Anatomic Variation of the Middle Cerebral Artery in the Sprague-Dawley Rat

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Background and Purpose: The aim of this study was to record and analyze the anatomy of the middle cerebral artery in the rat in an attempt to identify a potential source of variation of quantitative infarct measurements in commonly used models of focal cerebral ischemia.

Methods: Over a period of 22 months, as part of ongoing studies, we routinely induced focal cerebral ischemia in 263 Sprague-Dawley rats. This was achieved by permanent occlusion of a length of the right middle cerebral artery approximately 1 to 5 mm distal to the point at which it traverses the rhinal fissure combined with 60 minutes of bilateral common carotid occlusion. The branching pattern of the middle cerebral artery was recorded for each animal by drawing and photography before occlusion and at the time of death.

Results: Six distinct branching patterns were observed. The majority (82.9%) of middle cerebral arteries studied conformed to the typical bifurcating vessel commonly referred to in the literature. However, the remaining 17.1% were far more variable with major, atypical branching, introducing, we believe, unnecessary and avoidable variation into quantitative measurements of ischemia in this and similar models.

Conclusions: Middle cerebral artery anatomy should be routinely recorded in models of focal cerebral ischemia in which this artery is occluded distal to the olfactory tract, thus allowing improved analysis of data. (Stroke. 1993;24:2087-2093.)

Key Words: animal models • cerebral arteries • cerebral ischemia • rats

Focal models of cerebral ischemia in the rat have been used since 1975 when Robinson et al.1,2 occluded the middle cerebral artery (MCA) to measure subsequent effects on catecholamine levels and behavior. Since then, numerous models incorporating occlusion of the MCA at one or more points along its length have emerged in an attempt to produce a more reproducible lesion that would be amenable to pharmacologic intervention.3-11

Occlusion of the MCA proximal to the olfactory tract and the origin of the lateral lenticulostriate arteries3 and occlusion of the MCA at or just distal to the point where the MCA traverses the rhinal fissure7 are essential components of probably the two most commonly reported techniques for inducing focal cerebral ischemia in the rat.

Yet, apart from a small number of limited studies,12-14 little, if any, reference is made to the potential for error in calculating infarct size as a consequence of variation in the branching patterns of the MCA. Furthermore, because the cortical branches originating from the MCA distal to the olfactory tract tend to be variable and, generally speaking, no major cortical branches arise between its origin at the circle of Willis and the point at

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Received February 25, 1993; final revision received June 28, 1993; accepted July 6, 1993.
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which it traverses the olfactory tract, failure to note the anatomy of the vessel when occluded distally may have far greater implications for this particular model.

Materials and Methods

As part of ongoing studies, 263 adult male Sprague-Dawley rats (Charles River, 300 to 400 g) were maintained on a 12-hour light/dark cycle and allowed access to food and water ad libitum both before and after all procedures. Rats were anesthetized with 60 mg·kg⁻¹ sodium pentobarbitone (RMB Animal Health Ltd, Sagat) injected intraperitoneally, with topical application of lidocaine analgesic (Xylocaine, Astra Pharmaceuticals Ltd) as required.

Focal cerebral ischemia was induced as follows. Both left and right common carotid arteries were exposed and a ligature loosely placed around each to enable occlusion at a later stage. Tracheostomy was performed in nonrecovery animals only; in all other instances the animals were allowed to breath spontaneously. In all cases, a transtemporal approach was adopted to occlude the right MCA. The temporalsis muscle was divided midway between the right orbit and external auditory canal, avoiding transection of the facial nerve. The junction of the squamosal bone with the frontal bone and zygomatic arch was exposed under the operating stereomicroscope (×6.5 to 40 magnification), and, using a saline-cooled dental drill, a section of the frontal bone approximately 1 to 3 mm rostral and 1 to 3 mm dorsal to
the anterior fusion point of the zygomatic and squamosal bones was removed to a thin layer. This served to protect the dura mater and surface of the cortex from thermal and physical injury and was subsequently removed with a fine watchmaker’s forceps, leaving the MCA clearly visible through the dura mater. The dura immediately overlying the MCA was carefully removed with watchmaker’s forceps.

