

The Potential of Adipose-Derived Regenerative Cell Therapy for Peripheral Vascular Disease

Peripheral vascular disease (PVD) resulting in impairment in perfusion to the limbs is a growing problem that affects approximately 3-10% of the world's population¹. These patients fall into a progressive disease spectrum that includes asymptomatic disease, intermittent claudication, and chronic limb ischemia. Treatment of this disease can be separated into two approaches; risk factor reduction (including diabetes, hypertension, and smoking) and revascularization through approaches such as bypass grafting, endarterectomy, and stenting. Unfortunately there are few options for those patients for whom these approaches are either not available or have failed. For this reason many investigators have begun evaluating and applying cell therapy using cell populations that have demonstrated *in vitro* and *in vivo* potential for promoting angiogenesis, arteriogenesis (formation of collateral arterioles), and wound healing (see Attanasio and Snell² and Aranguren *et al*³ for recent reviews of this literature). The purpose of this document is to briefly summarize published clinical experience to date and to discuss the potential of cells derived from human adipose tissue in this context.

CLINICAL STUDIES IN PERIPHERAL VASCULAR DISEASE

The recent review by Aranguren *et al*³ contains a comprehensive list of 39 published studies encompassing 775 patients with PVD who have been treated with either bone marrow or peripheral blood mononuclear

cells (MNC). The authors of this review note that most studies are limited by inclusion of only a small number of patients, absence of randomization and controls, absence of blinding of investigators and/or patients, and relatively short duration of follow-up. They also note that most of the patients enrolled (>80%) suffered from chronic limb ischemia rather than intermittent claudication. Having acknowledged these limitations the authors go on to note that the majority of studies (35 of 39) showed clinically relevant improvement in endpoints such as ankle-brachial index, transcutaneous oxygen pressure, frequency of amputation, and pain. However, three studies using bone marrow MNC (total 30 patients) and one study using blood MNC (6 patients) showed adverse events that included worsening of symptoms and, in one patient with thromboangiitis obliterans, sudden

death. Despite this the data are, on the whole, encouraging though they demonstrate the need for care in selection of study inclusion criteria such as disease type and severity, cell source, and route of administration. For example, 33 of the 39 studies examined only the intramuscular route of administration; five applied dual intramuscular and intra-arterial administration, one used only intra-arterial delivery. This is important because there are several potential mechanisms by which benefit may be achieved (Table 1) including two primary mechanisms by which blood flow can be improved—angiogenesis which works in response to ischemia by development of new capillaries, and arteriogenesis which involves expansion of collateral vessel flow induced by shear force⁴. Delivery to the ischemic muscle eliminates (or at least substantially reduces)

Table 1: Potential Mechanisms by Which Improvement May Be Achieved

Effect	Mechanism of Action	Benefit
Arteriogenesis: expansion of arterioles that bypass the site of occlusion	Indirect: Growth factor-induced recruitment of effector cells Direct: Formation of new pericytes and/or vascular smooth muscle cells	Increased blood flow
Angiogenesis: expansion of the capillary bed within the ischemic tissue	Indirect: Growth factor-induced endothelial cell proliferation Direct: Formation of new endothelial cells	Increased blood flow
Myogenesis: formation of new muscle tissue	Direct: Formation of new skeletal muscle cells	Restoration of muscle
Anti-apoptosis: prevention or inhibition of programmed cell death	Indirect: preservation of myocytes by expression of anti-apoptotic growth factors	Preservation of muscle
Wound Healing	Indirect: stimulation of healing by modulation of inflammation and recruitment of regenerative cells	Healing of ischemic wounds

the ability of the cell population to contribute to arteriogenesis thereby eliminating a potential mechanism of action.

