Platelet Emboli in Rat Brain Cross When the Contralateral Carotid Artery Is Occluded

Gretchen E. Tietjen, MD; Nancy Futrell, MD; Julio H. Garcia, MD; and Clark Millikan, MD

The pathogenesis of embolic events ipsilateral to an occluded carotid artery is uncertain. To examine this question we combined occlusion of the left common carotid artery with embolism from the right common carotid artery in rats. Following ligation of the left carotid artery in 20 experimental rats, we irradiated the right carotid artery with a laser (632 nm, 200 mW/cm², 12–15 minutes) following the intravenous injection of 12.5 mg/kg of the photosensitizing agent Photofrin II. Controls had left carotid artery occlusion with (n=13) or without (n=6) Photofrin II. Fifteen of the 20 experimental rats survived to be perfused at 24 hours; cerebral infarcts were identified in 12 rats, with bilateral infarcts in 10. There were 112 infarcts (101 small [<2.5 mm] and 11 large [>2.5 mm] on the right and 103 (93 small and 10 large) on the left. Emboli were seen in association with some infarcts and were evenly distributed in the two hemispheres (37 emboli on the right and 40 on the left, with the midline azygous artery occluded in four animals). Left carotid artery occlusion did not produce infarcts or emboli in the controls. We conclude that cerebral infarcts in the distribution of an occluded common carotid artery may be caused by emboli from the contralateral carotid artery in rats. (Stroke 1991;22:1053–1058)

Transient ischemic attacks and cerebral infarcts in the distribution of an occluded carotid artery are well-recognized events.1–7 Proposed mechanisms of embolic cerebral infarction associated with established carotid artery occlusion include embolization of material from the distal end of the internal carotid artery (ICA) thrombus and embolization from the nonobliterated proximal ICA remnant, the “stump,” via the external carotid artery and its anastomotic channels.1–8–10 The crossing of emboli from the contralateral carotid artery to the territory distal to the ICA occlusion has been considered an unlikely mechanism of cerebral ischemia and infarction.8 Atherosclerosis, a generalized disease process, plus thrombosis account for the majority of carotid artery occlusions.2 In most cases there are pathological changes in other intracranial and extracranial cerebral vessels, including the contralateral carotid artery.6,11 as well. We hypothesize that emboli may be carried wherever blood flows and that, in the case of carotid artery occlusion, an infarct in the homolateral cerebral hemisphere may be caused by an embolus from the contralateral carotid artery. We tested our hypothesis by producing platelet emboli in the right common carotid artery (CCA) after occluding the left CCA in Wistar rats, which have a complete circle of Willis.12

Materials and Methods

We used 39 male Wistar rats (20 experimental, 19 controls) weighing 260–560 g. All animals were anesthetized with 12 ml/kg i.p. chloral hydrate 4.5%. In each rat the left CCA was exposed through a midline incision and separated from the vagus nerve. Two sutures were tied tightly around the vessel, and it was severed between the sutures. In the 20 experimental rats 12.5 mg/kg Photofrin II, a photosensitizing agent, was injected into the tail vein over 1 minute. The technique for carotid irradiation has been described in detail.12 Briefly, the right CCA was surgically exposed, and 30 minutes after the injection of Photofrin II a 5-mm segment of the vessel was irradiated with red light at 632 nm for 12–15 minutes with a laser power of 200 mW/cm². The rectal temperature was maintained between 35.5° and 37.5°C throughout the procedure.

Five experimental rats were eliminated from the study because of respiratory distress requiring early sacrifice (two) or death before 24 hours (three). The 15 surviving experimental rats were all killed under chloral hydrate anesthesia between 20 and 28 hours after irradiation by transcardiac perfusion with 250...
ml normal saline followed by 500 ml of 10% neutral buffered formalin at a pressure of 100 mm Hg.

One set of controls (13 rats) received 12.5 mg/kg Photofrin II via tail vein injection after left CCA ligation and transection, but the right CCA was not isolated or irradiated. A second set of control animals (six rats) underwent left CCA ligation and right CCA isolation without Photofrin II injection. The right CCA was exposed to room light and air for 45–60 minutes, the approximate time needed to isolate and irradiate the artery during the experimental protocol. All controls were killed 24 hours later by transcardiac perfusion as described above.

Immediately after perfusion the rat's head was removed and placed in 10% neutral buffered formalin. At least 8 hours after perfusion the brain was removed from the cranium and placed in formalin for an additional 24 hours before sectioning into 3-mm-thick coronal blocks. Tissues were processed and embedded in paraffin, and 7-μm-thick sections were cut every 56 μm and stained with hematoxylin and eosin.

The diagnosis of cerebral infarction was based on shrinkage and condensation of neuronal perikarya, nuclear pyknosis, cytoplasmic eosinophilia, development of a "perineuronal halo," and vacuolation of the neuropil in a circumscribed area. Infarct size and embolus diameter were measured using a reticle. Embolus length was estimated if the same occluded vessel was visible on consecutive sections. The longest dimension of each infarct was measured, and the lesions were classified by size, territory, and laterality. Large infarcts were arbitrarily designated as those with a longest dimension of ≥2.5 mm, and small infarcts, as those with a longest dimension of <2.5 mm.

