Human Endothelial Cell Collection from the Middle Cerebral Artery in Acute Ischemic Stroke

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Background: Endovascular treatment for large-vessel acute ischemic stroke (AIS) has rapidly emerged. However, the understanding of the complex biology involving endothelial cells (ECs) remains scarce. *Methods:* Using stent retrievers during endovascular thrombectomy (ET) in patients with AIS, ECs were segregated, centrifuged in a dissociation buffer, and suspended in endothelial specific antibody solution. Subsequently, fluorescence-activated cell sorting (FACS) and microscopic analyses were performed. *Results:* Three stent-retriever devices (2 Solitaire, 1 Trevo) were collected as separate deployments. Of 5.0% (±.48%) total events using FACS, 6.8% (±.68%) of cells were specific for ECs using fluorescent markers and were further visualized on fluorescence microscopy for consistence with the positive controls. *Conclusions:* We describe a novel, minimally invasive biopsy technique to collect and harvest ECs from stent retrievers during ET and validate the approach in the treatment of AIS. Further work for detailed characterization and viability assessment of ECs is needed to compare their biology with in vitro and animal models. **Key Words:** Endothelial cell—biopsy—stroke—thrombectomy—stent retriever.

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Introduction

Although our ability to treat cerebral arterial occlusions continues to improve at a rapid pace, our understanding of the complex biology of acute ischemic stroke (AIS) remains rudimentary. The cerebral endothelial cell (EC) has emerged as a key player in stroke pathogenesis. As a part of the Virchow triad, EC injury due to oxidative stress at the vessel wall contributes to blood–brain barrier (BBB) permeability and thrombosis.[1-3] These findings however, have remained relegated to animal models, and due in part to the differences between rodent and human strokes, none of these discoveries have resulted in a clinically applicable treatment. In the present study, we develop and validate a technique to recover ECs adherent to endovascular stent retrievers during endovascular thrombectomy (ET) for patients with AIS.

Methods

We identified patients with AIS treated with stent retrievers within the middle cerebral artery (MCA) during ET. The stent retrievers were used in their standard fashion for intracranial occlusions without any alterations during the ET. After the devices were dragged through the MCA, they were recaptured in a distal access catheter at the terminal segment of the internal carotid artery to ensure that only ECs from the MCA were harvested. The devices were retrieved from the patient and all the adherent thrombus was removed. As such, the cells that were captured did not originate from the thrombus. Devices were then cut from the delivery wire into a centrifuge tube containing 10 mL of EC dissociation buffer (Gibco, Grand Island, NY). After agitation for 5 minutes at room temperature, the device was removed and discarded. The solution was centrifuged at 1500 rotations per minute for 10 minutes, and the supernatant was aspirated. The cells were then suspended in phosphate-buffered saline with 3% bovine serum albumin with 1:200 dilution of primary conjugated antibodies CD31-AF488 (Cat# 303110; BioLegend, San Diego, CA) and CD45-PE (Cat# 304014, BioLegend). CD31 is present on ECs, certain leukocytes, and platelets. CD45 is present on all CD31+ leukocytes but not on ECs. After staining, the cells were washed with phosphate-buffered saline and fixed with 1% paraformaldehyde. Concurrent fluorescence-activated cell sorting (FACS) and fluorescence microscopic analysis were performed with the ImageStream Mark II device (EMD Millipore, Darmstadt, Germany). This protocol is outlined in Figure 1. Staining and FACS analysis were also performed for cultured human cerebral endothelial cells (chECs) (Cat# ACBRI 376; Cell Systems, Kirkland, WA) as positive control. FACS analysis was performed with 3 technical replicates to assess for variability within the sample. Mean (±standard deviation) yields of the sorted fractions were recorded. The present study was approved by the institutional review board of the local institution and was conducted in compliance with the Health Information Portability and Accountability Act.

Results

Three stent-retriever devices were collected as separate deployments through the segments of MCA. Two Solitaire (Medtronic Inc., Dublin, Ireland) and 1 Trevo (Stryker Neurovascular, Fremont, CA) devices were used. FACS was initially used to sort by size and morphological characteristics to define the cellular population. This cellular fraction was approximately 5.0% (±.48%) of all the triggered events. Fluorescent markers were used to sort the cells, and approximately 6.8% (±.68%) of the cells were $CD31^{+}CD45^{-}$ with morphological and size characteristics consistent with ECs. ECs were further determined by comparing their morphology, as visualized on brightfield and 2-channel fluorescence microscopy, to be consistent with the positive control chECs. Representative images of a captured cerebral EC, along with positive and negative control images for comparison, are shown in Figure 1, B.

Discussion

We describe the first successful endovascular biopsy technique to harvest cerebral ECs from patients with AIS. Our technique to capture ECs from the ischemic tissue bed during AIS treatment with ET conferred no additional risk. Notably, our capture protocol involved cutting the stent retriever into an EC dissociation buffer and centrifugation that could be readily accomplished in any clinical laboratory. Although previous studies have described cell capture techniques from animals and peripheral arteries,[4-6] we believe our work represents the first demonstration of cerebral EC capture from the ischemic bed of patients suffering from AIS and allows validation of in vivo endovascular biopsy techniques.

A robust method for the capture of chECs from patients with AIS is lacking. Prior works in animal models have demonstrated the role of endothelial dysfunction in the inflammatory signaling at the early phase of ischemia, and thus play a key role in stroke pathogenesis. Thrombotic disruption of pulsatile cerebral flow over EC results in BBB disruption, with a resultant reduced EC expression of cadherin and B-catenin.[7] Activated by the onset of hypoxia, EC injury results in a cascade of proinflammatory signals that result in fibrin–platelet clumps and microvascular occlusions. Ultimately, these injury mechanisms result in increased permeability of the BBB, macrophage and mast cell activation, and inflammatory cell infiltration. In addition, oxidative injury in ECs reduces the production of nitric oxide, an important vasodilator and inhibitor of platelet aggregation.[2] Reduced production of nitric oxide further affects distal collateral flow that controls the penumbral tissue in large-vessel occlusion strokes.[8]

Our study has few limitations that need to be considered. The EC capture protocol requires several ex vivo manipulation steps, including cell detachment, suspension in media, and FACS. The stent deployment and dragging procedure during capture protocol could deform the native ECs and thereby provoke functional changes in the captured ECs. The ability of neurointerventional devices to disrupt the EC layer has been previously discussed[7]; however, our technique would safely allow isolating, harvesting, and studying ECs. One such avenue of exploration is aneurysm pathogenesis. ECs from the aneurysmal domes can be captured on standard neurointerventional coils, and the genetic profiles of these patients can be compared with nonaneurysmal cohorts.[6] More generally, the ability to harvest ECs as a by-product of endovascular procedures may allow for