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Non-reconstructable peripheral vascular disease of the lower extremity in ten patients treated with adipose-derived stromal vascular fraction cells

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ABSTRACT

We present a series of ten patients with non-reconstructable peripheral vascular disease (PVD), secondary to arteriosclerosis (AS) and/or diabetes mellitus (DM), treated with local injection of non-expanded autologous, adipose-derived stromal vascular fraction (SVF) cells for the purposes of enhancing neovascularization and chronic wound healing. Adipose tissue was surgically harvested and processed to yield the heterogeneous SVF cells for immediate point-of-care injection. The gastrocnemius muscles and ulcers or wounds where present were locally injected with the resulting SVF. Response to treatment was evaluated both clinically based on pain-free ambulation, wound healing capacity over time and ankle/brachial index (ABI) measurements, and by imaging using MRI-based angiography. All patients exhibited clinical improvement (reduction in rest pain and claudication and improvements in ABI), with imaging signs of neovascularization in the majority (5 of 6) of patients in whom the evaluation was feasible. Similarly, 5 of 6 chronic wounds healed without further surgical intervention. This series highlights the utility of non-expanded adipose-derived heterogeneous SVF cell population processed at the point-of-care, to treat patients with end-stage PVD as an alternative to palliation or amputation.

1. Introduction

Peripheral vascular disease (PVD) due to arterial insufficiency is a common problem, affecting up to 6.9% of individuals >45 years of age (Stoffers et al., 1996). The most common causes in the U.S. population are macrovascular lesions due to arteriosclerosis (AS) (70%–80%), and microvascular lesions due to diabetes mellitus (DM) (20%–30%) (Marso and Hiatt, 2006), with a large proportion of patients presenting concomitant infrapopliteal arteriosclerotic disease (Chen et al., 2013). Local and regional ischemia follow a clinical progression, starting with claudication, referred as muscle pain distal to the occlusion site brought on by predictable levels of exertion. Later, critical limb ischemia (CLI) presents with rest pain typically at the level of the metatarsal foot, unrelenting, and relieved by dangling the foot, and when blood flow falls below a critical perfusion pressure, ulceration or frank ischemic necrosis ensues. This symptomatic progression can be classified using the criteria of Fontaine and Rutherford (Nehler et al., 2003; Rutherford et al., 1997).

The diagnosis is confirmed by vascular examination including pulse doppler and the ankle/brachial index (ABI) calculation (the ratio between the systolic pressures at the ankle and the mid-arm). Normal ABI or stage I is ≥1.0. Stage II occurs at an ABI of 0.9, while rest pain at stage III is characterized by an ABI of 0.5. Additional imaging-based modalities, such as computerized tomography (CT) angiography and/or magnetic resonance (MRI) angiography, localize sites of occlusion and help to determine if surgical bypass is feasible.

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Thirty percent (30%) of patients with CLI present with non-reconstructable disease, a situation particularly critical for diabetic patients, due to calcific and fibrocalcific disease in the distal vasculature (Bishop et al., 2008; Dormandy and Rutherford, 2000; Norgren et al., 2007). Furthermore, 40% of diabetic patients diagnosed with CLI will progress to tissue necrosis and/or gangrene, versus 9% of patients with AS disease (Lévigne et al., 2013; Tobalem et al., 2015). Given these numbers, the need exists for novel cost-effective treatments for CLI. Current treatment alternatives such as percutaneous transluminal angioplasty (PTA) have a variable therapeutic efficacy, with patients resulting in partial revascularization and higher rates of restenosis, especially in infrapopliteal arteries (Dormandy and Rutherford, 2000). In addition, endovascular revascularization procedures are precluded in about 30% of patients with CLI due to high operative risks and/or unpropitious vascular anatomy (Mamidi et al., 2012). In an effort to reduce the already high incidence of amputations and other complications, alternative approaches have been proposed. Gene therapy was introduced as a way to provide angiogenic growth factors (e.g.: VEGF, HIF1a, HGF, FGF1), based on their known inductive capacity to form vascular structures (Sedighiani and Nikol, 2011), a fact initially appreciated in cardiac ischemia (Isner and Asahara, 1999). Cell-based therapies have also been proposed as promising alternatives, on the basis of providing cell progenitors capable of fabricating new blood vessels in ischemic areas. For instance, bone marrow-derived and peripheral blood circulating (with or without previous mobilization) mononuclear cells (MNCs) containing a population of endothelial progenitor cells (EPCs) have been used in various clinical trials for vascular insufficiency (evaluating safety and efficacy) with variable degrees of success (reviewed in Raval and Losordo, 2013). In those studies, intra-muscular (IM) injections of unselected or selected ($CD34^+$) cells ameliorate the symptoms associated with poor distal blood supply.

