Four-Vessel Occlusion Model/Furlow

SUMMARY This investigation describes a surgical approach for ligation of the middle cerebral artery (MCA) in the young rat and evaluates consequences of the occlusion with a neurologic exam for motor deficits, Evans blue test for blood-brain barrier leaks, and light microscopy for histologic changes after 3 days. Evans blue extravasation and the lesion were limited to cortex at the burr hole site in occluded and sham operated rats. MCA occlusion beyond the point of origin of the striate branches in the young rat results in neither neurological deficits, dye markings, nor histologic changes in the distal vascular field to indicate an infarct. Apparently, the young rodent collateral supply maintains the tissue in a viable state.

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RAPID OCCLUSION of the middle cerebral artery (MCA) almost always results in a cerebral infarct.1-3 The lesion may be precipitated by an insufficient collateral supply due to too few collaterals,4 inappropriate regulation of existing ones, altered hemodynamics or failure in metabolism.5,6 A literature search disclosed neither a method for rapid occlusion of the MCA nor a description of its consequences for the young rat. Arterial collaterals are known to exist in this animal where numerous anastomotic junctions join distal branches of the three major cerebral vessels.5 Even so, the collateral supply may not protect against infarction. If an infarct should occur, the responsible mechanisms and possible preventive interventions could be studied. Conversely, the young rat may develop a cerebral collateral circulation without complications imposed by an infarct. In this case interventions that promote infarction could be investigated.

Objectives for this study were 1) to introduce a feasible surgical approach for rapid occlusion of the MCA at a standardized location, 2) to compare sizes of lesions marked intravitally in MCA occluded and sham operated animals, 3) to identify histologic changes in the cortex at the craniotomy site and 4) to characterize the infarct, if any, distal to the ligation after 3 days.

Methods

Surgical Exposure of MCA

Eleven 36 day old normal Wistar rats of either sex were anesthetized with ketamine hydrochloride (136-150 mg/kg body weight, i.m.) Five animals were sham operated. The MCA was occluded on the right side in 6. Skin of the temporal-parietal region was shaved and an incision was made over the right eye (fig. 1A). The incision above the zygoma was deep, cutting through the temporalis muscle to the squamosal bone. Deep surgery was performed with aid of a Bausch and Lomb StereoZoom 7 Microscope and Nikon MK II

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References


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FIGURE 1. A. Thirty-six day old normal Wistar rat marked above eye to indicate side, location and length of surgical incision line. B. Burr hole target region for exposure of MCA.

Light. Enough muscle on each side of the incision was excised to allow observation of the rostral end of the zygoma where fused to the squamosal bone. Muscle was scraped from this surgical landmark for locating the MCA. Often it could be seen through the bone. A 1–2 mm diameter (0.785–3.142 mm² in area) craniotomy was drilled with a #6 dental burr about 1 mm rostral to the fusion point (fig. 1B). To prevent the drill from going through the dura mater, the burr hole was not drilled completely through the skull. Bone remaining at the depth of the hole after drilling was removed with forceps. Dura mater was carefully pierced with a #11 scalpel blade taking care to avoid branches of the middle meningeal artery. A probe made from an Anchor Brand taper point 1833-2 needle was used to extend the dural opening in order to bluntly dissect the MCA from its surrounding pia-arachnoid. (Figure 2A).

MCA Occlusion

Monofilament nylon thread, about 35 μm in diameter, was obtained from ⅜ inch nylon rope (K Mart Stores), made visible with a black Magic Marker, and stiffened at one end by fingernail polish (fig. 2A). This facilitated passage of thread tip behind the vessel (fig. 2A) using fine point forceps that were also utilized for tying purposes. A square knot with dimensions less than the diameter of the vessel secured occlusion of the MCA (fig. 2C) about 700 μm dorsal to the rhinal fissure but nearly 1300 μm ventral to MCA bifurcations distributing to frontal, parietal and occipital cortical regions.

Post Surgical Procedures

Following wound closure with #4 silk suture, 0.5 ml of 2% Evans blue dye made with physiologic saline was injected into the abdominal cavity, but not the gut. The rat turned blue after several hours, but not the cerebral cortex unless the Evans blue-albumin blood-brain barrier was broken as occurs with severe ischemic injury, cerebral infarction in rat or due to local surgical trauma incurred during exposure or ligation of the MCA.

Regular rat chow, water and lettuce were available for ad lib consumption. Water intake was not measured but appeared depressed on the first postoperative day. On postoperative days 2 and 3 neurological tests for motor deficits were evaluated after placing the rat on the narrow (7mm) edge of a meter stick. Station, gait, and digits were observed for asymmetry.

