

Title: PECAM-1 Blockade Reduces Neutrophil Infiltration Into the Subcortex Post-Stroke

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Background and Purpose: Current therapies for ischemic stroke focus on reperfusion but do not address the acute inflammatory response that results in significant reperfusion injury. To advance future therapies, a thorough understanding of the precise spatiotemporal underpinnings of leukocyte extravasation and infiltration is necessary. We describe the evolution of the inflammatory response in a mouse transient middle cerebral artery occlusion (tMCAO) stroke model at several time points after reperfusion and the modulation of this response with PECAM blockade.

Methods: The transient Middle Cerebral Artery Occlusion model (90 minutes of ischemia followed by reperfusion) was used to simulate large vessel occlusion stroke and recanalization. We used wide field and confocal immunofluorescence microscopy to examine the exact distribution of neutrophils with close examination of the leukocyte position with regard to the brain vasculature and the perivascular space. Flow cytometry of single cell suspensions was used to confirm cell identity at different time points post-stroke.

Results: At 12 and 24 hours, neutrophil recruitment and extravasation was predominated localized to the cortical surface. This contrasts with other organs where there is considerable migration of neutrophils into the inflamed tissue by 24 hours. Over 48 to 72 hours, neutrophils were increasingly found deeper into the subcortex. Throughout the infarct (determined with triphenyl tetrazolium chloride staining), neutrophil recruitment was not uniform but rather organized in clusters. Disrupting leukocyte diapedesis with PECAM function-blocking antibodies restricted leukocytes to within 500 microns of the surface when compared to control; and this was still evident at 72 hours (n=3 mice per group, $p < 0.01$, Control $46\% \pm 4.0\%$; PECAM-1 Ab $62\% \pm 5.0\%$). High-power wide-field microscopy confirmed limitation of TEM by PECAM-1 blockade at 24h. Flow cytometry confirmed that a majority of the infiltrating cells were neutrophils both at 24h and 72h.

Conclusions: Our findings demonstrate that neutrophil infiltration into a stroke evolves over several days following reperfusion. The use of PECAM blockade modulates the natural progression of neutrophils into the infarcted stroke bed. A better understanding of neutrophil spatiotemporal infiltration and its regulators could help inform the next generation of therapeutic interventions.