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An Ultrastructural Assessment of an Embolic Method of Producing Cerebral Ischemia

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SUMMARY This study presents ultrastructural confirmation of the embolic method of producing an ischemic lesion. The embolic method was shown to produce more advanced parenchymal changes than those reported for the same postocclusion period following vascular clipping.

With this method there was less alteration in the morphological integrity of occluded extraparenchymatous areas of the arterial bed, especially in the associated nerve bundles.

TWO OF THE MOST reproducible animal models used to study cerebrovascular-related changes involve the surgical occlusion of cerebral vessels with surgical clips or introduction of an embolic material into the cerebrovascular system. 

Ultrastructural characterizations of parenchymal response following ischemic insults induced by the clip model have been reported, as well as those alterations that occur within the arterial wall and accompanying nerve bundles. However, to our knowledge no ultrastructural evaluation of parenchymal response following embolization with plastic materials has been carried out, nor has determination been made of arterial response in the embolic area. In this com-

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Another catheter was inserted into the left brachial artery to draw the arterial blood into the extracorporeal circulation system. Arterial and cerebral venous blood were propelled through the cuvette and Guyton analyzer at a constant rate (5 ml/min) and were returned to the systemic circulation via the femoral vein.

Dissection of the neck permitted insertion of a catheter into the left lingual artery. The catheter served as the site of injection of a 2-ml hydrogen bolus. An additional catheter was placed in the left lateral sinus via the facial vein and provided a source of inducing cerebral venous blood into the extracorporeal circulation.

The insertion of a specially made T-shaped catheter into the left common carotid artery permitted the injection of emboli without the interruption of carotid blood flow. The left external carotid was ligated, and the vagus nerve and cervical sympathetic chains were carefully preserved intact during the surgical manipulations.

Intracranial pressure was measured through the catheter which remained in the cisterna magna after laminectomy while intracranial venous pressure was monitored through the catheter which was wedged into the anterior part of the superior sagittal sinus.

Prior to involving the extracorporeal circulation, 3,000 IU heparin were injected intravenously.

Blood pressure, intracranial pressure, and superior sagittal sinus pressure were continuously recorded during the study with Statham pressure transducers. Cerebral (A-V) oxygen differences were also continuously measured with the Guyton analyzer. EEG and ECG were recorded during the experiment with a Grass 4-channel electroencephalograph. The previously described hydrogen bolus technique was used in determining cerebral blood flow values. The clearance curves were recorded by a hydrogen electrode in the cuvette and calculated by stochastic analysis.

After preparations for physiological monitoring were completed, the embolus, prepared according to the method of Molinari and consisting of a cylindrical mass of Microfil (7 mm × 1.6 mm), was injected through the left carotid catheter in accordance with the Molinari procedure for selective segmental occlusion of the stem of the middle cerebral artery.

After an interval of two hours, during which continuous monitoring was carried out, the animals were prepared for ultrastructural evaluation by being subjected to whole body intracardiac perfusion with a fixative of 3% glutaraldehyde and 1% osmium tetroxide in 0.1 M phosphate. The brains were removed from the animals and coronally sectioned, and specimens were taken from the frontal, parietal, occipital, and insular cortices, putamen, globus pallidus, and caudate nucleus, as well as the areas of the artery proximal, under, and distal to the embolus. The specimens were placed in the fixative for an additional 12 hours, after which they were washed in two phosphate buffer rinses and placed in 1% osmium tetroxide/phosphate buffer postfixing solution for a 4-hour interval.

The tissue was then subjected to standard ethanol dehydration and embedded in Spurr plastic. Semithin plastic sections stained with toluidine blue were prepared for light microscopy determination of microvascular clearing, lesion zones, and specimen orientation.

**Figure 1.** This region of the left globus pallidus contains extensive edematous involvement (E). The microvasculature is unbroken, although edematous changes are evident in the pericytic elements (arrows). × 6,500

Selected areas were thin sectioned and stained with 3.5% uranyl acetate/lead citrate.

