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A novel atherothrombotic model of ischemic stroke induced by injection of collagen into the cerebral vasculature

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HIGHLIGHTS

- We developed a stroke model similar in pathophysiology to atherothrombotic stroke.
- Collagen was injected directly into the cerebral circulation of mice and rats.
- Cerebral blood flow remained depressed for at least 1 h after collagen injection.
- Platelet and fibrin rich thrombi formed in macro- and microvascular cerebral arteries.
- Cerebral infarcts and neurobehavioral deficits were observed after 48 h.

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ABSTRACT

Background: Most ischemic strokes in humans are caused by ruptured arterial atheroma, which activate platelets and produce thrombi that occlude cerebral vessels.
Methods: To simulate these events, we threaded a catheter through the internal carotid artery toward the middle cerebral artery (MCA) orifice and injected collagen directly into the cerebral circulation of male C57Bl/6 mice and Wistar rats.
Results: Laser-Doppler flowmetry demonstrated reductions in cerebral blood flow (CBF) of ~80% in mice and ~60% in rats. CBF spontaneously increased but remained depressed after catheter withdrawal. Magnetic resonance imaging showed that ipsilateral CBF was reduced at 3 h after collagen injection and markedly improved at 48 h. Micro-computed tomography revealed reduced blood vessel density in the ipsilateral MCA territory at 3 h. Gross examination of excised brains revealed thrombi within ipsilateral cerebral arteries at 3 h, but not 24 h, after collagen injection. Immunofluorescence microscopy confirmed that platelets and fibrinogen/fibrin were major components of these thrombi at both macrovascular and microvascular levels. Cerebral infarcts comprising ~30% of hemispheric volume and neurobehavioral deficits were observed 48 h after ischemic injury in both mice and rats.
Comparison with existing methods: Collagen injection caused brain injury that was similar in magnitude and variability to mechanical MCA occlusion or injection of a pre-formed clot; however, alterations in CBF and the mechanism of vascular occlusion were more consistent with clinical ischemic stroke.
Conclusion: This novel rodent model of ischemic stroke has pathophysiologic characteristics consistent with clinical atherothrombotic stroke, is technically feasible, and creates reproducible brain injury.

1. Introduction

Stroke is now the second leading worldwide cause of death and the third leading cause of disability-adjusted life-years (Murray and Lopez, 2013). More than 85% of human strokes are ischemic in nature, and of these, the majority are atherothrombotic (non-cardioembolic) in origin (Roger et al., 2012). Anti-platelet agents, such as aspirin and clopidogrel, are standard of care for primary

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and secondary prevention of atherothrombotic stroke, whereas anticoagulant drugs, such as warfarin, provide protection against cardioembolic stroke (Warden et al., 2012; Lansberg et al., 2012). Thrombolytic therapy is standard of care for treatment of acute ischemic stroke that has either an atherothrombotic or cardioembolic origin, but it requires administration within a few hours of symptom onset (Lansberg et al., 2012). Unfortunately, none of the currently available therapies is completely effective at reducing brain injury. Despite decades of intensive research and successful intervention in animal stroke models, novel laboratory therapies have not translated to the clinical environment. This failure can be attributed in part to shortcomings in animal stroke models, which do not faithfully recapitulate the pathophysiologic events that occur in most human strokes (Macrae, 2011; Fisher et al., 2009).

Most ischemic strokes in humans are caused by the rupture of unstable atherosclerotic plaque present in large extracranial and intracranial arterial vessels, with the carotid artery being a particularly common site (Nieswandt et al., 2011; Badimon and Vilahur, 2007; Warden et al., 2012; Hennerici, 2004). Components of plaques that remain on the arterial surface and that embolize distally activate platelets and produce thrombi that occlude large cerebral vessels and distal microvessels, causing ischemic injury, the magnitude of which depends on the size of the vessel and duration of occlusion (Davi and Patrono, 2007; Hennerici, 2004). The spectrum of clinical events, from transient ischemic attack to hemispheric stroke, reflects these pathologic events, which commonly include spontaneous or therapeutic recanalization of the occluded vessel and subsequent reperfusion injury. Much of the experimental stroke literature has focused on searching for neuroprotective strategies using models that include mechanical occlusion of the middle cerebral artery (MCA) and intra-arterial injection of a preformed blood clot, or on repair strategies with a permanent photothrombotic occlusion model of the distal MCA or its branches. However, these models do not lend themselves well for the study of atherothrombotic mechanisms, which involve platelet activation and thrombus formation in situ in cerebral macro- and microvessels. To more closely mimic human events, we modified the standard transient filament occlusion model of ischemia/reperfusion injury. Rather than threading a filament to mechanically occlude the MCA or injecting a preformed clot, we thread a narrow catheter near the MCA origin but do not occlude blood flow. Instead, we perform a series of intra-arterial injections of collagen, a potent platelet activator and component of ruptured atherosclerotic plaque (Fuster et al., 1990). The collagen induces platelet-rich clot formation and consequent vascular occlusion.

