A New Model of Brainstem Ischemia in Dogs

Satoshi Kuwabara, MD, Junji Uno, MD, and Susumu Ishikawa, MD

A new model of brainstem ischemia in dogs is described. The perforating arteries arising from the posterior cerebral arteries as far as the bilateral junctions with the posterior communicating arteries were occluded using a subtemporal approach to the region of the interpeduncular cistern. Infarction of the posterior thalamus, subthalamus, midbrain, and upper pons was consistently produced. The dogs survived for > 1 week while exhibiting such clinical symptoms as disturbance of consciousness, tetraparesis, oculomotor paralysis, respiratory abnormalities, bradycardia, and arrhythmia. The clinical features and extent of infarction observed in this model closely resemble those of rostral brainstem infarction in human stroke. The model may be useful in studying the pathophysiology of brainstem ischemia. (Stroke 1988;19:365–371)

Experimental studies of cerebral ischemia have recently made remarkable progress with the introduction of new methods, development of different ischemic stroke models, and improvement in the quantitative analysis of physiologic and biochemical measurements that permit more precise and detailed studies. The majority of investigations to date have primarily assessed the carotid system, especially the middle cerebral artery territory. Few studies have used the vertebrobasilar system and brainstem, leaving uncertainty as to whether the pathophysiologic processes differ in this vascular territory. We have developed a model of brainstem ischemia in dogs by selectively occluding the perforating arteries supplying the rostral brainstem. Our model invariably produces infarction in the posterior thalamus, subthalamus, midbrain, and upper pons.

This article describes our method for producing this model and the neurologic symptoms, changes in vital signs, and extent of infarction observed.

Materials and Methods

Twenty-four mongrel dogs (8–14 kg) were anesthetized with 25 mg/kg i.v. sodium pentobarbital and immobilized with 0.1 mg/kg i.v. pancuronium bromide. The dogs were then intubated and ventilated with a Harvard respirator (South Natick, Massachusetts). Cannulas were inserted into femoral vessels. PaO₂, PaCO₂, arterial pH, and rectal temperature were monitored and altered when required. Electrocardiogram, systemic blood pressure, and respiratory movement were simultaneously recorded throughout the operation and postoperatively for 20 hours.

The dog was placed in the left lateral position. After removal of the right zygomatic arch, a 2 × 2 cm temporal craniectomy was made as close as possible to the base of the skull. The dura was opened, and the posterior half of the circle of Willis was approached by gently retracting the temporal lobe upward with the use of an operating microscope. Following microdissection of the arachnoid of the interpeduncular cistern, cerebrospinal fluid was gently aspirated. The free margin of the tentorium was then cut, and the apex of the petrous bone was removed using a high-speed drill. This surgical procedure provided visualization sufficient to approach the ventral aspect of the midbrain and upper pons. Bilateral exposure was produced of the posterior communicating, posterior cerebral, and superior cerebellar arteries and the basilar artery. The arachnoid over these vessels was dissected. In 20 dogs, 8–10 perforating arteries arising from both posterior cerebral arteries to their junction with the posterior communicating arteries were carefully coagulated and cut (Figure 1). Care was taken to avoid vasospasm or stenosis of the parent arteries. Systemic blood pressure was maintained within the normal range during the operation.

Sham Operation (Four Dogs)

The perforating arteries were exposed and manipulated but not occluded. The dogs were maintained for 1 week. These dogs were used as the control group.

Acute Experiments (Six Dogs)