**Results**

The animals were confirmed to be normotensive during the study. The other metabolic/hemodynamic parameters which were measured in the experiment will be reported in a separate manuscript. On dissection the embolus was seen to obstruct grossly the middle cerebral artery in the same anatomical zone described by Molinari and coworkers. Ultrastructural assessment of the model will be discussed on the basis of the observations of parenchymal and arterial response.

**Parenchymal Findings**

Changes in the brain parenchyma were much more advanced and widespread than those reported after the same ischemic period following cerebral artery clipping. As
reported, after vascular occlusion by clipping for such intervals (two-hour occlusive insult), the resultant changes were predominantly intracellular and consisted of astrocytic swelling limited to the perivascular foot processes within the basal ganglia, while minimal changes were seen in the cortical areas.

In the embolization model of the present study, widespread edematous changes were observed in both the cortical and basal ganglia areas of the ipsilateral hemisphere. Focal areas of greatest tissue involvement within the basal ganglia contained both extracellular and intracellular changes affecting all parenchymal cell types (fig. 1).

The cortical regions in the ischemic territory, including the frontal cortex (orbitofrontal involvement), contained loci of normal morphology as well as adjacent areas of focal patterns of perivascular astrocytic involvement (fig. 2). In other cortical areas the edematous influence was recognizable within neuronal and oligodendroglia components, as reflected in the loss of cytoplasmic background density and increased vacuolization (fig. 3). Extracellular swelling was also present in the involved cortical areas.

An assessment of the occipital cortex revealed no ultrastructural modifications (fig. 4).

In the more necrotic regions within cortical and basal ganglia, luminal compression or crenation was noted within the microvascular tree (fig. 5). This change was not apparently related to detectable endothelial cell alteration but was usually accompanied by a pericytic response consisting of edematous cytoplasm and loss of formed elements. Even though the luminal configuration would indicate an increased resistance to flow, it was of interest to note the vascular clearing which had occurred (per fusate flow had cleared the lumen of vascular elements), supporting the concept that the changes were representative of the ischemic influence as opposed to being induced from inadequate fixation.

Arterial Findings

In the clip model the arterial wall response was shown to occur after very brief periods of insult. The related nerve
fibers were reported to be of particular susceptibility. In the present study, the only arterial alterations observed were in the area of embolism and consisted of central vacuolization of cytoplasmic density within the smooth muscle elements of the medial layer as well as a breakdown of the endothelial components (fig. 6). Dense granular material, presumed to be derived from embolic interaction with the endothelial interface, was present within the less dense granular residue of the endothelial layer (fig. 6). Unlike our findings with the clip model, the nerve bundles of the embolized artery were not appreciably altered at this stage of occlusion (fig. 7). The arterial regions proximal to the embolus were spared from major involvement, but some central loss of cytoplasmic granulation and fibrillar compactness were present within the distal region of the smooth muscle cells (fig. 8).

Discussion

In the present study the adaptability of the embolism model to ultrastructural studies in cerebrovascular disease was confirmed. It was of interest that even in areas of advanced necrosis and presumed microvascular stasis, the deformed capillary lumina were cleared of contents by the low viscosity perfusate.

The embolic method utilized in this study was found to produce a more advanced and widespread response within parenchyma than was produced for the same occlusion time with the clip method.11, 14, 22 This was attributable to the greater blockage of collateral vessels of middle cerebral origin obtained with the embolism method, whereas with the clip method a relatively small area of the middle cerebral artery is selectively occluded, thereby permitting more collateral participation.