ADIPOSE TISSUE AS A SOURCE OF CELLS FOR PVD CELL THERAPY

While there are, as yet, no clinical studies using adipose tissue-derived cells in the treatment of PVD there are good reasons for considering adipose tissue as a potentially valuable source of cells for this purpose. Indeed, both freshly isolated and cultured adipose cells have been shown to improve perfusion in animal models of limb ischemia⁵⁻⁹. Interestingly, in the one study that performed a head-to-head analysis, animals treated with cultured human Adipose-Derived Stem Cells (ADSC) had better blood flow than those treated with marrow mesenchymal stem cells (MSC)⁸. ADSC have been shown to be capable of mediating each of the activities listed in Table 1. They express arteriogenic factors including Placental Growth Factor and Hepatocyte Growth Factor^{10,11}, angiogenic factors including Vascular Endothelial Growth Factor^{11,12}, they have been shown to be capable of differentiating into cells of the endothelial and skeletal muscle lineages^{5,13,14}, and they express factors that inhibit apoptosis and modulate inflammation^{10,11,15,16}. *In vivo* studies have also demonstrated the ability of ADSC to stimulate arteriogenesis and angiogenesis⁵⁻⁸, to promote myogenic recovery *in vivo*^{17,18}, to inhibit apoptosis^{6,19}, to modulate inflammation^{15,16,20,21}, and to improve wound healing²²⁻²⁴.

ADSC are generated by digesting adipose tissue, and culturing the non-buoyant (adipocyte-depleted) fraction. A number of groups have now shown that the frequency of stem cells within this non-buoyant fraction is on the order of 1% of nucleated cells²⁵⁻²⁷, a number that is considerably higher than the 0.0004% present within age-matched marrow^{28,29}. The freshly isolated population also contains EPCs⁵. Further, Kondo *et al*

have shown that infusion of ADSC in animals with surgically-induced hind limb ischemia leads to an increase in circulating EPC⁷.

This fact, combined with ability to obtain relatively large volumes of tissue with minimal morbidity through liposuction, suggests that this population (referred to as Adipose-Derived stem and Regenerative Cells; ADRCs) may be a superior source of cells for the treatment of PVD. As mentioned above, the ability of freshly isolated cells to mediate improvement in limb ischemia has been demonstrated in an animal model⁵. Further, non-cultured adipose cells have been shown to be effective in preclinical models of wound healing³⁰ and myocardial ischemia^{31,32}, and have been used in human clinical case reports of periorbital wrinkle repair³³, tracheomediastinal fistula repair³⁴, and calvarial repair³⁵. Clinical studies with freshly isolated adipose-derived cells are currently underway in several disease indications including Liver Cirrhosis (Clinicaltrials.gov Study Identifier NCT00913289), Acute Myocardial Ischemia with ST segment elevation (Study Identifier NCT00442806), Non-Revascularizable Myocardial Ischemia (Study Identifier NCT00426868), and Breast Reconstruction following partial mastectomy (Study Identifier NCT00616135).

PRACTICAL ISSUES ASSOCIATED WITH CLINICAL USE OF FRESHLY ISOLATED CELLS

While the data described above demonstrate a clear potential for freshly isolated adipose-derived stem and regenerative cells (ADRC) in PVD there are factors that need to be considered before initiation of clinical studies. First, as with almost all preclinical models, the results described above were obtained using otherwise healthy animals subjected to acute limb ischemia. This is a distinctly different setting than that in the clinic where risk factors such as diabetes and age may negatively impact the ability of the

cells to act or the treated limb to respond. However, it should be noted that bone marrow and peripheral blood-derived cells and the preclinical models used to test them are subject to the same limitations. Despite these limitations the early clinical studies summarized above have shown that patients treated with marrow or blood-derived MNC have shown improvement.

Second, adipose tissue is a solid organ rather than a suspension of single cells like marrow or blood. Consequently, cells from adipose tissue must be obtained by enzymatic digestion. This requires application of clinical grade enzymes and reagents in a tissue processing method that minimizes the risk of contamination of the cells during processing. Further, for intra-arterial delivery the systems and methods used to generate and deliver these cells should be validated to ensure that delivery can be achieved without risk of embolism or other adverse event.

SUMMARY

A majority of studies with bone marrow or cultured peripheral blood mononuclear cells showed clinically relevant improvement in endpoints such as ankle-brachial index, transcutaneous oxygen pressure, frequency of amputation, and pain. Cells obtained from human adipose tissue have also been shown to improve perfusion in animal models of limb ischemia. Data suggests that cell culture may not be necessary in order to obtain a clinically effective population of cells, and that the process of cell culture may even be deleterious by eliminating other cell populations that can contribute to a positive outcome. By eliminating cell culture, clinical use of freshly isolated adipose cells provides a simpler and less expensive alternative to other cell sources. However, such use requires application of systems and methods for tissue collection and processing that are validated to provide a suitable cell output.

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