High-Efficiency Targeting of Glial Precursor Cells to Inflammatory Brain Lesions Using the VLA4-VCAM1 Cell Adhesion Pathway: Real-Time MR Monitoring of Instant Cell Engraftment

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Introduction: Cell-based therapies promise effective treatment for neurodegenerative disorders. However, a major challenge to the clinical translation of these therapies is the efficient delivery of therapeutic cells to the diseased tissues. Unfortunately, direct stereotactic injection - the standard delivery method – has various limitations, such as needle-induced tissue damage or limited areas of cellular engraftment. The later is of particular concern in the treatment of diseases characterized by multifocal or diffuse lesions, which require a broad distribution of the transplanted cell graft. Intra-arterial cell delivery presents a novel approach to enable widespread delivery of stem cells to tissues of the central nervous system. In this study, we investigated whether targeted, intra-arterial delivery of stem cells to inflamed cerebral endothelium could be achieved. Here we genetically engineered neural stem cells to mimic immunologically active lymphocytes, which home to inflamed endothelial cells via the VLA-4/VCAM-1 adhesion pathway. To mitigate intra-arterial cell delivery's associated risk of microembolism, which results from excessive cell engraftment; we employed real-time MRI cell monitoring in order to have better control over the cell adhesion and to assure the safety of this approach. We hypothesized that over-expression of VLA-4 would allow intra-arterially delivered cells to bind specifically to activated cerebral endothelium and that this binding could be monitored non-invasively and in real-time with MRI.

Methods: **Cell Engineering**: Human glial restricted precursors (hGRPs) were genetically engineered to transiently express both subunits of VLA-4 ($\alpha4$ & $\beta1$) via lipofectamine transfections. Engineered hGRPs were labeled with red fluorescent iron oxide nanoparticles (Rhodamine B Molday ION) for MR imaging. **In Vitro Adhesion Assay:** A Microfluidics cell adhesion assay was employed for in vitro assessment of hGRP cell adhesion following VLA-4 integrin overexpression. A microfluidics chamber containing multiple 200x100µm channels was fabricated; the channels were coated with human brain endothelial cells (HBECs); the HBECs in some channels were exposed to Tumor Necrosis Factor alpha (TNFα) containing media to activate VCAM-1 expression; and either naïve or VLA-4 expressing hGRPs were separately perfused through individual channels. Time-lapse microscopy was used to quantify cell adhesion. **Animal Studies**: To explore the feasibility of targeted, intra-arterial cell delivery to inflamed brain endothelium; adult Lewis rats (n=6) were given i.p. injections of lipopolysaccharide (LPS, 6mg/kg) to induce global endothelial expression of VCAM-1. Control rats (n=6) did not receive LPS. The internal carotid arteries of the rats were cannulated and the arteries supplying blood to regions outside of the brain were ligated. Once cannulated, the rats were placed in a 9.4T Bruker MRI scanner to monitor the engraftment of SPIO-labeled hGRPs. T2*-weighted images (TE/TR=4/300 ms, AV=4, RES=200x200 µm) were obtained for each animal prior to cell injection, after administering either $1x10^6$ VLA-4 overexpressing or naïve cells, after administering an additional $1x10^6$ cells, and then 10 and 20 minutes after administering the final $1x10^6$ cell injection. Within one hour after obtaining the final image, rats were transcardially perfused and brains were processed for immunohistochemistry. Statistical analysis of the MR images was performed using a pixel-by-pixel t-test comparing the signal intensity of the images obtained before and after cell injection. The average number of significant pixels per animal in each experimental group was quantified and compared.

Results: Immunohistochemistry and fluorescent microscopy demonstrated that hGRPs were successfully transfected to express both $\alpha4$ (Fig. A) & $\beta1$ (Fig. B) subunits of VLA-4 and confirmed that hGRPs were efficiently labeled with iron oxide nanoparticles. The the in vitro microfluidics cell adhesion assay showed that the experimental group with VLA-4 expressing hGRP and VCAM-1 overexpressing endothelium (+VLA-4/+TNFα) had a significantly higher amount of adherent cell than any control groups (-VLA-4/+TNFα, +VLA-4/-TNFα, & -VLA-4/-TNFα [Fig. C]). In vivo experiments following intra-arterial cell delivery demonstrated that VLA-4 expressing hGRPs infused into LPS-treated rats had a significant number of persistent hypointense pixels in the ipsilateral hemisphere of the brain (Fig. E), suggesting a robust, widespread binding of cells. While control groups (-VLA-4/-LPS [Fig. D] -VLA-4/+LPS, +VLA-4/-LPS) displayed some hypointense pixels in the ipsilateral hemisphere immediately after cell infusion, they cleared over time, suggesting only a transient binding in these groups. Immunohistochemistry validated the imaging results, with the rhodamin B+ cells in the ipsilateral hemisphere co-localizing with the hypointense pixels on MRI.

Conclusion: We demonstrated that targeted intra-arterial delivery of VLA-4 expressing hGRPs to inflamed endothelium is feasible and can be monitored in real time using MRI. Immunohistochemical data corroborated that persistent hypointense pixels in MRI data correspond to the presence of VLA-4-expressing hGRPs throughout the ipsilateral hemisphere. Future work will investigate whether VLA-4 expressing stem cells can cross the blood brain barrier and home to areas of brain pathology, with the work described here representing a first critical step to achieve this goal.

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