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Mesenchymoangioblast-derived mesenchymal stromal cells inhibit cell damage, tissue damage and improve peripheral blood flow following hindlimb ischemic injury in mice

JILL M. KOCH[1], SARITHA S. D'SOUZA[2], DENISE J. SCHWAHN[3], IAN DIXON[4] & TIMOTHY A. HACKER[1]

[1]*Department of Medicine, University of Wisconsin–Madison, Madison, WI, USA,* [2]*Wisconsin National Primate Research Center, University of Wisconsin–Madison, Madison, WI, USA,* [3]*Research Animal Resources Center, University of Wisconsin–Madison, Madison, WI, USA, and* [4]*Cynata Therapeutics Limited, Armadale, Victoria, Australia*

Abstract

Background aims. Existing treatments have limited success in modifying the course of peripheral artery disease, which may eventually lead to limb-threatening ulcers and amputation. Cellular therapies have the potential to provide a new treatment option for this condition, but isolation of cells by conventional means has limitations with respect to reproducibility and scalability. *Methods.* Induced pluripotent stem cells (iPSCs) were differentiated into precursor cells known as mesenchymoangioblasts (MCAs) and subsequently into mesenchymal stromal cells (MSCs). Hindlimb ischemia in mice was created by ligating both the iliac and femoral arteries of one hindlimb. Immediately after surgery, each animal received intramuscular injections of 5×10^6 cells or media in the ischemic limb. Toe necrosis was assessed visually, and hindlimb blood flow was measured by laser Doppler using a set region of interest (ROI) and by tracing the entire foot. Myofiber heterogeneity, nuclear centralization, fatty degeneration, fibrosis and capillary angiogenesis in the gastrocnemius muscle were assessed histologically. *Results.* Blood flow in the MCA-derived MSC-treated animals was higher at each day ($P < 0.006$), and these mice recovered faster than control animals (3.6 vs. 2.5 for set ROI; 7.5 vs. 4.1 foot tracing; slope; $P < 0.001$). There was significantly less myofiber heterogeneity, nuclear centralization, fatty degeneration and fibrosis in MCA-derived MSC-treated animals, indicating less tissue damage. *Discussion.* MCA-derived MSCs improved limb blood flow, reduced necrosis and maintained muscle mass and gross muscle appearance. We conclude that MCA-derived MSCs have a significant and protective effect against ischemic insults.

Key Words: *critical limb ischemia, hindlimb ischemia, induced pluripotent stem cells, mesenchymal stem cells, peripheral arterial disease, stem cell therapy*

Introduction

Peripheral artery disease (PAD) is described by impaired or reduced blood flow to the lower extremities as a result of narrowing vessels and/or atherosclerosis [1]. PAD affects 3–12% of the U.S. population and is implicated as an independent risk for cardiovascular disease, myocardial infarctions and stroke [1–3]. The degree of PAD depends on the health of the vessels, amount of arterial occlusion and the metabolic demand and lack of nutrient supply to the ischemic tissue in the lower extremities [4]. Current treatments for PAD include lifestyle modification, medication, arterial bypass, angioplasty, stent placement and/or amputation if tissue damage is severe enough [1]. Improving blood flow to the ischemic extremities decreases the severity of PAD and reduces a patient's risk for other cardiovascular diseases. However, existing treatments have had limited success in halting or reversing the course of the disease, and the condition frequently progresses to critical limb ischemia (CLI), tissue necrosis, and loss [1,3]. Consequently, there remains a significant unmet need for new therapies for this debilitating condition.

In recent years, there has been extensive interest in the development of novel cell-based therapies for a wide range of conditions, including PAD and CLI. Numerous studies have shown efficacy of mesenchymal stromal cells (MSCs) in the treatment of rodent models of CLI via increased limb perfusion and

Correspondence: **Timothy A. Hacker**, PhD, Department of Medicine, University of Wisconsin–Madison, 1300 University Avenue, Madison, WI 53706, USA.
E-mail: th2@medicine.wisc.edu

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capillary density and decreased necrosis and limb loss [5]. Various other cell-based therapies, including bone marrow mononuclear cells, peripheral blood mononuclear cells and endothelial progenitor cells, have also previously shown positive effects in animal models of CLI [6]. A number of clinical studies of cell therapies for CLI and related conditions have also been conducted, with promising results [5,6].

