SPECIES VARIATIONS IN VASCULAR REACTIVITY/Sasaki et al


A VARIETY OF ISCHEMIC BRAIN MODELS have been developed to study ischemic brain disorders, including our own dog models. Each model has its own merits and demerits, and the selection of a suitable model often holds the key to the success of a study. Large animals, such as the dog, are not the model of choice when conducting autoradiographic studies because they need much dose of an expensive radioisotope. Undoubtedly, small animals like the rat serve as a better model in such cases. The use of small rodents in the study of brain ischemia, however, has been often hampered by technical difficulties and inconsistent results. The recent development of a four-vessel occlusion model provided a new scope for the study of ischemic rats as a model of global ischemia. The relative ease of the procedure has resulted in wide acceptance of this model. The inconsistent results obtained with this model, however, were debated in a recent issue of this journal.

In this communication, we describe experimental brain ischemia produced in rats by occlusion of the common carotid arteries and the basilar artery (designated as a three-vessel occlusion model). This method results in better control of the cerebral blood flow than occlusion of carotid and vertebral arteries. The results obtained in this model are compared with those obtained by the four-vessel occlusion model.

A New Model of Bilateral Hemispheric Ischemia in the Rat — Three Vessel Occlusion Model

MOTONOBU KAMEYAMA, M.D., JIRO SUZUKI, M.D., REIZOU SHIRANE, M.D., AND AKIRA OGAWA, M.D.

SUMMARY A new model of bilateral hemispheric ischemia was created in the rat by occluding the common carotid arteries and the basilar artery; this resulted in consistent and severe impairment of the cerebral blood flow. The procedure for producing this model is described, and the results of EEG and autoradiography obtained by this model are compared to those obtained by the four-vessel occlusion model.

Materials and Method

1. Production of Animal Models

Male Wistar rats, weighing 200-250 gm, were used throughout the experiments. Under light halothane anesthesia, a 3 cm skin incision was made on the anterior neck region and a subsequent operation was performed using a surgical microscope.

After dissecting the sternohyoid and omohyoid muscles, the base of the occipital cranium, where the muscles longus capitis terminates, was exposed. A small bone window (3 x 3 mm) was opened, and through the window, the basilar artery was freed from the adjacent tissue, electrocauterized and severed (transcervico-transclival approach). The wound was closed and the rats were allowed to awaken.

On the day following occlusion of the basilar artery, the rats were subjected to occlusion of the common carotid arteries after the absence of neurological deficits had been confirmed. Approximately 20-30% of rats revealed neurological deficits following the basilar artery occlusion. The neck wound was reopened under halothane anesthesia, the trachea was intubated, and the rats were immobilized with pancuronium bromide. Controlled respiration was instituted using a Harvard Rodent Respirator (Model 681). The common carotid arteries were then clipped bilaterally (referred to hereafter as the three-vessel occlusion model) (fig. 1).

In some rats, an additional surgical procedure assured the blockade of blood flow to the brain through collateral pathways. A thread was carefully passed around the neck sparing the jugular veins, the vagus nerves and the trachea, and the loop of the thread was approximated with caution so as not to disturb venous blood flow and respiration (referred to as the four-vessel occlusion + neck ligation model).

For comparative studies, a group of ischemic rats
FIGURE 1. Site of vascular occlusion of the three-vessel occlusion model.

was prepared according to the method of Pulsinelli and Brierley. In brief, electrocauterization of the bilateral vertebral arteries was followed on the next day by bilateral clipping of the common carotid arteries (referred to as the four-vessel occlusion model). For all animals in these three groups, blood pressure (Telemed, ICP 77030), arterial blood pH, PO$_2$, and PCO$_2$ (Instrumental Laboratory System 1303, AVL 939) and body temperature (San'ei rectal thermister) were monitored and maintained within physiological limits.

2. EEG Recording

Epidural electrodes were placed over the fronto-parietal region of the rat brain to monitor electrical activities of the brain. EEGs were recorded in 20 rats prepared for three-vessel occlusion, 6 rats for three-vessel occlusion + neck ligation and 20 rats for four-vessel occlusion, using a Nihon Koden Electrocencephalograph (Model 7902-S).

The electrical activity of ischemic brains was classified into four groups based on EEGs taken one minute after the completion of the vessel occlusion: i) No change, where the activity was similar before and after the vascular occlusion, ii) Moderate, where decreases in both voltage and frequency were apparent, iii) Severe, where a marked decrease in voltage was accompanied by very low frequency, and iv) Flat, in which virtually no electrical activity was detected (fig. 2).

