A Novel Fluorescent Imaging Technique for Assessment of Cerebral Vasospasm after Experimental Subarachnoid Hemorrhage

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Introduction

Various techniques have been developed to study changes in cerebral vasculature in numerous neuropathological processes including subarachnoid hemorrhage (SAH), vasospasm, and delayed cerebral ischemia (DCI). In the laboratory setting, one of the most widely employed techniques uses India ink-gelatin casting to visualize cerebral vasculature. This technique, however, presents numerous challenges with high viscosity, rapid solidification, and immunohistochemistry. To overcome these limitations, we developed a novel technique for assessing cerebral vasculature using cerebrovascular perfusion with ROX SE, a fluorescent labeling dye.

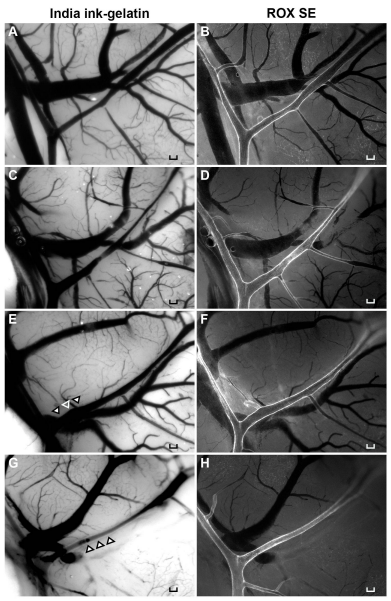
Methods

The endovascular perforation method at the ACA-MCA bifurcation was utilized to induce SAH in mice. Vasospasm was assessed by measuring the MCA diameter. Immunohistochemistry was utilized to assess other neuropathological endpoints of DCI.

Conclusions

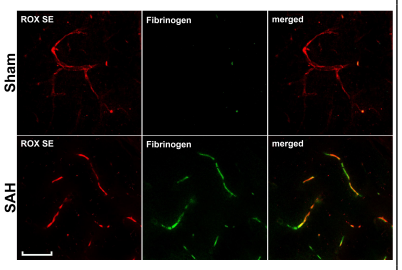
The ROX SE perfusion technique can be successfully utilized to evaluate SAH-induced cerebral vasospasm with results comparable to India ink-gelatin casting. This technique also offers multiple advantages including better precision and the ability to use ROX SE-perfused tissue for histological studies to assess other pathophysiological process occurring after SAH.

Comparison of imaging techniques



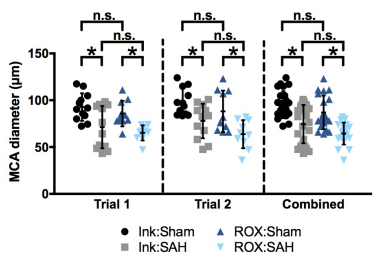
ROX SE perfusion showed improvement of poor visualization of overlaying vessels (E) and poor perfusion (G) with India ink.

Microvessel thrombi formation



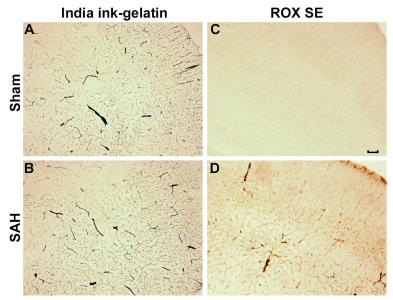
Anti-fibrinogen immunofluorescence reveals increased microvessel thrombi formation after SAH.

Comparison of vasospasm assessment



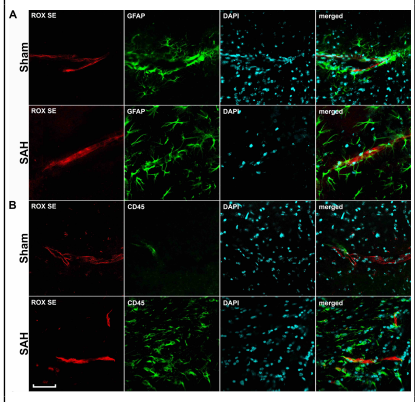
Quantitative comparison between ROX SE perfusion and India ink-gelatin casting techniques. In two trials, Sham or SAH surgery was followed by either ROX SE perfusion or India ink-gelatin casting. In both trials, both techniques detected significant vasospasm after SAH ($p<0.05$).

Improvement of immunohistochemical analysis



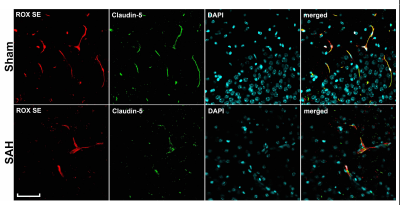
Anti-fibrinogen DAB staining demonstrates improvement of immunohistochemical analysis using ROX SE perfusion. No difference was seen in the India ink-gelatin casted brain sections due to obscuration of vessel lumen by solidified India ink-gelatin.

Neuroinflammation after SAH



Immunofluorescence using anti--glial acidic fibrillary protein (GFAP) and anti-CD45 antibodies reveals marked increase in astrocyte (A) and microglial activation (B) in mice that underwent SAH. This demonstrates increased neuroinflammation after SAH.

Blood-brain barrier disruption after SAH



Immnofluorescence using antibodies targeting claudin-5, a blood-brain barrier protein. Discontinuity of claudin-5 staining along cerebral vessel walls is seen in mice that underwent SAH, indicative of blood-brain barrier disruption after SAH.