At this stage, the artery was examined closely under ×40 magnification to reveal all the major and many of the minor branches from the main trunk of the MCA. The anatomy of the MCA was carefully recorded by drawing and/or photography, as viewed through the cranial window (Fig 1). The use of vasodilators to visualize the anatomy was not necessary. The right common carotid, previously exposed, was permanently ligated and the left common carotid temporarily occluded with a small artery clip for 60 minutes. The right MCA was then immediately occluded with low-power micropipolar coagulation approximately 1 to 2 mm distal to the point where the main trunk traverses the rhinal fissure and at further points distally until all the major branches (visible through the cranial window) supplying the cortex were occluded.

Care was taken to perform the surgery under suitably clean conditions, and all wounds were sutured and treated with oxytetracycline HCl and hydrocortisone acetate (Terra-Cortril, Pfizer Limited) as a matter of course. Body temperature was maintained within normal limits (37 to 38.5°C) with a heated operating table during the procedure, and, for rats allowed to recover after surgery, a small animal incubator was used until each was fully recovered from anesthesia (usually within 120 minutes).

In acute studies, rats were not allowed to recover from anesthesia and were killed 2 to 6 hours after right MCA occlusion. In studies in which rats were allowed to recover for more than 24 hours, food, water, and body weight were continuously monitored, and the rats were killed within 7 days. At the end of each study, the brain was rapidly removed from the cranium and the MCA anatomy observed under ×40 magnification and reevaluated by comparison with initial drawings and/or photographs. Further drawings and/or photographs were taken as required. Although some constriction of the artery is evident after death, the branching pattern is easily discernible under the microscope, even in cases where only small amounts of blood remain in the artery.

To further visualize the cerebrovascular anatomy of the rat, six animals were studied after injection of a blue epoxy resin (Alec Tinteriti Ltd, UK). Immediately after death, the cerebral circulation was perfused with 0.5 mL of resin for 5 minutes via both common carotid arteries.

**Results**

The main trunk of the MCA in the rat originates in the circle of Willis and proceeds at an angle anteriorly to the point where it traverses the olfactory tract. Lateral and medial lenticulostriate (end arteries) branches originate proximal to the olfactory tract. It is in this region that the MCA is most commonly occluded in “proximal” MCA occlusion models of focal cerebral ischemia. Few major branches supplying the frontal or parietal cortex from the MCA trunk in this region were observed in any of our studies.

As the MCA trunk proceeds dorsally, it reaches a point where it traverses the inferior cerebral vein (ICV), which runs in the rhinal fissure. It is at or just distal to this point that the MCA is most commonly occluded in distal MCA occlusion models of focal cerebral ischemia. This type of occlusion offers some advantages over the proximal occlusion models in that the surgery required is less involved and recovery experiments can be performed with less discomfort to the animals. However, it is in this region that we experienced the greatest variability in MCA branching, and six distinct patterns are subsequently described (Table).

No discrepancies were found between premortem and postmortem observations of all MCA patterns analyzed at the craniectomy site. It was found that the MCA usually bifurcated 1 to 5 mm above the ICV, forming two major branches, one each supplying the frontal and parietal cortex. These were observed to further subdivide into smaller branches, which were more variable and not recorded in our study.

In the majority of animals observed (82.9%), the MCA exhibited a standard Y (bifurcation) configuration as described above (Fig 2). Vessels included in this group also contained at least one (but not more than three) branches supplying the frontoinsular and/or temporal cortex just proximal to the bifurcation. We termed this collection of MCA patterns Class I.

A second group of MCA branching patterns emerged that was essentially the same as Class I with the exception of the presence of a major branch, usually originating from between the olfactory tract and the ICV. This was observed supplying cortical regions above the rhinal fissure in six animals. This major branch often remained undetected during the surgical procedure because it was below the level and to one side of the cranial window. It was recorded at the time of death.