3. Autoradiography

One minute after clipping the common carotid arteries, 100 µCi/Kg of $^{14}$C-iodoantipyrine (IAP) (Amer- sham) was injected into the femoral vein according to the method of Sakurada et al. Immediately following the injection, rats were decapitated and brains were quickly frozen. 20 µm thick frozen sections were made with a cryostat (American Optical, HISTOSTAT). Cerebral blood flow images were obtained by 10 days exposure to X-ray film (Kodak, NMC-1). For calibration, $^{14}$C standards (Amersham) were included. The autoradiographic study was performed on 11 rats from each of the three groups.

The degree of darkening of the X-ray film was quantitatively assessed using a densitometer (Sakura, PDA-15). After calculating the regional radioactivity from the $^{14}$C standards at each site of the same film, relative cerebral blood flow was estimated from the ratio of regional radioactivity of the fronto-parietal cortex to that of the cervical spinal region, which was assumed not to be affected by the vascular occlusion. When the densitometer could not detect a difference from the background activity, blood flow at the region was assumed to be zero.

As a control, sham operations, in which a bone

Patterns of EEG Changes

control

no change

moderate

severe

flat

FIGURE 2. Patterns of EEG changes following ischemia. Moderate: both voltage and frequency decreased. Severe: electrical activity further decreased but was still preserved. Flat: electrical activity virtually disappeared.
BILATERAL HEMISPHERIC ISCHEMIA IN THE RAT
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TABLE 1  EEG Changes Following Ischemia

<table>
<thead>
<tr>
<th>Rat model</th>
<th>No change</th>
<th>Moderate</th>
<th>Severe</th>
<th>Flat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Four-vessel occlusion model</td>
<td>1</td>
<td>7</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>(n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three-vessel occlusion model</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>19</td>
</tr>
<tr>
<td>(n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Three-vessel occlusion + neck</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>ligation model</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 6)</td>
<td></td>
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</tr>
</tbody>
</table>

*p < 0.001.

window was opened and the basilar artery was exposed but not occluded, were performed in three rats.

4. Hydrogen Clearance

Regional cerebral blood flow in the parietal cortex was measured in 10 rats prepared for three-vessel occlusion by the hydrogen clearance method. A needle type platinum electrode 0.3 mm in diameter insulated except for the final 0.3 mm was implanted into the parietal cortex through a small bone window. The value of regional cerebral blood flow was determined from the initial slope method of the clearance curve following 3 minutes of inhalation of 5-10% hydrogen gas.

Results

1. EEG

The results of EEG recordings are summarized in table 1. Among 20 rats that underwent three-vessel occlusion, the EEG was flat in all rats but one. Likewise, among 6 rats of three-vessel occlusion + neck ligation, 5 were flat and one was severe. In contrast, 20 rats that had undergone four-vessel occlusion showed a variety of EEG changes. In only 12 out of 20 rats were EEGs either flat or significantly slow. Seven showed moderate change and one was unchanged. The difference between the three-vessel occlusion group and the four-vessel occlusion group was statistically significant (p < 0.001) as measured by a chi square test.

2. Cerebral Blood Flow Images Obtained from Autoradiography

In the three-vessel occlusion group, almost no \(^{14}\)C-IAP activity was detected in the cerebrum and cerebellum of 9 out of 11 rats, indicating severe ischemia everywhere except the brainstem (fig. 3,b). In the remaining two rats, mild or moderate \(^{14}\)C-IAP accumulation was found in the brain (fig. 3,c). Almost no radioactivity was seen outside the brainstem in all 11 rats belonging to the three-vessel occlusion + neck ligation group.

In contrast, various degrees of radioactivity were found in the cerebrum and cerebellum of 11 rats from the four-vessel occlusion group. In some rats, radioactivity was as high as that of the control, although in others, only a trace could be found, suggesting a wide variation in the severity of ischemia. In 2 rats with flat EEGs, a substantial amount of radioactivity was found in the brains, especially high in the thalamic region (fig. 3, d and e).

With regard to the relative CBF (i.e., the ratio of blood flow of the fronto-parietal region to that of the unaffected upper cervical cord), the control group had a ratio of 118.3 ± 20.1%, the three-vessel occlusion group had one of 6.4 ± 14.9%, the three-vessel occlusion + neck ligation group had one of 0 ± 0%, and the four-vessel occlusion group had one of 61.6 ± 38.6%. The difference between the two three-vessel occlusion groups and the four-vessel occlusion group were highly significant (in both p < 0.001) as measured by a Student's t test (fig. 4).

3. Hydrogen Clearance

In all 10 rats that underwent three-vessel occlusion, the intracranial diffusion of hydrogen was insufficient in quantity to define a clearance curve.

