

Functional Activation of Human Adipose-Derived Stromal Cells by Hypoxia for Adhesion to Vascular Proteins

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INTRODUCTION: A number of studies have shown that human adipose-derived stromal cells (hASCs) are able to affect microvascular growth and remodeling at sites of inflammation or injury when injected into the circulation *in vivo*, however; the mechanisms by which hASCs localize to these areas are unknown. It has also been shown that hypoxia can be used to activate hASCs in this regard. Therefore, the goal of this study is to identify possible molecular mechanisms by which hASC homing might occur *in vivo* and to determine whether hypoxic preconditioning is an effective means of increasing therapeutic cell delivery.

METHODS: Liposuction- and panniculectomy-isolated hASCs were grown on culture plastic and introduced into a parallel-plate flow chamber at a flow rate of 1 dyn/cm² (P=3). 48 hours prior to use, cells were either kept in normal culture conditions or pretreated by culture in an atmosphere of less than 2% O₂. Human proteins (type I collagen, fibronectin, VCAM-1, ICAM-1, E-selectin, P-selectin, and L-Selectin) were individually adsorbed to the bottom surface of the flow chamber, and hASCs were assayed for their ability to adhere both under laminar flow conditions and after 6 minutes of no-flow conditions. Alternately, rat heart microvessel endothelial cells (RHMVECs) were cultured on fibronectin and used as the bottom surface of the flow chamber instead of individual proteins. Endothelial cells were pretreated with either PBS or TNF-alpha prior to introduction of hASCs. Interactions were interpreted as being either positive or negative, while the frequency of adhesion events was quantified.

RESULTS: Our results show that significantly more hASCs adhere to surfaces coated with type I collagen, VCAM-1, ICAM-1, and fibronectin under static (no-flow) conditions and to VCAM-1-coated surfaces under laminar flow conditions compared to TWEEN controls. After 48 hours of hypoxic culture, only cells isolated by liposuction showed an increase in frequency of adhesion events (increases were observed on VCAM-1 substrates in static adhesion assays and both VCAM-1 and ICAM-1 substrates in laminar adhesion assays). Preliminary adhesion testing on endothelial monolayers indicate that hypoxic preconditioning increases adhesion of hASCs to RHMVECs, and that this trend may be further increased by exposing the endothelial cells to TNF-alpha.

CONCLUSIONS: These results show that hASCs have the ability to bind several proteins present in areas of injured or inflamed endothelium and can use them to adhere under post-capillary venular flow conditions. Hypoxia has also been shown to be an effective means of increasing hASC binding to these proteins.