A third type, Class III, resembled neither Class I nor Class II. MCAs included in this group had a typically “sparse” branching pattern (visible through the cranial window) that was characterized either by the presence of the standard bifurcation but with no visible branches supplying the insular and temporal cortex or by one branch only supplying either of these cortical areas but with no obvious bifurcation present. Again, a small subgroup of animals (seven in total) were observed with an MCA branching pattern fitting this description. At the time of death the anatomy was reexamined to
Frequency of and Criteria for Inclusion in the Six Branching Patterns of the Middle Cerebral Artery in Sprague-Dawley Rats

<table>
<thead>
<tr>
<th>MCA Type</th>
<th>Incidence (n=263)</th>
<th>Main Criteria for Inclusion in Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I</td>
<td>218</td>
<td>82.9% Bifurcation with &lt;4 temporal and frontoinsular branches in total</td>
</tr>
<tr>
<td>Class II</td>
<td>6</td>
<td>2.3% As for Class I except for presence of cortical branch, originating below level of cranial window, and supplying frontal, parietal, frontoinsular, or temporal cortex</td>
</tr>
<tr>
<td>Class III</td>
<td>7</td>
<td>2.7% Bifurcation with no major branches supplying frontoinsular or temporal regions of the cortex in the region of the cranial window; when bifurcation not evident, MCA branching patterns with &lt;2 such branches are included</td>
</tr>
<tr>
<td>Class IV</td>
<td>13</td>
<td>4.9% Multiple major branching within and around region of cranial window; pattern variations included may contain ≥4 temporal and frontoinsular branches proximal to bifurcation, trifurcation, or even quadrifurcation</td>
</tr>
<tr>
<td>Class V</td>
<td>9</td>
<td>3.4% Bifurcation originating proximal to the ICV</td>
</tr>
<tr>
<td>Class VI</td>
<td>10</td>
<td>3.8% Anastomoses of major and/or minor branches with other branches or with the MCA trunk itself</td>
</tr>
</tbody>
</table>

MCA indicates middle cerebral artery; ICV, inferior cerebral vein.

A fourth pattern found in 13 rats was characterized by multiple major branching within the region of the cranial window. Pattern variations included in this type, Class IV, contained 4 or more temporal and insular branches in any combination (eg, one temporal and three insular) proximal to the bifurcation, trifurcation, or even quadrifurcation of the main trunk.

A fifth type of branching pattern, Class V (Fig 3, bottom panel), was observed in nine cases. Here, the

discount some animals in which the bifurcation was present just above the cranial window (and therefore not visible to the surgeon at the time of occlusion).

Fig 2. Diagrammatic representation of the six middle cerebral artery (MCA) branching patterns that emerged from this study. Criteria for inclusion in each class are summarized in the Table and explained in more detail in the text. Dotted outline indicates extent of cranial window; thick broken line, zygomatic arch; MCA colored orange, segments not visible to surgeon at time of MCA occlusion; MCA colored red, segments visible through cranial window and occluded; Fr, frontal bone; Sq, squamosal bone; and ICV, inferior cerebral vein. Numerals represent major branches to different areas of the cerebral cortex: 1, frontal; 2, parietal; 3, frontoinsular; 4, temporal; 5, may supply any of these regions; and 6, frontal or parietal.
Fig 3. Top, Photograph shows example of Class VI middle cerebral artery (MCA) branching pattern. The parietal branch is seen to anastomose with the main trunk of the right MCA (arrow). In this instance, the MCA was visualized by injecting a dyed resin into the arterial blood supply to the brain immediately after death. Bottom, Photograph shows example of Class V pattern. Unusually, the MCA has bifurcated, just on the medial border of the olfactory tract (arrow), into main branches observed to supply the frontal and parietal cortex. This photograph was taken within 30 seconds of the brain being extracted from the cranium. Bar = ~0.25 mm for both panels.
main bifurcation of the MCA trunk was not visible through the window but was observed at the time of death to occur proximal to the ICV. In some instances two slightly diverging major branches were viewed through the craniectomy; in other instances one of these branches remained undetected until the end of the experiment.

