disease processes.6-8 Importance and primary role of the platelet in the production of arterial thrombi are well established.6 Studies of platelet survival and turnover are virtually the only available measures of the activity of the platelet in vivo, and may provide some information on vascular disease and thrombosis as well as on the fate of the platelets in circulation. Therefore, the present investigation was undertaken to evaluate platelet survival and consumption in the relatively young SHRSP, which were thought to be in the prethrombotic state and were destined to have a stroke spontaneously in the future, and stroke-resistant SHR (SHRSR), in which the incidence of stroke was less than 10%; normotensive rats of Wistar-Kyoto (WK) strain were used as the control.
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