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Current Opinion in Pharmacology

Cell therapy for peripheral artery disease

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Patients with severe peripheral artery disease (PAD) who are not candidates for revascularization have poor prognosis. Cell therapy using peripheral blood-derived or bone marrow-derived mononuclear cells, mesenchymal stem cells, or marker-specific subsets of bone marrow cells with angiogenic properties may hold promise for no-option PAD patients. Injected cells may exert beneficial actions by enhancing local angiogenesis (either through maturation of endothelial progenitors, or through secretion of angiogenic mediators), or by transducing cytoprotective signals that preserve tissue structure. Despite extensive research, robust clinical evidence supporting the use of cell therapy in patients with critical limb ischemia is lacking. Larger, well-designed placebo-controlled clinical trials did not support the positive results of smaller less rigorous studies. There is a need for high-quality clinical studies to test the effectiveness of cell therapy in PAD patients. Moreover, fundamental cell biological studies are needed to identify the optimal cell types, and to develop strategies that may enhance homing, survival and effectiveness of the injected cells.

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Current Opinion in Pharmacology 2018, **39**:27–34

This review comes from a themed issue on **Cardiovascular and renal**

Edited by **Dimitris Tousoulis** and **Evangelos Oikonomu**

https://doi.org/10.1016/j.coph.2018.01.005

Introduction

Lower extremity peripheral artery disease (PAD) is a major health burden, representing the third-leading cause of cardiovascular morbidity related to atherosclerotic disease after coronary disease and stroke. The prevalence of PAD rises sharply with age, affecting almost 20% of the US population at the age of 80 [1,2]. Epidemiologic studies have highlighted the global impact of the disease, suggesting dramatic recent increases in PAD prevalence in low and middle-income countries, and supporting the notion that we are faced with a global PAD pandemic, affecting more than 200 million men and women in both high-income countries and in the developing world [3]. Considering the mortality, morbidity and disability associated with PAD, there is an urgent need to develop new therapeutic strategies in order to prevent development and progression of the disease, and to treat life-threatening or limb-threatening complications. Experimental studies and early stage clinical trials have suggested that cell therapy may be a promising new approach for patients with PAD [4]. The current review manuscript discusses the potential role of cell therapy approaches in the treatment of PAD.

The pathophysiologic basis of PAD

The clinical manifestations of PAD reflect the consequences of a mismatch between blood supply and demand [5,6]. The typical symptom of PAD is intermittent claudication, a characteristic squeezing leg pain associated with walking and relieved by rest. In normal subjects, exercise is associated with marked increases in peripheral artery blood flow and limb oxygen uptake, driven by increased metabolic demand. In contrast, in PAD patients, fixed stenotic lesions in peripheral arteries limit blood flow, reducing the supply of the affected territory and leading to ischemia. Although the main cause of supply and demand disequilibrium in PAD patients is structural, excessive vascular tone due to activation of neurohumoral pathways, or impaired vasodilatory responses due to endothelial dysfunction may increase vascular resistance, further limiting blood flow in the extremity [7].

Repetitive limb ischemia followed by reperfusion causes mitochondrial dysfunction in skeletal myocytes and triggers generation of reactive oxygen species (ROS), leading to chronic structural changes in the skeletal muscle. ROS-driven apoptosis of skeletal myocytes leads to a reduction in skeletal muscle mass and is accompanied by fatty infiltration, impaired peripheral nerve function and fibrosis [8,6,9,10]. These pathologic alterations are associated with chronic skeletal muscle dysfunction and significant functional impairment. In a subset of patients, chronic ischemia follows an aggressive clinical course that culminates in the development of rest pain and significant tissue loss, a condition termed critical limb ischemia (CLI). Traditional treatment strategies in patients with CLI are focused on surgical bypass or endovascular interventions, aimed at restoring perfusion to prevent amputation of the affected limb [11]. However, a significant percentage of CLI patients do not have revascularization

options; these patients have poor prognosis and often require amputation.