Finally, a sixth distinct branching pattern, Class VI (Fig 3, top panel), observed on 10 occasions, was characterized by the anastomosing of particular branches of the standard Class I configuration, eg, anastomoses of temporal with parietal branches. The size of the circuit created by such anastomoses varied, in some cases being relatively large (Fig 3, top panel) and in some instances quite small (eg, between two smaller branches of the main frontal branch).

Discussion

Great variations of MCA division patterns have been found in anatomic studies of the human brain microvasculature. Vincentelli et al reported that the main division of the MCA trunk may occur close to its origin in the circle of Willis and may exist as a bifurcation, trifurcation, or even as a quadrifurcation. Sometimes, the position of the bifurcation of the human MCA may be difficult to determine because of the origin and size of some branches from the trunk supplying the cerebral cortex. Knowledge of such anatomic variations is important to the neurosurgeon to help reduce the risk of accidental damage to certain blood vessels, such as the lenticulostriate arteries of the patient during surgical procedures.

In the research laboratory, occlusion of one MCA in animal models (principally rodent) of focal cerebral ischemia has been repeatedly used in attempts to model the human condition of stroke. The main aim of this study was to describe and categorize, retrospectively, the different, quite distinctive branching patterns of the MCA in the Sprague-Dawley rat. The existence of these differences became apparent to us at a very early stage of our research, using animal models of focal cerebral ischemia. Indeed, Rubino and Young found some similar patterns in their study on the effects of occluding individual MCA branches on resulting infarct size. Yet the potential for error that this may create, especially in focal models relying on distal occlusion of the MCA, has largely been ignored in the many other articles published in this area, with authors tending to concentrate on the effects of temperature, blood pressure, and blood gases.

For example, the possibility exists that in a particular experiment an MCA branching pattern consistent with Class V criteria (Fig 2 and Fig 3, bottom panel) may present itself in a drug-treated animal. Because the importance of distal MCA anatomy may be reduced where the MCA is occluded proximally (the resulting cerebral damage does not depend on occluding branches from a region of the blood vessel with a high degree of variation in its branching), the resulting infarct may not be dramatically different from other treated animals with Class I MCA anatomies. This may partly explain the lack of description of the MCA anatomy by groups working with proximal techniques of MCA occlusion. If the focal model selected necessitates a more distal occlusion, ie, distal to the olfactory tract, then the situation becomes more complex.

First, the surgeon may notice the abnormal anatomy either through the cranial window (if visible) or at the end of the experiment, at which point he may choose to exclude data from this animal from the study.

Alternatively, the surgeon may only see one of the two branches through the window and mistake it for the main MCA trunk, which is then occluded in the mistaken belief that the blood supply to the cortical region of interest has been successfully limited. Only one of the main frontal or parietal branches was occluded in four of the nine rats observed to have this MCA pattern in our study. Indeed, in the example shown in Fig 3, bottom panel, the full anatomy only became apparent at the time of death. Thus, on analysis, unless the aberrant anatomy is recorded, the result from this animal is clearly compromised because either the frontal or parietal cortex is still receiving blood via the MCA.

Similar situations could arise in cases of animals with Class II or Class VI MCA anatomies, depending on the location and extent of the branch(es) or the site and degree of anastomosing, respectively. It is entirely conceivable that the point at which the frontal/frontoinsular branches anastomose with the main MCA trunk in the example of Class VI given in Fig 2 may be below the level of the cranial window and that anastomoses between these branches themselves may be to one side of the window. This would result in a somewhat distorted view of the MCA, with the surgeon not realizing that the frontal cortex is still receiving blood from the MCA.

