

Characterization of Inflammatory Cells after EDAS surgery in patients with Intracranial Arterial Stenosis

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Introduction

Indirect revascularization via encephaloduroarteriosynangiosis (EDAS) establishes collateral flow through new vessels formed from external carotid branches in patients with moyamoya disease and intracranial arterial stenosis (IAS) of non-moyamoya origin. A better understanding of the process of neovascularization is key to improve the potential therapeutic effects of these techniques. The objective of this study was to determine the type of inflammatory cell response after EDAS surgery in subjects with intracranial arterial stenosis of atherosclerotic origin, and to compare that response with a control group.

Methods

We performed a prospective case control cohort study to evaluate the changes in circulating inflammatory cells after EDAS surgery in comparison with a group of patients undergoing intracranial interventions for non-vascular, tumoral, or traumatic indications. Circulating blood cells were extracted from peripheral blood samples in EDAS patients and controls at baseline (before surgery) and days 1 and 3. In EDAS patients, additional samples were collected at days 5 and 7. Fresh blood was mixed with DMSO (10% by volume) and stored at -80C overnight. Samples were tagged with CD45, CD33, CD14, CD15, CD68, and CD206 antibodies. Fluorescence Activated Cell Sorting (FACS) was used to separate the subpopulation of WBCs.

Results

An increase in CD45+ cells was noted at 72 hrs in all cases. However, a particularly impressive elevation in the CD14+ and CD33+ was noticed in the EDAS group. The mean rate increase in cell count in the EDAS group was 3.14 for CD14+ and 2.68 for CD33+ cells (SD 2.5 and 1.72 respectively). The mean rate increase in cell count in the control group was 1.46 for CD14+ and 1.63 for CD33+ cells (SD 0.38 and 0.90 respectively) as shown in figure 1. The quantitative FACS analysis (Figure 2) demonstrated statistically significant increases in both CD14+ and CD33+ cell counts 72 hrs after EDAS surgery but not after surgeries on the control group (Figure 3). This difference was not significant in the between groups comparison.

Figure 1. FACS Analysis Rate of Increase Summary Plot

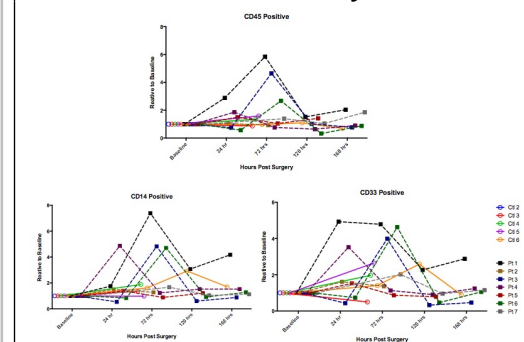
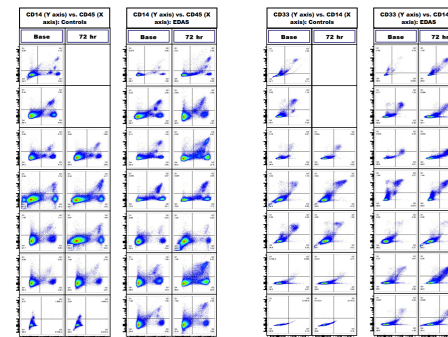
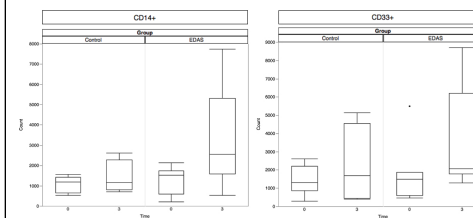


Figure 2. Quantification of FACS Data Controls vs. EDAS



Left: CD14 (Y axis) vs. CD45 (X axis),
Right CD33 (Y axis) vs. CD14 (X axis)

Figure 3. Comparison of EDAS vs. Control CD14+ and CD33+ at 72 hrs



CD14+ EDAS mean cell count
baseline:1286.14, 72 hrs: 3233.57
($p=0.03$). CD14+ controls mean cell count
baseline: 970.75, 72 hrs: 1423.25 ($p=0.20$).
CD33+ EDAS mean cell count
baseline:1764.14 72 hrs: 3549.14 ($p=0.03$).
CD33+ controls mean cell count baseline:
1263.25, 72 hrs: 2231.5 ($p=0.26$).

Conclusions

Experimental evidence has shown that bone marrow derived circulating cells play a major role in the establishment of angiogenesis. The ability to induce growth of new conduits from pre-existing blood vessels has been attributed to subpopulations of mononuclear cells existing in the adult bone marrow and circulating in peripheral blood.

Our findings are in accordance with these mechanisms. This preliminary results constitute the first reported evaluation in humans of the potential pro-angiogenic role of macrophages during vascular expansion and repair in the setting of cerebral ischemia and surgical synangiosis. The findings are consistent with the concept that CD14+ and CD33+ cells might constitute an important subset of monocyte/macrophages poised to mediate vascular repair after stroke. Further evaluation of these cell populations can represent a clinically valuable tool to potentiate or accelerate the effects of indirect revascularizations with EDAS surgery in patients with intracranial arterial stenosis of atherosclerotic origin.

References

1. Alev C, Ii M, Asahara T. Endothelial progenitor cells: a novel tool for the therapy of ischemic diseases. *Antioxid Redox Signal* 2011;15:949-65.
2. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med*. 2000;6:389-395.
3. Dusick JR, Liebeskind DS, Saver JL, Martin NA, Gonzalez NR. Indirect Revascularization for Nonmoyamoya Intracranial Arterial Stenoses: Clinical and Angiographic Outcomes. *J Neurosurg*