Cell therapy as a therapeutic approach in PAD

Considering the limited treatment options for patients with severe PAD, the rationale for cell therapy approaches is sound. In patients with severe atherosclerotic disease of the native arterial circulation, administration of cell populations capable of activating an angiogenic program may result in formation of neovessels, improving perfusion of the affected limb. Increased blood supply may prevent ischemic episodes and may even contribute to restoration of normal skeletal muscle structure. It should be emphasized that any beneficial effects of cell therapy in PAD may not be necessarily due to incorporation of the cells into the vascular network, but may involve paracrine effects mediated through secretion of angiogenic mediators. Cell therapy may also activate yet unidentified cytoprotective and regenerative pathways that may improve limb function through effects independent of neovessel formation.

A growing body of experimental and clinical evidence suggests that cell-based therapy may hold promise in patients with severe PAD. Experimental investigations have used models of hindlimb ischemia to study the effectiveness of cell therapy approaches in promoting angiogenesis and in attenuating skeletal muscle injury. On the other hand most clinical studies investigating the effectiveness of cell therapy in patients with CLI are small phase I or II clinical trials. Considering the variable approaches used by different groups, the wide range of cell types used, and the absence of standardized protocols for characterization of the cells and for evaluation of clinical outcome, there is substantial uncertainty regarding the effectiveness of various cell types in PAD patients.

The therapeutic potential of endothelial progenitor cells (EPCs)

The identification of EPCs, as bone marrow-derived progenitors, that home to sites of injury and may contribute to angiogenesis [12] provided a strong rationale for the use of cell therapy in PAD patients. It should be noted that, despite progress in understanding the mechanistic basis of the angiogenic response, the contribution of blood-derived progenitors in neovessel formation following injury remains controversial. In a mouse model of hindlimb ischemia, both marrow-derived and non-marrow derived endothelial progenitor populations have been implicated in formation of neovessels [13]. Despite the recent use of lineage tracing approaches in mouse models, the origin of neovascular endothelial cells in sites of injury remains controversial. Studies in the ischemic myocardium suggested a significant contribution of mesenchymal cells that undergo conversion into endothelial cells through a p53-dependent mechanism [14]. In contrast, other investigations suggested that practically all neovessels in the injured myocardium are derived from pre-existing endothelial cells, and not through lineage transdifferentiation [15]. It is plausible that the relative contributions of various cellular sources in the angiogenic response may be dependent on the pathophysiologic context and on the site of injury. Unfortunately, lineage tracing studies investigating the cellular origin of angiogenic vascular cells in ischemic skeletal muscle have not been performed.

Regardless of the origin of endogenous angiogenic endothelial cells in the ischemic limb, local injection of circulating endothelial progenitors would be expected to enrich the ischemic site with a pool of angiogenic cells, promoting neovessel formation and improving function. To achieve this goal, several different approaches have been used, injecting unselected or marker-specific mononuclear cells from the bone marrow, or the peripheral blood. These populations may contain a subset of bona fide endothelial progenitors that incorporate to the vascular network forming new vessels, and other cell types that may contribute to the angiogenic process by secreting cytokines, angiogenic growth factors, matrix metalloproteinases, matricellular proteins, or miRNA-containing exosomes (Figure 1) [16•,17,18].

Bone marrow-derived mononuclear cells (BM-MNC) and peripheral blood-derived mononuclear cells (PB-MNC) in the treatment of PAD

Unselected mononuclear cells harvested, derived either from the bone marrow or the peripheral blood, represent a mixture of monocytes, non-hematopoietic stromal cells (including mesenchymal stem cells), and EPCs and have been used in both experimental models of limb ischemia and in patients with PAD. In the TACT (Therapeutic Angiogenesis using Cell Transplantation) study, injection with autologous BM-MNCs in the gastrocnemius of the ischemic limb reduced rest pain and increased transcutaneous oxygen pressure in patients with CLI; improvement was sustained for at least 24 weeks [19]. Over the last few years, several additional clinical trials suggested that intramuscular or intra-arterial injections of BM-MNCs or PB-MNCs in patients with CLI are safe, and may reduce rest pain and improve ulcer healing (Table 1) [20,21]. In many studies, improved clinical outcome was associated with objective evidence of enhanced perfusion. However, in most studies, effects on amputation rates did not reach statistical significance. Some of the larger, more rigorous, and well-designed studies failed to support the beneficial effects [22•], suggested by smaller nonplacebo controlled investigations [20]. The conflicting findings may reflect the clinical improvement observed in placebo-treated patients [22•], and emphasize the importance of rigorous design, large population size and accurate blinding in order to test the