Because of the retrospective nature of this analysis, animals with the same MCA classification have inevitably received differing treatments or were part of separate studies and so cannot be reliably compared for lesion size. However, it is not too difficult to imagine the contribution different anatomies may make to variability in the size of the lesion produced. For example, animals with an MCA branching pattern from Class IV (with all branches occluded) may produce larger areas of cerebral damage than those with the standard (Class I) MCA configuration. This would compare with MCA anatomies falling into Class III, which may result in smaller areas of damage. Similarly, animals having MCA patterns best described by Class V criteria, in which only one of the main branches has been occluded, may produce a lesion that covers a smaller area than would have been expected for that animal if it had been a control with Class I MCA anatomy in the same study.

Reasons for Class III MCA occlusions possibly resulting in less damage in distal models are speculative. It is possible that the MCA in such cases is supplying a smaller area of cortical tissue than average and that the anterior and posterior cerebral arteries are supplying a greater area than normal. The opposite may be true for Class IV. We anticipate that corrosion casting studies of such examples of MCA anatomy may provide some answers in the near future (eg, Class VI, Fig 3, top panel).

An additional complicating factor may be that of the number and development of collaterals in the cerebral circulation as a whole. Distal branches of the MCA have been shown to anastomose with branches of the anterior and posterior cerebral arteries. Permanent occlusion of one MCA has been shown by Coyle and Heistad.20,21
to cause an increase in retrograde blood flow through anastomosing branches into the region of the occluded artery. Such anastomoses may enlarge with time after MCA occlusion, thus increasing blood flow to the potentially ischemic regions supplied by the branches of the MCA. Variability in the number and type of cortical branches originating from the main trunk of the MCA may therefore have an effect on the frequency and size of anastomosing with the other main arteries supplying the cortex, which may in turn influence the size of the lesion after MCA occlusion.

Recently, we have begun to divert animals (approximately one in six) that fail to conform to the standard MCA configuration from ongoing studies into anatomic studies, which allows us to compare the influence of the different patterns on infarct quantization. However, this is a longer-term study in that because of the relative infrequency of individual classes, it may be some time before we can divert enough animals from each class to show any degree of significance between them. Meanwhile, until this issue has been satisfactorily resolved, it would seem wise to observe MCA anatomy more closely, especially in rat focal models involving distal occlusion. Individual categories may account for less than 3% of the total, but when taken together approximately 17% of all MCA patterns analyzed may produce predictably larger or smaller areas of cerebral damage, thus possibly producing false positives (or negatives) in drug trials.

Acknowledgments

The authors wish to thank Mr Brian Petch not only for his surgical expertise in developing focal models of cerebral ischemia at Pfizer but also for the excellent illustrations he produced for this manuscript. We would also like to thank Mrs Stephanie Swift for her preparation of the latter and Dr Jonathon Pryke for his assistance with the resin work.

References


Editorial Comment

The main point of the accompanying article is that variability in middle cerebral artery (MCA) branch patterns may contribute to variability of infarct size after MCA occlusion. I will comment on issues related to branch pattern recognition at occlusion time, the length of MCA occluded, and the area at risk for infarction.

First, another feature can aid recognition of pattern variability in vivo. MCA luminal width(s) and branch locations provide information about some patterns identified in this article. For example, Classes V and VI consist of two major MCA branches, each usually smaller (see Fig 3 in the article by Fox et al) in luminal width than the typical MCA, and one branch is located rostral to and the other caudal to the position normally occupied by the Class I MCA. Within a small craniectomy having a standard size and location the MCA of Class I is observed, but because branches in Classes V and VI are displaced, neither branch may be viewed (unless the craniectomy is made larger, often much larger) and both branches are narrower than normal. Thus, MCA luminal width and location relative to a standard craniectomy are useful for differentiating some patterns, rat elimination, or an appropriate occlusion strategy.

Second, an important issue is whether MCA occlusion is done only between adjacent side branches (point occlusion) or over a segment (segmental occlusion) having several attached branches. Since the segmental
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Stroke. 1993;24:2087-2092
doi: 10.1161/01.STR.24.12.2087
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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