In assessing the negative aspects of this model, one is prohibited from carrying out studies involving reflow and resultant tissue response15, 18 which are possible with the clip model. However, an important advantage of the embolic method applicable to acute studies involving assessment of autoregulatory function is evident from the level of morphological stability within the arterial nerve bundles in contrast to the rapid neuronal degeneration which is reported to occur after short intervals of clipping.18

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FIGURE 7. An apparently intact periadventitial nerve bundle is shown in this section. The integral components including myelinated fibers (M), unmyelinated fibers (UM), and Schwann cells (S) are normal in appearance. × 5,000

FIGURE 8. This region of the artery distal to the embolus contains changes within the medial layer consisting of central degranulation of cytoplasm and loss of filamentous structure (X). However, the endothelial layer (E) of the intima appears intact. × 6,000
Cerebral Ischemia

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SUMMARY The effect of ischemia on catecholamine and 5-hydroxytryptamine (5-HT) levels in brain cortex was examined in the gerbil stroke model.

Unilateral common carotid artery occlusion produced bilateral decrease in cortical dopamine levels in gerbils both symptomatic and asymptomatic of cerebral ischemia. The 5-HT progressively decreased only in the occluded hemisphere of ischemic animals. In ρ-chlorophenylalanine (PCPA)-treated gerbils, dopamine decreased only in the occluded hemisphere of symptomatic animals, but norepinephrine became decreased bilaterally compared with controls. The 5-HT decrease was twice that seen in untreated animals. It is suggested that these results indicate initial release together with reduced synthesis of monoamines in ischemic brain.

The incidence of ischemia induced by carotid occlusion decreased from 44% to 26% in PCPA-treated animals, which also suggests that depletion of 5-HT available for neuronal release prior to the induction of ischemia may reduce stroke incidence by limiting impairment of collateral vasocapacitance. PCPA pretreatment did not influence the development of edema in the occluded hemisphere of ischemic animals once ischemia was established.

Catecholamine and 5-Hydroxytryptamine Levels in Ischemic Brain

Influence of ρ-Chlorophenylalanine

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PIAL VESSEL CONSTRICION associated with foci of spreading cortical pallor observed in the primate cerebral cortex after major cerebral arterial occlusion might be due to release of some chemical agent or agents from ischemic brain tissue. The validity of this hypothesis has been supported in part by measurement of 5-hydroxytryptamine (5-HT) release from brain into cerebral venous blood after the induction of acute cerebral ischemia in baboons. Increased 5-HT and catecholamine levels in lumbar cerebrospinal fluid have also been recorded in clinical patients early after the onset of cerebral infarction. Although these studies strongly suggest some abnormality of monoamine neurotransmitter metabolism during cerebral ischemia, both the responsible mechanisms and the roles they may play in the pathogenesis of cerebral infarction remain to be established.

To examine further the possible disorder of 5-HT and catecholamine metabolism in cerebral ischemia, we have measured tissue levels of 5-HT, dopamine, and norepinephrine in the cerebral cortex of gerbils. Unilateral common carotid ligation in this animal consistently induces cerebral hemispheric ischemia in 40 to 50% of animals, the incidence of ischemia and eventual infarction apparently depending on vascular anatomical variation.

Since the incidence of eventual infarction in the gerbil seems so dependent on adequacy of collateral circulation, it seemed appropriate to use this model to test the hypothesis that neuronal release of 5-HT with subsequent vasoconstriction may limit collateral vasocapacitance in foci of cerebral ischemia, thereby contributing to the progression of ischemia. Therefore, a separate animal group was pretreated with the tryptophan-hydroxylase inhibitor, ρ-chlorophenylalanine (PCPA), in order to deplete cerebral tissue 5-HT content and determine if the stroke incidence rate was thus modified.

Finally, because a relationship between accumulation of 5-HT in brain tissue and the development of cerebral edema has also been observed in some recent studies, cerebral hemispheric swelling and water content were measured in both the untreated and PCPA-pretreated series of animals.

Methods

Adult male and female Mongolian gerbils (Meriones unguiculatus) weighing 50 to 80 gm were studied. Animals were caged (three per unit) at constant temperature in simulated day and night conditions and allowed free access to drinking water and chow.

In 188 gerbils the right common carotid artery was dissected free of its accompanying vagus nerve and vein and...
An ultrastructural assessment of an embolic method of producing cerebral ischemia.
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