A key advantage of MSCs is that they can be administered to allogeneic recipients without matching because they do not express HLA Class II antigens and therefore do not elicit alloreactive lymphocyte responses [7]. Furthermore, although MSCs have the potential to differentiate into a range of cell types, the mechanism of action of allogeneic MSC therapy is believed to be primarily mediated via paracrine activity, direct interactions with endogenous cells and immunomodulatory effects, rather than via differentiation and engraftment [5,8]. These attributes give rise to the possibility of manufacturing large batches of MSCs for use in an "off-the-shelf" manner, which would have significant logistical and commercial advantages over patient-specific therapies. In contrast, cell types that express HLA Class II antigens are generally only suitable for autologous use.

Previously investigated MSCs have typically been isolated from donated tissue (either autologous or allogeneic). Although impressive data have been generated using MSCs derived in this way, there is a limit to the culture expansion capacity of MSCs, and hence the number of clinical doses that can be generated from a single donation [9]. Consequently, to produce a large number of MSC doses using donated tissue as starting material, an ongoing supply of new donations would be required. Reliance on donors has several major limitations, including logistical challenges, cost, donor-to-donor variability, intra-population heterogeneity, limited expansion potential and potential immunogenicity [10]. The impact of these limitations would be magnified when manufacture at commercial scale is required.

Pluripotent stem cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have the potential to serve as a limitless starting material for the production of cell-based therapies. For example, a recent publication described a system capable of GMP-compliant 10^{72}-fold expansion of iPSCs, without loss of the cells' ability to differentiate into all three germ-layers [11]. Consequently, a single iPSC bank offers the potential to generate an effectively infinite supply of therapeutic cells, without changing the starting material.

It has previously been shown that ESC- and iPSC-derived endothelial cells [12,13] and vasculogenic pericytes [14] had positive effects in murine models of hindlimb ischemia. However, the authors of all three of those studies suggest that the clinical potential of these cell types lies in autologous use.

iPSC-derived MSCs have also been shown to be effective in attenuating hindlimb ischaemia in a murine model [15]. The method used to derive MSCs from iPSCs in that study relies on spontaneous iPSC differentiation, followed by incubation with CD24-phycoerythrin and CD105-fluorescein isothiocyanate and fluorescence-activated cell sorting (FACS) to isolate CD105 + and CD24– cells. Although the resulting cells generally display MSC-like characteristics, expression of CD90, a marker typically associated of MSCs [16], appears to be low [17]. There are two major sources of MSCs: neural crest [18] and mesoderm [19]. It is unclear which source spontaneously differentiated iPSC-derived MSCs arise from. This is potentially significant because there is evidence that MSCs from different sources have distinct, specialized roles [20]. Furthermore, because this method relies on cell sorting, there may be a risk of inadvertent retention of non-target cell types. Consequently, we believe there remains a need to develop a method of producing MSCs of defined mesodermal origin in a consistent and commercially scalable manner.

The mesenchymoangioblast (MCA) has been identified as a common mesodermal precursor for endothelial cells and MSCs in human pluripotent stem cell cultures [21]. In response to fibroblast growth factor-2 (FGF-2), MCAs generate mesenchymal colonies, which give rise to MSCs of defined, mesodermal origin. These MCA-derived MSCs have a typical CD73 + CD105 + CD90 + CD31–CD45–MSC phenotype and have the capacity to differentiate into bone, cartilage and adipose tissue, which is consistent with the International Society for Cellular Therapy's minimal criteria for defining multipotent MSCs [16].

This novel approach to generating MSCs has several important advantages:

1. A homogenous population of clonal MSCs with well-defined origin and enormous expansion potential is generated; each MCA can yield up to 10^{22} MSCs.
2. Contamination with immune cells is completely excluded since cells are induced from mesodermal progenitors before immune cells are formed in cultures.
3. Inter-donor and intra-donor variability is eliminated because a single donation can be used to generate a bank of iPSCs, which can then be used to produce a limitless quantity of MCA-derived MSCs.

Because several previous studies have shown positive effects of MSCs in murine models and in human subjects, we decided to investigate whether

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