The Four-Vessel Occlusion Rat Model: Method for Complete Occlusion of Vertebral Arteries and Control of Collateral Circulation

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Four-vessel occlusion in rats was introduced approximately 10 years ago to provide a rodent model of reversible forebrain ischemia. The model presented several advantages, which included ease of preparation, a high rate of predictable ischemic neuronal damage, a low incidence of seizures, and the absence of anesthesia. However, two issues concerning the efficient use of the four-vessel occlusion rat model as originally described by us require clarification. First, several groups have reported difficulty assuring complete occlusion of the vertebral arteries by electrocauterization through the alar foramina of the first cervical vertebra. Because this difficulty may stem partly from our failure to have adequately described details of this method in our original article, the following is provided to facilitate more effective use of the model.

It is important to position the anesthetized rat’s head in stereotactic ear bars with the head tilted down at approximately 30° to the horizontal. The cervical spine is gently stretched by placing tension on the rat’s tail with a rubber band anchored to the table. These steps stabilize and rotate the first cervical vertebra such that the viewing angle of the alar foramina can therefore be seen. The alar foramina are visualized with minimal electrocautery needle tip cauterization. Once the alar foramina are visualized, a small (<1 mm) electrocautery needle is inserted vertically through the alar foramina and down into the bony tunnel of the first vertebra, through which the vertebral arteries travel. Brief, intermittent application of current to the needle tip will cauterize the vertebral arteries and avert bleeding in the majority of rats. In those rats in which bleeding occurs, a pipette connected to a wall vacuum provides a clear surgical field. Continued electrocauterization clots the blood in the vertebral tunnel and quickly seals the vertebral artery.

Complete vertebral artery occlusion in 100% of the rats can be assured by direct visualization of the bony perimeter of the tunnel in which the vertebral arteries travel. If this tunnel is empty and if one can visualize its perimeter, it is impossible for the vertebral arteries to be intact. Direct observation of the tunnel is accomplished by pivoting the dissecting microscope and placing torque on the first cervical vertebra so that the viewing angle of the microscope is in line with the tunnel’s axis. This requires no more than a minute for each tunnel. We have successfully taught this procedure to many investigators from different laboratories, and we invite any individual who wishes to learn the technique to call our laboratory at (212) 472-5745 and arrange for an appointment to learn the procedure.

The modification suggested by Sugio et al for occluding the vertebral arteries in the soft tissues between the first and second cervical vertebrae, while theoretically valid, is unnecessary and increases the chance of excessive hemorrhage from a torn vertebral artery. When we first developed the four-vessel occlusion method in rats we approached the vertebral arteries at precisely the same level that Sugio et al describe, that is, between the first and second vertebrae. We were quickly persuaded that this approach was impractical because we encountered frequent, excessive bleeding when the vertebral arteries were electrocauterized at this level. In contrast, electrocauterization of the vertebral arteries in the vertebral tunnel of the first cervical vertebra allows for easy control of hemorrhage by clotting the blood and by tamponade of the vessels within the tunnel.

The second issue concerns the source of the blood that reaches the brain in rats that fail to become unresponsive following occlusion of the carotid and vertebral arteries. It was suggested that a still-patent vertebral artery is responsible for this condition. While it is true that a patent vertebral artery can supply sufficient blood to the brain to...
prevent the unresponsive state, there are strains of rats in which complete occlusion of the carotid and vertebral arteries will not produce an unresponsive animal. In fact, rats from the same strain but different suppliers or different shipments of rats from the same supplier may vary in their response to four-vessel occlusion. We base these statements on our experience gained from performing this procedure on many thousands of rats over a 10-year period. As described in our original article and redefined in a letter to the Editor of Stroke, the source of continued cerebral blood flow in rats subjected to successful occlusion of the common carotid and vertebral arteries is the anterior spinal and the collateral arteries in the cervical and para-vertebral muscles.

To increase the percentage of rats that meet the criteria for successful four-vessel occlusion, that is, complete and lasting loss of the righting reflex even after innocuous stimulation, we developed and reported a simple modification of the model in 1983. This modification entails the placement of a 15-cm length of 0-gauge surgical silk through the cervical region of the rat such that the suture lies posterior to the trachea, esophagus, external jugular veins, and common carotid arteries but anterior to the cervical and paravertebral muscles. This suture exits the skin on both sides, and the two ends are secured loosely to the nape of the rat's neck with adhesive tape. After occlusion of the common carotid arteries (the vertebral arteries having been permanently occluded 24 hours earlier), the cervical suture is tied and slowly tightened to encircle and occlude the collateral blood vessels in the neck muscles. The degree to which the suture is tightened depends on two factors: the rat's unresponsiveness as measured by the righting reflex and the ability of the rat to breathe spontaneously. If the suture is maximally tightened, the majority of rats will die as a result of severe brainstem ischemia and respiratory arrest. The goal then is to tighten the suture sufficiently to cause complete unresponsiveness but not so tight as to cause respiratory failure.

In a recent but unpublished study of the effects of surgical deafferentation on ischemic damage to the hippocampus (A.M. Buchan and W.A. Pulsinelli, unpublished observations), 90% of the rats subjected to the modified four-vessel occlusion met the criteria for unresponsiveness throughout the ischemic period. Of the 211 consecutive rats entered into this study, 141 (67%) survived 3 days free of convulsions. Four-vessel occlusion in rats remains a very effective model for examining the consequences of transient but severe forebrain ischemia.

References

Key Words • vertebral artery • arterial occlusive diseases • collateral circulation • rats
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Stroke. 1988;19:913-914
doi: 10.1161/01.STR.19.7.913

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1988 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/19/7/913.citation

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