Immediately after occlusion of the perforating arteries, the dog was heparinized. The chest was opened, the ascending aorta was cannulated, the descending aorta was ligated, the superior vena cava was cut, and the brain was perfused at 120 mm Hg pressure with 1,200 ml 6% microcarbon (6% carbon black) in 800 ml formalin or 500 ml microbarium (50% barium sulfate) in 5% gelatin. For microbarium perfusion, the brain was perfused first with 2,000 ml normal saline. The brain was then removed and fixed in 10% formalin for 1 week. Sagittal and/or serial coronal sections were prepared. The extent of defective perfusion indicated by microcarbon was studied in each brain section, and microangiograms of the coronal sections were prepared to examine the structural alterations in the microvasculature indicated by microbarium.
whereas the lesion in the latter dogs similarly extended, but from the midportion of the bilateral cerebral peduncles (Figures 3, bottom and 4). Disturbances of consciousness were related in general to the total size of the infarct. Persistent motor disturbances were related to the extent of infarction of the cerebral peduncles. Neural structures that were either partially or entirely infarcted included the medial dorsal nucleus, central medial nucleus, and paraventricular nucleus of the thalamus, the subthalamic nucleus, oculomotor nucleus, trochlear nucleus, interpeduncular nucleus, red nucleus, substantia nigra, dorsal tegmental decussation, medial longitudinal fasciculus, decussation of the superior cerebellar peduncle, the cerebral peduncle, pontine nuclei, and pontine fibers.

Histopathologic changes. Macroscopic observation showed anemic infarction in 10 dogs and hemorrhagic infarction in four. Microscopic examination revealed numerous macrophages with eccentric nuclei and foamy cytoplasm in the infarct. Ischemic changes in nerve cells, capillary proliferation, and extravasation of microcarbon were observed at the edge of the infarct. In the four dogs with hemorrhagic infarction, many extravascular red cells and macrophages containing hemosiderin were present chiefly at the edge of the infarct.

Discussion

Two major approaches are available and mainly used for production of experimental models of focal cerebral...
ischemia: extravascular arterial occlusion by clips, ligatures, or electrocautery; or intravascular occlusion by the intra-arterial injection of various embolic materials. These two approaches have also been applied to the production of experimental ischemia in the brainstem. However, few models thus produced were satisfactory in reproducibility of the lesion, clinical effects, and survival rate after the surgical procedure.

Extravascular occlusion of the basilar artery at various levels by the subtemporal or transclival approach in dogs, cats, or rabbits did not result in neurologic deficits or infarction. Recently, Yamada et al reported a model of hindbrain ischemia involving occlusion of the vertebrobasilar junction in gerbils. However, the model is essentially not that of brainstem ischemia because of the involvement of the cerebellum, and furthermore, long-term follow-up studies may not be possible. For intravascular methods, iron powder or air foam was injected into the vertebral artery of dogs, killing the majority soon after the procedure due to severe and multifocal ischemia of the brainstem. Oki et al produced brainstem infarction in dogs by segmental embolization of the basilar artery using a silicone rubber cylinder. However, unlike the middle cerebral artery, the vertebrobasilar arterial system of dogs has many branches as well as normal variations. The site of occlusion, therefore, is variable, and a
degree of variation in lesion size and location is inevitable with this embolic method.

We selectively occluded only the perforating arteries supplying the rostral brainstem while preserving the circulation of the main cerebral arteries and succeeded in producing infarction, almost uniform in both location and size, involving the posterior thalamus, subthalamus, midbrain, and upper pons in all the chronic dogs. This method has a high reproducibility because in dogs, first, the perforating arteries of the brainstem, with diameters of up to 20 μm, are usually end arteries with no anastomoses in the brain parenchyma; second, the posterior perforating arteries originating from the basilar bifurcation and paramedian branches arising from the proximal portion of the posterior cerebral artery supply the median and paramedian regions of the posterior thalamus, midbrain, and upper pons; and finally, the distribution of perforating arteries of the brainstem has the same zone pattern as in humans.

Yonas et al have shown in baboons that selective occlusion of the lateral lenticulostriate arteries consistently results in infarction of the caudate, putamen, and anterior limb of the internal capsule. Vajda et al also in baboons produced an ischemic lesion confined to the posterior thalamus by occluding the thalamoperforating and posterior choroidal arteries. These results suggest that infarction with a uniform effect can be
Acute Group

Bilateral junctions with posterior communicating arteries were arteries arising from posterior cerebral arteries as far as occluded perforating arteries in dogs. Only perforating arteries in dogs were occluded.