Discussion

The rat has been widely used in experimental brain ischemia for a number of reasons: i) It is easy to handle and the surgical procedures can be carried out in a short period of time. ii) It is large enough to monitor physiological parameters such as blood pressure, blood gases and EEG. iii) It has been the source of a great deal of

![Figure 3. \(^{14}\)C-IAP autoradiography. a) control, b,c) three-vessel occlusion model and three-vessel occlusion + neck ligation model. This type of severe ischemia (b) was seen in 9 out of 11 three-vessel occlusion and all 11 three-vessel occlusion + neck ligation animals, though residual blood flow (c) was detected in the remaining 2 rats undergone the three-vessel occlusion. d,e) four-vessel occlusion model with flat EEG showing the highest grade of ischemia. Note the preservation of residual blood flow particularly in the thalamic region.](https://example.com/image)
Among various rat models for global brain ischemia, the four-vessel model of Pulsinelli and Brierley and of Kägstrom et al.,10 and the compression ischemia model of Ljunggreen et al.,4 have gained popularity, although each model entails problems which limit their usefulness. For example, the injection of artificial CSF in the compression ischemia model increases intracranial pressure to the level of 40 mm Hg above the normal systolic pressure; analysis of the results obtained by this technique is complicated by the effects of increased intracranial pressure on brain tissue. Conversely, a constant level of ischemia in Kägstrom’s model can be attained only when the vascular occlusion is accompanied by lowered blood pressure.

In contrast, the four-vessel occlusion model of Pulsinelli and Brierley is relatively easy to produce and it does not require the manipulation of systemic blood pressure or intracranial pressure. This model has been used in a variety of experimental studies.14-16, 20-22 However, as Pulsinelli and Brierley have admitted themselves a constant level of ischemia in this model is attained only when the animals are selected based on neurological signs after occlusion of the vessels.6 We also found considerable variation in the degree of ischemia in this model as presented in the Results. The principal reasons for inconsistency of the ischemic state in this model appear to be: i) the difficulty in confirming complete electrocauterization of the vertebral artery and ii) the existence of collateral pathways mainly from the anterior spinal artery.

The occlusion of the basilar artery in our model not only allows for verification of the occlusion of the trunk arteries to the brain, but also prevents collateral circulation from the anterior spinal artery, and thus provides a constant level of ischemia. All the parameters used in the present study clearly indicate that cerebral blood flow is consistently and severely impaired in rats after occlusion of the three major feeding arteries to the brain. Of 20 rats subjected to the three-vessel occlusion, 19 showed flat EEG records, and in our autoradiographic study, cerebral blood flow was virtually absent in 9 out of 11 rats. Moreover, the measurement of blood flow in the parietal cortex by a hydrogen clearance method demonstrated the immeasurably low cerebral blood flow in all 10 rats examined. When three-vessel occlusion was followed by ligation of the neck, autoradiographical image analysis disclosed the development of severe ischemia in the entire brain, sparing only the brain stem, in all 11 rats. It should be kept in mind, however, that the neck ligation procedure has a potential risk of including autonomic disorders. Thus, the application of neck ligation depends on the type of experiment being planned.

Nevertheless, three-vessel occlusion with or without neck ligation offers consistent results without additional manipulation or selection of the animals. It is particularly important to have such models when the temporal sequence of ischemic events is studied. The usefulness of this model has already been shown in the study of sequential changes in the astrocytic cell membrane by a freeze fracture technique.23 This model of ischemia may be useful to study the sequence of events taking place during acute ischemia and after restoration of blood flow.

Acknowledgments
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References
A Model of Selective Experimental Ischaemia in the Primate Thalamus

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SUMMARY A model for studying changes in local CBF and evoked potentials in selective thalamic ischaemia has been developed. The arterial supply to the posterior thalamus (mainly from the posterior choroidal arteries) was occluded in the baboon using a transorbital approach to the region of preoptic and ambient cisterns. Local CBF was measured by the hydrogen clearance method using electrodes introduced into the nucleus ventralis posterior lateralis of thalamus as well as cortex on both sides. The production of focal ischaemia was demonstrated by a significant decrease in thalamic CBF and confirmed by examination of the brain perfused with carbon particles.

Despite continuing advances in cerebrovascular research and increased understanding of brain function under ischaemic conditions, there is still a fundamental need for reliable animal models of focal ischaemic brain regulated with the perfusion method. Surg Neurol 16: 167–172, 1981


13. Kayama T, Mizos K, Suzuki J: A canine model of a completely cerebral ischemia. The first models were developed to investigate the most common clinical cerebrovascular lesions and used occlusion of the relevant arteries in different animals. Carotid and particularly middle cerebral occlusions made it possible to achieve a better understanding of changes of brain function in response to ischaemia such as development of brain oedema, failure of autoregulation and the threshold of different dysfunctions occurring after cerebral ischaemia. Middle cerebral occlusion in particular simulates a clinical stroke in most respects. The predominant injury, however, is cortical with an admixture of caudate and putaminal damage and considerable loss of substance in...
A new model of bilateral hemispheric ischemia in the rat--three vessel occlusion model.
M Kameyama, J Suzuki, R Shirane and A Ogawa

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