Tissue Preparations

On day 3 following MCA occlusion, the rats were anesthetized with ether then injected with papaverine hydrochloride (40–50 mg/kg rat in sterile water) to produce maximal vasodilation and death. Tissue fixation was initiated by perfusion of 50 ml 10% neutral formaldehyde.

FIGURE 2. A. Blunt dissection of vessel with needle probe to allow passage of nylon thread behind middle cerebral artery (MCA). Hook probe made from wire used to clean polygraph pen. B. Middle cerebral artery (MCA) and cortical vein (V) as viewed through dura mater. C. After dura mater (D) reflection and ligation of MCA with nylon thread (T). Squamosal bone (B). Solid circles indicate edge of reflected dura mater.
buffered formalin into the thoracic aorta. Brains were stored in the fixative until placed in 25% sucrose-formalin several days before section. Frozen brains were cut with a microtome for alternating thin (25–50 μm) and thick (300–500 μm) sections stained with basic fuchsin or hematoxylin and eosin.

Photography and Computer Assisted Measurements

Gross brains were photographed with blue sensitive film at 1.5 X magnification. Negatives were projected at 15 X magnification for tracing Evans blue mark borders on paper. In order to compute mark areas within the borders, coordinate points along the boundaries were digitized with a Summagraphics Corporation Bit Pad Digitizer interfaced to a Commodore Microcomputer. Areas were computed for MCA occluded rats and sham operated controls then compared with an unpaired t test.

Results

Neurological Signs

Other than slight lateral displacement of the lower incisor teeth on occlusion, no abnormal neurological signs were detected in the young rats following MCA occlusion. Neither circling nor motor seizure behavior was observed after the anesthetic period. No rat placed on the meter was observed to have contralateral asymmetry in fore- or hind-foot posture or placement during walking. Digits grasping the stick edge during walking were bilaterally symmetric in place and action. When suspended by the tail there was bilateral symmetry in limb extension and when held by the nape limb flexion was symmetric.

Evans Blue Leakage

Cortex stained with Evans blue was localized to the burr hole region where the MCA was ligated (fig. 3A). There was variability in size of the marked tissue (table) but in no case did the marking extend across the tissue field to anastomoses of the MCA with either the anterior or posterior cerebral arteries located at least 6 mm and as much as 15 mm distal to the tie. Mark size was not significantly different for occluded rats compared to sham operated ones that underwent the surgery including dissection of the MCA from the pia-arachnoid and passage of the thread deep to the MCA but excluding occlusion of the vessel (table). For both sham and ligated rats, the MCA vessel wall was often, but not invariably, grossly stained blue for one to several millimeters proximal and distal to the tie (fig. 3A).

Histologic Findings

The lesion observed in the histologic sections (fig. 3B) of occluded or sham operated rats was localized to the cortex (fig. 3B) next to the burr hole. There was reduced staining of the neuropil (fig. 3B). Shrunken neurons with darkened cytoplasm were present. Macrophages and mitotic figures were evident, hemorrhage and vascular-like cords were present. These features were localized to the region where the MCA was dissected and ligated. This lesion was in the region marked blue in MCA occluded and sham operated rats. Precise spatial limits of the mark were not accurately determined in tissue sections for the blue was not seen in thin frozen sections after storage in formalin where diffusion occurred.

In no case did the cortical lesion characterized by altered histology nor the Evans blue mark extend to the region of the lesion.
dorsal arterial collaterals known to be located in greatest number between 2 and 3 mm lateral to the midline. Frank necrosis with reduced eosinophilic staining and sponginess of the neuropil, irregular or nonstaining of neuronal or glial cell cytoplasm, cellular disintegration, vacuolations and other features characterizing an infarction 72 hours following MCA ligation in monkey were not observed in the basal ganglia, the other hemisphere or cortex beyond the burr hole site.

Discussion

Surgery

Surgical approach to the rat MCA via the transtemporal route was without complications. The amount of muscle removed did not incapacitate eating, the ophthalmic artery was not encountered as in an orbital route, 2, 3 no retraction of cerebral tissue was necessary and young rats recovered well. Retraction and ligation of the MCA must avoid rupture or transection which may be fatal or produce an extensive hemorrhage. Disadvantages of the method include a craniotomy with possible alterations in intracranial pressure, 3 bacterial invasion and pathologic evaluation of the tissue. Nerves in the MCA adventitia were probably blocked by the ligature. This neural involvement probably did not cause Evans blue leaks in the MCA vascular wall since the finding was both proximal and distal to the tie and was present in sham operated rats.