On the other hand, transplantation of adult mesenchymal stem cells (MSCs) has great promise based on their regenerative capacity. These cells secrete a wide range of growth factors and cytokines acting in a paracrine fashion. Many of these factors are proangiogenic (e.g.: VEGF), inducing the formation of new blood vessels, while others assist in the repair process of injured tissues (Caplan and Correa, 2011; Caplan and Dennis, 2006). MSCs can be obtained from a variety of tissues including bone marrow and adipose tissue, the latter being particularly rich in MSCs (Zuk et al., 2001). MSCs are readily obtained, as a component of the adipose tissue-derived multicellular Stromal Vascular Fraction (SVF), after enzymatic digestion and centrifugation of lipoaspirate (Bourin et al., 2013). SVF is a heterogeneous population of MNCs that includes adipose-derived stem cells (ADSCs) of mesenchymal phenotype (analogous to MSCs), endothelial progenitor cells (EPCs), hemopoietic progenitors, monocytes, leukocytes and pericytes (Amos et al., 2008; Nguyen et al., 2016; Guo et al., 2016). Pericytes represent the perivascular phenotype of native MSCs (Crisan et al., 2008; da Silva Meirelles et al., 2008, 2006; Sacchetti et al., 2007) and constitute a key cell component of SVF during angiogenesis, as they stabilize nascent blood vessels (Armulik et al., 2005, 2011; von Tell et al., 2006).

A number of animal models of CLI, including the hindlimb ischemia model after femoral artery ligation in rats (Rochester et al., 1994) and rabbits (Hao et al., 2014), have been proven useful to assess the effects of various cell types and to study potential mechanisms of action. For instance, IM injections of culture-expanded ADSCs increased flow and induced a higher systemic presence of EPCs (Kondo et al., 2009). Iwase et al. (2005) demonstrated the superior angiogenic potential of bone marrow-derived MSCs over MNCs; the former were able to differentiate into both endothelial cells and vascular smooth muscle cells. Finally, Hao et al. (2014) reported the neovascularization effect of both ADSCs and bone marrow-derived MNCs. In these and other studies, ADSCs came to be recognized as a source for angiogenic factors acting through a paracrine mechanism, and in concert with other cellular players (e.g.: EPCs and macrophages) (Nakagami et al., 2005; Rehman et al., 2004; Sumi et al., 2007).

Pre-clinical data have prompted multiple groups to explore the feasibility, safety and efficacy of bone marrow-derived cell-based therapy for PVD, through the design and execution of small clinical trials (summarized in Lawall et al., 2011, 2010; Liew and O'brien, 2012; Raval and Losordo, 2013). Powell et al. (2011) reported the interim results of the RESTORE-CLI trial, where IM injections of tissue repair cells (analogous to a MNC mixture) proved no serious adverse effects, with increased amputation-free survival of patients and improved wound healing. In addition to the IM route, intra-arterial (IA) administration (through a femoral artery catheter) has been also safely and efficaciously used to inject allogeneic, expanded, bone marrow-derived MSCs (Das et al., 2013).

In sum, adult stem cell transplantation constitutes a paradigm shift in the treatment of chronic limb ischemia (CLI), especially for diabetic patients (O'Neill et al., 2012; Powell, 2012; Weck et al., 2011). The safety and efficacy of culture-expanded ADSCs derived from SVF for the treatment of CLI has been documented (Bura et al., 2014; Lee et al., 2012) and reviewed (Zhi et al., 2014), although further studies with more rigorous designs, including randomization, standard-of-care or placebo controls, are still needed. However, to the best of our knowledge, no report has been made so far with *fresh*, *non-fractionated*, *un-cultured*, *point-of-care administered SVF* in CLI. Therefore, an open label, non-randomized study to assess the safety and efficacy of non-culture-expanded adipose-derived SVF cells administered IM to ten patients with nonreconstructable CLI was designed, approved, and executed at the National Autonomous University of Nicaragua in Leon.

2. Materials and methods

2.1. Ethics

This study (not registered in clinicaltrials.gov) was approved by the Medical Ethics Committee of UNAN-Leon and by the Ministry of Health of Nicaragua (MINSA). The procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national, Universidad Nacional Autónoma de Nicaragua — León) and the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all participants in accordance with standards of MINSA and the World Health Organization, and included consent to publish this study in all formats.

2.2. Patients' enrollment

The criteria for inclusion were: rest pain or claudication at least of one half block or less, non-healing ulcer or surgical amputation site for greater than three months, and inoperable PVD due to medical reasons. Given the limited resources available in Nicaragua for endovascular intervention or bypass, all patients at this advanced stage of disease were considered candidates for amputation. Criteria for exclusion were: age < 40 years, unstable cardiovascular disease at the moment of enrollment, smoking and/or the presence of chronic pulmonary disease, ongoing infection and/or sepsis, and uncontrolled diabetes.

2.3. Surgical procedure and SVF preparation

The SVF cells were obtained after enzymatic digestion of surgically harvested adipose tissue. Liposuction was performed from the flanks and abdomen with the yield of dry fat per case ranging from 250 to 350 cm^3. The lipoaspirate was collected directly into a sterile tissue-processing canister (GID SVF-1, Louisville, CO, USA) for tissue dissociation and processing under closed conditions at all times and following the manufacturer's instructions. It was first washed three times with sterile Lactated Ringer's Solution inside the canister to remove red cells and residual oils, and then dissociated with GMP-grade collagenase (GIDzyme, GID, Louisville, CO, USA) in 125 ml of Lactate Ringer's Solution, at a concentration of 200 CDU/ml of total volume. The mixture was dissociated