One-Stage Anterior Approach for Four-Vessel Occlusion in Rat

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Background and Purpose—We report a modified 4-vessel occlusion (4VO) rat model.

Method—We used a 1-stage anterior approach for making bilateral hemispheric ischemia.

Results—Modified 4VO method decreased cerebral blood flow to 12% to 14% of baseline levels.

Conclusion—This modified 4VO method is a minimally invasive, quick, reliable procedure for producing ischemic changes. (Stroke. 2005;36:2212-2214.)

Key Words: cerebral blood flow ■ cerebral ischemia, global

The 4-vessel occlusion (4VO) model described by Pulsinelli is the most used technique to create global ischemia. However, incomplete occlusion occurs, and the 2-stage surgery at the 24-hour interval may lead to preconditioning effect. Several modified models produce a more consistent reduction in cerebral blood flow (CBF), but the surgery itself is invasive and technically demanding. In the present study, we show a 1-stage anterior approach to occlude the common carotid arteries (CCAs) and vertebral arteries (VAS) to be a safe, easy, less invasive technique for making bilateral hemispheric ischemia.

Materials and Methods

This protocol was approved by the animal care and use committee at Louisiana State University Health Sciences Center in Shreveport. A total of 33 male Sprague-Dawley rats weighing between 340 and 390 g were assigned to 3 groups: sham operated (n=6); arteries occluded for 10 minutes (10-minute group; n=12); and arteries occluded for 30 minutes (30-minute group; n=15).

Rats were anesthetized with α-chloralose (40 mg/kg IP) and urethane (400 mg/kg IP) and placed in the supine position. A 2.5-cm midline skin incision was made on the neck, and with the aid of a surgical microscope, the subcutaneous connective tissue and muscles were gently retracted to expose the trachea and thyroid (Figure 1Ba). The bilateral CCAs were isolated and 2-0 silk surgical sutures were loosely placed around the arteries as landmarks for applying microvascular clips (No. 18055-04; Fine Science Tools). The longus colli muscle was then exposed by retracting the trachea and esophagus to the right side (Figure 1Bb). This allowed for the anterior tubercle of the atlas to be identified through the muscle, thereby verifying the operating location (Figure 1Aa, 1Ad, and 1Ae). By exposing the cervical vertebral bodies from the atlas to the upper half of the fourth cervical vertebral body, the bilateral VAs were visualized between the second and third transverse processes (Figure 1Aa, 1Ab, and 1Ad). The VAs were then isolated carefully without interrupting blood flow (Figure 1Bc). The right CCA, left CCA, right VA, and left VA were occluded in turn with microvascular clips. The arteries were allowed to reperfuse 10 or 30 minutes after occlusion. Animals with sham operation underwent the same procedures with the exception of the application of microvascular clips.

The CBF in the right hemisphere was measured continuously with a laser Doppler flowmeter (PF5010; PERIMED; n=3) as described previously. Morphology (Nissl staining) was conducted as described, and the number of intact cells was counted at 10 regions of interest in each hemisphere. Neurological deficits were measured before the surgery and 24, 48, and 72 hours after bilateral hemispheric ischemia with the modified Garcia scoring system.

Results were expressed as mean±SEM. The neurological score was performed with the Kruskal–Wallis ANOVA, followed by the Games–Howel post hoc test. The count of cells was analyzed by 2-way ANOVA, followed by the Tukey post hoc test. A P value of <0.05 was considered statistically significant.

Results

The modified 4VO surgery did not affect blood gas values, hematocrit, hemoglobin, or rectal temperature, nor did the surgery cause injuries to the esophagus or trachea or result in excessive hemorrhage.

A decrease in CBF to 12% and 14% (Figure 2A) of the baseline occurred after the completion of 4VO, and a slight overflow was seen immediately after the completion of declamping the arteries (recirculation). The neurological scores of the 3 groups are shown in Figure 2B. The 10-minute and 30-minute groups showed a significant decrease in neurological score at 24, 48, and 72 hours (P<0.05 versus sham).

Significant cell damages were seen in the 10-minute group at CA1 and CA3 and in the 30-minute group in the following regions: RGb, S1, white matter, thalamus, CA1,
CA2, CA3, and dentate gyrus ($P<0.05$ versus sham; Figure 3A2 through 3H2).

**Discussion**

Early rodent global bilateral hemispheric models included: ligation of the bilateral carotid arteries and withdrawing blood to cause a reduction in blood pressure; increasing intracranial pressure with injecting artificial cerebrospinal fluid; and injecting KCl intracardially to induce a cardiac arrest. These models are less invasive but lack clinical relevance. The original 4VO model by Pulsinelli contributed greatly to the research of global ischemia, but the required 2 surgical approaches may lead to the development of collateral circulation or a preconditioning effect. Modifications increased technical demands and surgical complications including leakage of cerebrospinal fluid, resulting in a decompression effect and excessive tissue damage, which may render neurological damage that is not associated with global ischemia.

Our modified 4VO model achieves consistent results through a minimally invasive surgical procedure. The procedure consists of a 1-stage anterior approach whereby the VAs and CCAs can be completely occluded at the same time for a desired duration. Therefore, consistent neurological deficits and morphological changes in RGb, S1, white matter, thalamus, CA1, CA2, CA3, and dentate gyrus were obtained. Those ischemic lesions in this modified 4VO, supported by the reduced CBF, are consistent with the lesions in the original 4VO.7

**Acknowledgments**

This study was partially supported by grants from American Heart Association Bugher Foundation awards for stroke and from National Institutes of Health grants NS45694, HD43120, and NS43338 to J.H.Z.
References


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Stroke. 2005;36:2212-2214; originally published online September 15, 2005;
doi: 10.1161/01.STR.0000182238.08510.c5
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

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/36/10/2212

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