Neurological Findings

Others described motor deficits or signs usually contralateral to the MCA occlusion in cats 1-3 or primates, 2 where there was variability in infarct size and deficits. Neither motor deficits nor asymmetries in gait on a narrow surface nor postural abnormalities were observed in rat. There was a slight lateral shift of the lower incisors during occlusion. Most likely removal of masticatory musculature results in occlusal asymmetry. 17

Evans Blue Marking

Others suggested that surgical trauma was responsible for the intravital staining of monkey cortex at the vessel exposure site. 2 Dye marking of the sham operated rats reported here supports that notion and indicates that MCA occlusion was not necessary for the staining. The trauma, which can be minimized but not eliminated, included drilling of the burr hole, dissection of the vessel and passage of the ligature beneath the MCA, usually with invasion into the cortex. This all occurred long before the rat became overtly blue and suggests either the dye barrier remained opened for hours or that it opened, perhaps briefly, during or following arrival of dye in the circulation.

Evans blue leaks in cerebral infarcts in rats with circulation times as short as 5 minutes. 14 Since after 1 day there was roughly 70% clearance of albumin bound Evans blue in plasma following a comparable intravenous injection in cat, 11 the 3 day postocclusion period for rat would seem to be an adequate circulation time for tissue marking with the more slowly administered dye, if an infarct should occur and if the barrier should open during or after its development under the experimental conditions. Neither neurological evaluation, the dye test nor histologic analysis gave any supportive evidence for an infarct 3 days after MCA occlusion. One can only reasonably conclude there was no infarct.

Age and Site of Occlusion

Unlike the young rat, sudden MCA occlusion in adult cats and primates nearly always results in an infarct although considerable variation in lesion sizes have been reported. 1-5 This is not unexpected since the distal collaterals in adult primates including human are neither uniform in distribution nor as numerous as for the young rodent. 9 Vascular structural change resulting in regression of these collaterals with age occurs most prominently in human and other primates during fetal and neonatal development but less so for nonconvoluted brains which includes the rat. The amount of regression that occurs before an occlusion is most likely an important variable governing the outcome even in rat.

Location of an arterial occlusion determines the collateral field that requires supply if tissue is to remain viable. MCA occlusion proximal to the striate branches enlarges the collateral field to include components of the basal ganglia in cat and monkey. 2, 8 In several cases the infarct was only near the midline and basal ganglia, 1 or in striatal components and did not include the more proximal cortex indicating cortical collateral supply was adequate. For rat, the occlusion was distal to the striate branches. Since only a cortical supply was required, the capacity of existing collaterals was adequate to protect against infarction.

This arterial occlusion in normal young rats gave no evidence of having produced a circulatory defect. The observations establish that collateral supply to the cortical vascular bed of this middle cerebral artery protects against infarction and neurological motor deficits. The technique provides a tool to study interventions that may promote infarction or to evaluate the regression of collaterals with age.

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References

Interference Between Central Dopaminergic Stimulation, and Adrenal Secretion in Normoxic or Hypobaric Hypoxic Rats

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SUMMARY Previous data have established that postsynaptic stimulation of central dopaminergic receptors was mainly involved in the protective action of apomorphine against the comportamental consequences of hypobaric hypoxia in rats: disturbances in a conditioned avoidance response. We confirm this notion by showing that domperidone (a peripheral dopaminergic blocking agent) does not antagonize the protective effect of apomorphine. Furthermore, we establish that the action of apomorphine is at least partially mediated by adrenal glands since it is no longer seen in adrenalectomized rats. In normal rats, apomorphine showing that domperidone (a peripheral dopaminergic blocking agent) does not antagonize the protective effect of apomorphine against the comportamental consequences of hypobaric hypoxia in rats: disturbances in a conditioned avoidance response. We confirm this notion by showing that domperidone (a peripheral dopaminergic blocking agent) does not antagonize the protective effect of apomorphine. Furthermore, we establish that the action of apomorphine is at least partially mediated by adrenal glands since it is no longer seen in adrenalectomized rats. In normal rats, apomorphine is suppressed by a centrally-acting dopaminergic antagonist (Pimozide) at doses that block the post-synaptic receptors. In order to confirm that the protection afforded by apomorphine is mainly centrally mediated we have studied the interaction of apomorphine with a dopaminergic antagonist which does not cross the blood brain barrier: domperidone. It is well known that hypobaric hypoxia induces a stress, resulting in an increased cerebral amnergic turnover and in a modification of the adrenal metabolism. We then compared the effects of apomorphine in normal and adrenalectomized rats to see
Middle cerebral artery occlusion in the young rat.
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