Results

The experimental protocol, including 24 hours' survival, was completed in 15 of the 20 experimental animals; 12 had cerebral infarcts. Infarcts were present only on the right in one, only on the left in one, and bilaterally in 10 experimental rats killed 1 day after irradiation. There were 112 infarcts on the right and 103 on the left. Of the 215 infarcts, 90% (194) were small and well circumscribed (Figure 1, top). Vessels occluded by material having the granular neuropil in a circumscribed area. Infarct size and embolus diameter were measured using a reticle. Embolus length was estimated if the same occluded vessel was visible on consecutive sections. The longest dimension of each infarct was measured, and the lesions were classified by size, territory, and laterality. Large infarcts were arbitrarily designated as those with a longest dimension of ≥2.5 mm, and small infarcts, as those with a longest dimension of <2.5 mm.

Another explanation for the infarcts in the left hemisphere would be that they were caused by embolization of stagnated blood from the ligated ends of the artery, by mechanical manipulation of either CCA, or by absorption of the photosensitizing dye. Ligation of the left CCA, with and without Photofrin II injection, did not cause cerebral infarction in our control animals. Other investigators have reported that unilateral carotid artery ligation does not produce stroke in rats.

Ninety percent of the brain lesions in our experimental rats were small and well-circumscribed, often associated with occluded vessels, consistent with the presumed mechanism of embolism. Evidence that cerebral infarcts are caused by emboli following photochemical damage to the carotid artery has been previously reviewed.

Emboli were found in association with infarcts in both cerebral hemispheres, although the number of infarcts greatly exceeded the number of emboli detected. This study was not designed to locate all of...
the emboli, which could be done only with serial sections. Studies of autologous clot models of cerebral embolism suggest that emboli are not detectable near all experimental embolic infarcts, presumably because emboli spontaneously fragment and move distally. Studies of human autopsy material suggest that some emboli produce cerebral infarction before disintegrating or migrating to a vessel distal to the infarct.

Embolic cerebral infarcts following photochemical damage to the right CCA in rats have measured up to 1.6 mm in diameter. Addition of contralateral CCA ligation to the experimental protocol resulted in the unexpected production of large and even massive cerebral infarcts. It might be expected that large infarcts would occur predominantly in the hemisphere ipsilateral to the arterial occlusion, but the large cortical and caudoputaminal infarcts were relatively evenly distributed (nine on the right and seven on the left). The presence of occluded arteries and arterioles in association with six of the 16 large infarcts suggests that emboli may be part of the mechanism producing large infarcts. Mechanisms in addition to embolism are likely to have been involved

**FIGURE 1. Photomicrographs of sections of rat brain.** Top: Bilateral small, well-circumscribed cortical infarcts. Scale marker 1 mm. Bottom: Arteriole occluded with granular material, probably platelets, at periphery of infarct. Scale marker 50 μm.
in the generation of some of the large cerebral infarcts, possibly related to disruption of potential collateral flow patterns when most of the blood supply to both hemispheres must be provided by a single carotid artery. The laminar pattern seen in some infarcts (Figure 3, bottom) might suggest systemic hypotension, except that in three rats the laminar pattern was unilateral, and in one of these animals the laminar pattern was contralateral to the occluded CCA. Blood pressure was not monitored during the experiment, but previous work with Photofrin II is notable for the absence of hypotension after injection.12

In addition to the difference in the laterality and size of the infarcts produced in this model compared with the model of carotid irradiation alone, we noted a difference in the distribution of infarcts within the ipsilateral hemisphere. Only 3.6% (four) of the 112 right-sided infarcts involved hippocampal structures, whereas the original experiment of platelet embolism without extracranial vessel occlusion produced 27.5% (22 of 80) infarcts in the hippocampal region.12 This difference is significant ($p<0.001$). Redistribution of embolic infarcts in rats with an occluded left CCA may imply redistribution of blood flow following left CCA occlusion. When the left CCA is occluded and the right CCA must supply parts of both hemispheres, perfusion pressure on the right may decrease, promoting collateral blood flow to the hippocampus from the vertebrobasilar system.
The results from this study may have mechanistic implications for humans with atherosclerotic cerebrovascular disease. Patients with transient ischemic attacks and cerebral infarcts in the setting of carotid artery occlusion are more likely to have significant contralateral stenosis than those with asymptomatic occlusions.6 There are also patients who improve after carotid endarterectomy contralateral to their ischemic symptoms and the site of occlusion.21 These findings have been explained by alterations in collateral blood flow, but the possibility that the stenotic carotid artery serves as an embolic source should be considered. It might also be noted that neonates undergoing CCA ligation for extracorporeal membrane oxygenation are observed to have both ipsilateral and contralateral cerebral infarcts.22 The etiology of infarcts in this population is multifactorial, but documentation of blood flow reversal through the circle of Willis23 allows for speculation that emboli might cross into an area of impoverished blood flow.

We conclude that, in rats, cerebral infarcts in the distribution of an occluded CCA may be caused by emboli from the contralateral CCA. The possibility of a similar phenomenon in humans warrants further consideration.

Acknowledgments

The authors gratefully acknowledge the technical assistance of Nancy Allar and Alexandra Halvorsen and preparation of the manuscript by George Potts.
References


KEY WORDS • carotid arteries • cerebral infarction • embolism • rats
Platelet emboli in rat brain cross when the contralateral carotid artery is occluded.
G E Tietjen, N Futrell, J H Garcia and C Millikan

doi: 10.1161/01.STR.22.8.1053

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 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/22/8/1053

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in
*Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office.
Once the online version of the published article for which permission is being requested is located, click Request
Permissions in the middle column of the Web page under Services. Further information about this process is
available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org/subscriptions/