Chronic Experiments (14 Dogs)

A surgical procedure identical to that in the acute experiments was carried out under sterile conditions. The dural opening was covered with the temporalis muscle fascia, and the wound was closed in three layers. All dogs received transfusion and 20 mg/kg cefalotin sodium and were carefully examined daily for 7–10 days for neurologic alterations and changes in vital signs. Each dog was subsequently subjected to microcarbon perfusion. Coronal sections of the fixed brain were stained with hematoxylin and eosin for histopathologic examination.

Results

Control Group

All the sham-operated dogs recovered without any neurologic deficit and showed no evidence of pathologic lesions. Microangiographic study of the rostral brainstem revealed good filling of the paramedian and circumflex arteries, which arise from the basilar and posterior cerebral arteries. These perforating arteries appeared to converge into the mesencephalic aqueduct (Figure 2, top).

Acute Group

The area showing no perfusion of microcarbon centered around the midbrain, extended from the posterior thalamus to the upper pons, and was wedge-shaped at the level of the midbrain and upper pons. Microangiograms showed no paramedian perforating arteries and a wedge-shaped area of nonfilling (Figure 2, bottom), which corresponded to the extent of infarction observed in the chronic experiment dogs.

Chronic Group

One dog died from generalized seizures within 72 hours after occlusion of the perforating arteries; the remaining 13 dogs survived for 7–10 days. Neurologic symptoms and changes in vital signs were examined in these 13 dogs. The brains of all 14 dogs were examined for histopathologic changes.

Neurologic symptoms. The 13 dogs could be divided into those with severe neurologic deficits (seven dogs) and those with moderate neurologic deficits (six dogs). All six dogs with severe neurologic deficits remained recumbent in a semicomatose state without recovery from anesthesia for >1 week. Spastic tetraparesis was also observed in all six dogs, abnormal salivation in four, and transient nystagmus in the horizontal plane in three. On the other hand, all seven dogs with moderate neurologic deficits showed spontaneous running movements of all extremities in the lateral recumbent position from 10 hours after the occlusion. As consciousness gradually improved, the dogs with moderate deficits made apparent efforts to gain standing posture, but none could stand or walk because of mild motor weakness and stiffness of all limbs. These dogs frequently assumed an abnormal posture of turning the head to the left, flexing the forelimbs, and extending the hindlimbs in response to painful stimuli. These neurologic deficits, including disturbances of consciousness ranging from somnolence to confusion, persisted over the entire period of observation, with some degree of improvement in all seven dogs. All 13 surviving dogs in the chronic group showed paralysis of both oculomotor nerves, and two dogs with moderate neurologic deficits had four or five attacks of general tonic convulsions.

Changes in vital signs. The systemic blood pressure, measured for 20 hours after occlusion, failed to show any remarkable change in any of the 13 dogs. Respiratory abnormalities were noted in eight dogs, of which two exhibited hyperpnea (30–60 breaths/min) for 9 hours after occlusion. The remaining six dogs had irregular breathing of Cheyne-Stokes pattern for 10–17 hours after occlusion. Normal respiration was eventually restored in all eight dogs. In the 10 dogs that showed both bradycardia and arrhythmia, the heart rate began to decrease from 6 hours after occlusion, with sinus arrhythmia, and remained at 40–60 beats/min after 24 hours. All six dogs with severe neurologic deficits showed respiratory disorders, bradycardia, and arrhythmia.

Location and size of infarction. Gross pathologic examination of the brain sections revealed well-defined infarctions that were consistently located in the posterior thalamus, subthalamus, midbrain, and upper pons in all 14 chronic dogs (including the dog that died within 72 hours after occlusion) (Figure 3). The lesion was butterfly-shaped in the paramedian portion of the posterior thalamus and subthalamus. At the level of the midbrain and upper pons, the infarction was symmetrically wedge-shaped, with the apex at the mesencephalic aqueduct and the base in the ventral portion of the midbrain and upper pons (Figure 4). The location and shape of the lesion were uniform in all dogs, but lesion size tended to increase with clinical severity of the neurologic deficits. At the level of the posterior thalamus, the size of the lesion in the dogs with moderate neurologic deficits was about two-thirds that in the dogs with severe neurologic deficits. At the midbrain level, the infarct in the former dogs extended from the inner third of the bilateral cerebral peduncles to the ventral part of the periaqueductal gray matter.


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