Enhanced Cerebral Targeting of Magnetically Labeled Glial Precursor Cells using the VLA-4/VCAM-1 Adhesion Pathway

P. Walczak[1,2], M. Levy[2,3], M. Gorelik[2,3], D. A. Kerr[2,3], and J. W. Bulte[1,2]

[1]Radiology, Johns Hopkins University, Baltimore, MD, United States, [2]Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD, United States, [3]Neurology, Johns Hopkins University, Baltimore, MD, United States

Introduction: Therapeutic intervention for neurodegenerative disorders remains one of the major challenges of modern medicine. A new promising approach to replace dead or defective endogenous cells is the administration of exogenous stem/progenitor cells. One of the most important issues in neurotransplantation, particularly in diffuse or multifocal diseases, is the appropriate delivery and efficient targeting of cells to the affected sites. Using a rat stroke model (*1*) it was recently shown that MRI is well-suited to monitor then cerebral engraftment of Feridex-labeled cells after intra-arterial (carotid) injection. For any application of stem cells in neurorepair of the brain following systemic delivery, the initial step of binding and "rolling" along the cerebral endothelium is critical for further entry into the brain parenchyma. We hypothesized that intracerebral docking of intra-arterially injected glial restricted precursor cells (CRPs) can be modulated by the use of the VLA-4/VCAM-1 adhesion pathway, a well characterized system involved in immune cell trafficking (*2*). Following transfection of GRPs to increase VLA-4 expression and labeling with Feridex, we evaluated the global cerebral distribution of intra-arterially injected GRPs in an LPS-induced rat brain model of inflammatory disease.

Methods: Rat GRPs were engineered to express α4 and β1 subunits of VLA-4 integrin (see Figure **A**). Briefly, inducible pSLIK-α4 was generated by subcloning in the human integrin α4 from a purified phagemid DNA (ATCC) into the pSLIK backbone. The human beta-1 integrin was linked to an IRES-GFP in a lentiviral vector obtained from Genecopoeia. GRPs were first transduced with pSLIK-α4 lentivirus and after G418 selection surviving cells were expanded and further transduced with Genecopoeia β1 lentivirus. The cells were expanded up to 5 million and sorted for GFP using a FACSVantage SE cell sorter. Confirmation of expression of α4 and β1 integrins on the cell surface was done using immunohistochemistry staining for α4 integrin with PS/2 antibody and the B1 integrin with an anti-β1 antibody. Before transplantation, FACS-sorted GRPs were expanded and labeled with the MR contrast agent Feridex. Adult Lewis rats were divided into two groups. One group was injected ip at 24 hrs before cell injection with lipopolysaccharide (LPS, a known inducer of endothelial VCAM-1 expression, see schematic Figures **C** and **D**). The second group was a control group without LPS injection. The extracranial right internal carotid artery (ICA) was cannulated with plastic tubing. VLA-4 expressing, Feridex labeled GRP cells were arterially infused at 1ml/min. Immediately after cell infusion, animals were sacrificed, transcardially perfused and tissue was collected for high resolution *ex vivo* MRI and histology. Brains were imaged using a Bruker 9.4 T horizontal bore magnet with T2*-weighted 3D gradient echo sequence (TE/TR=5/150ms, AV=4, RES=70x70x70μm).

Results: GRP cells stably expressed VLA-4 integrin as demonstrated by GFP fluorescence (Figure **A**). The efficiency of magnetic cell labeling with Feridex was verified with Prussian Blue stain showing that nearly 100% of cells were labeled (Figure **B**). MR imaging demonstrated that in control animals (without LPS treatment) there were no detectable cell-related hypointense signals in the brain (Figure **E**), indicating a lack of endothelial adhesion (i.e., as outlined in Figure **C**). In contrast, in LPS treated animals, extensive hypointense regions were detected, mostly in the right hemisphere, ipsilateral to the intracarotid cell infusion site (Figure **F**).

Conclusions: We have demonstrated that through overexpression of VLA-4 integrin, transplanted GRPs can be effectively targeted to inflamed brain with activated endothelium. This initial targeting step is likely to be critical for any attempt to use stem cells for intraparenchymal brain repair. We have shown that MRI cell tracking using Feridex-labeled cells is an excellent technique to monitor and evaluate this cerebral targeting and homing process. These proof-of-principle experiments are a first step towards using VLA-4 transfected neural precursor cells for improved cell therapy of inflammatory brain diseases such as multiple sclerosis following systemic administration.

References:(1). P. Walczak *et al.*, *Stroke* **39**, 1569 (2008). (2). M. J. Elices *et al.*, *Cell* **60**, 577 (1990).

Supported by: NMSS PP1491, NMSS RG3630, MSCRF 104062, TEDCO ESC-06-29-01.