2. Materials and methods

2.1. Rodent model of ischemic stroke

The investigational protocol was approved by the Johns Hopkins University Animal Care and Use Committee. Male C57Bl/6 mice (26–28 g; 3–4 months) were anesthetized with 1–2% isoflurane/30% O_2 in a temperature-controlled environment that maintained body temperature at 37.0 ± 0.5 °C. The right neck and carotid bifurcation were dissected. The external carotid artery was ligated and used as a stump for passage of a PE8 catheter (SAI Infusion Technologies, Lake Villa, IL), and the right common carotid artery was temporarily ligated. The PE8 catheter was advanced into the internal carotid artery (ICA) approximately 5 mm past its bifurcation with the external carotid. A 10-μl Hamilton syringe was used to inject 1 μg of collagen (1 μg/μl, Chrono-Log Corp., Havertown, PA) through the catheter six times at 5-min intervals ($N = 10$). In additional experiments, a single injection of collagen (5 μg or 1 μg) was used ($N = 5$ each dose). Cerebral blood flow (CBF) was measured with a laser-Doppler flow (LDF) (Moor Instruments Ltd., Wilmington, DE) apparatus fixed on a cranial window that was lateral and slightly posterior to the bregma and devoid of large vessels, as previously described (Alkayed et al., 1998). Carotid artery reperfusion was initiated 60 min after the first collagen injection by withdrawing the catheter and releasing the temporary carotid ligature. After necks were sutured, animals were placed into a humidity- and temperature-controlled chamber for the first 2 h of recovery.

The model was modified for use in male Wistar rats (300–400 g) ($N = 12$). We catheterized the right femoral artery to monitor blood pressure. In addition, with rats in the lateral position, we temporarily occluded both the left and right common carotid arteries with microvascular clips. The PE8 catheter was threaded through the ICA to the aperture of the MCA, as determined by a slight decrement (~10%) of LDF signal. Collagen was injected six times (10 μl per injection) at 5-min intervals. Carotid reperfusion was achieved 60 min after the first injection by removal of the catheter and removal of the microvascular clips placed on the common carotid arteries.

In additional experiments, we induced ischemia in mice by mechanical occlusion of the MCA ($N = 7$), and in rats by intra-arterial injection of a clot ($N = 12$). Briefly, we performed the filament occlusion model in the mouse as previously described (Eliasson et al., 1997) using a 6-0 nylon monofilament to mechanically occlude the MCA for 60 min. Occlusion was followed by 60 min of monitored reperfusion. We performed the embolic clot model in the rat by injecting a single preformed 25-mm-long blood clot into the MCA, as previously described by us and others (Zhang et al., 1997; Papangelou et al., 2013).

2.2. Measurement of cerebral perfusion in vivo by magnetic resonance imaging (MRI)

Three hours after collagen injection, we assessed cerebral perfusion and brain injury in mice by MRI using T2, diffusion, and perfusion-weighted images ($N = 3$). MRI was carried out on a horizontal bore 11.7 Tesla Bruker AVANCE 3 system equipped with a triple-axis gradient unit (maximum strength 740 mT/m) with modifications of protocols previously described by us and others (Wu et al., 2013; Duhamel et al., 2012). Briefly, the animal's respiration rate was kept at ~60 breaths/min by adjusting the dose of anesthesia (approximately 1–1.5% isoflurane with air and oxygen mixed at a 3:1 ratio). Body temperature was kept at approximately 35–37 °C by circulating warm water through the animal holder and was monitored constantly via a thermocouple placed under the body. Imaging acquisition was synchronized by respiratory gating signals. We used a fast spin echo sequence for T_2 MRI and a diffusion-weighted echo planar imaging (EPI) sequence for diffusion tensor imaging (DTI). We acquired T_2-weighted images with echo times (TEs) of 40 ms and a repetition time (TR) of 3000 ms, two signal averages, 0.4 mm slice thickness, and an in-plane resolution of 0.08 mm × 0.08 mm. For DTI, we used TE/TR = 30/3000 ms, 4 shots with navigator correction, two signal averages, 30 diffusion-weighted images with a maximum b value of 1000 s/mm^2, 0.4 mm slice thickness, and an in-plane resolution of 0.16 mm × 0.16 mm. Un-inverted flow-sensitive alternating inversion recovery (UNFAIR) arterial spin labeling was used with 1 mm slice thickness, 5 slices, and an in-plane resolution of 0.25 mm × 0.25 mm. Diffusion tensor was calculated using a log-linear fitting method implemented in DTIstudio (www.mristudio.org), and maps of apparent diffusion coefficient (ADC) and fractional anisotropy (FA) were also obtained (Basser et al., 1994; Basser and Pierpaoli, 1996). Maps of cerebral blood