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Autologous bone marrow mononuclear cell therapy improves symptoms in patients with end-stage peripheral arterial disease and reduces inflammation-associated parameters

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Abstract

Background aims. The purpose of this study was to evaluate the effect of autologous bone marrow mononuclear cells (BM-MNCs) on symptoms and perfusion indices in severely symptomatic patients with peripheral arterial disease (PAD) without further option for endovascular or surgical revascularization. *Methods.* Only patients with severe symptomatic PAD (Fontaine class IIb-IV, Rutherford category 3–6) not amenable for revascularization were treated. Bone marrow from both cristae iliacae was harvested; MNCs were isolated by the Ficoll density-gradient method and transplanted by means of intra-arterial and intramuscular injection in the index limb. Functional (pain score, ulcer healing, maximum walking distance) and perfusion indices such as ankle-brachial-index and transcutaneous oxygen pressure were documented before and after BM-MNC therapy. Additionally, serum concentration of C-reactive protein and interleukin-6 were measured as markers of inflammation before and after BM-MNC treatment. *Results.* Sixteen consecutive patients (four women; mean age, 63.0 ± 13 years) were treated with a mean dose of $4.2 \pm 2.2 \times 10^8$ BM-MNCs. At 6 months' follow-up, ankle-brachial-index, transcutaneous oxygen pressure and maximum walking distance significantly increased, whereas C-reactive protein and interleukin-6 conversely decreased ($P < 0.01$ versus baseline values), resulting in 88% limb salvage, 75% pain reduction and 71% complete wound healing and/or reduction of ulcer size. One major and one minor amputation were performed, both in patients with Rutherford category 6. *Conclusions.* Autologous BM-MNC therapy in patients with end-stage PAD improves tissue perfusion indices and decreases markers of inflammation. If our observations could be confirmed by large-scale, randomized controlled trials, BM-MNC transplantation could become an alternative therapeutic option for patients with end-stage PAD.

Key Words: *autologous mononuclear cells, cell-based therapy, critical limb ischemia, end-stage peripheral arterial disease, inflammation*

Introduction

Chronic critical limb ischemia (CLI) refers to an inadequate arterial perfusion of extremities caused by occlusive peripheral arterial disease (PAD). The disease-associated reduction of blood flow into the microcirculation of the downstream tissues results in rest pain and eventually in tissue loss. Approximately 1–3% of patients with PAD have CLI at initial presentation, and 5–10% of patients with asymptomatic PAD or claudication will progress to CLI within 5 years (1). In industrialized nations, the incidence of CLI has been estimated to add up to approximately 500–1000 per million individuals and year (1,2). A recent population-based analysis demonstrated a dramatic increase in PAD, particularly in the subset of CLI, mainly because of an excessive increase in atherosclerotic risk factors in a continuous aging population (3).

Currently, endovascular and surgical revascularizations are therapeutic standards for CLI. However, despite the marked advances in these techniques, a substantial part of patients with CLI (eg, with severe

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outflow obstructions or co-morbidity associated high perioperative risk) are not eligible for endovascular or surgical revascularization (4–6). The treatment of such "no-option" patients is limited only to a symptomatic therapy consisting of professional wound care, pain control and optimized secondary preventive measures to halt the aggravation of the underlying systemic vascular disease (7). Therefore, novel and better therapies are needed.

In this context, the intramuscular and/or intra-arterial application of autologous bone marrow–derived mononuclear cells (BM-MNCs) has emerged as a promising approach for neovascularisation in patients with end-stage PAD (8–12). Unlike mechanical revascularization of the conduit arteries, in which blood flow to the ischemic limb is restored by mechanical means (such as balloon angioplasty, stenting, atherectomy or bypass surgery) resulting in immediate increase of perfusion, cell-based therapy is thought to promote angiogenesis and arteriogenesis in ischemic tissue. Both processes involve a variety of different cellular and humoral interactions, whose mode of action has not been unraveled yet.

The fraction of BM-MNCs contains a heterogeneous mix of primitive and mature blood cells as well as non-hematopoietic stromal cells, for example, mesenchymal stromal and progenitors cells (MSCs) and endothelial progenitor cells (EPCs) (13). Even though it has been reported that BM-derived cells, for example, monocytes and macrophages, are attracted by ischemic areas, they rarely integrate into affected tissues (14). Because these cells were found to release a variety of different pro-angiogenic cytokines (eg, interleukin [IL]-1, IL-6 and IL-8; vascular endothelial growth factor [VEGF]; platelet-derived endothelial growth factor; transforming growth factor-β; basic fibroblast growth factor) (14–19), the observed clinical benefit after cell-based therapy may be attributed to paracrine rather than to cellular effects (8,14,16,20).

The importance of the release of paracrine factors in cellular therapies has just recently been documented: Several studies administered MSCs to treat myocardial ischemia (21–23). In this context, Lee *et al.* (24) demonstrated that the vast majority of transplanted MSCs embolized into the lungs and did not repopulate the ischemic areas of infarcted hearts. However, the authors provided compeling evidence that by release of cytokines and other secreted factors, lung-inhabiting MSCs exerted the benefical effect observed after MSC administration. Thus, even if transplanted cells do not intercalate into affected tissues, they appear to release a "cocktail" of factors that ameliorates symptoms in vascular and non-vascular diseases. Extracellular vesicles, such as exosomes and microvesicle, have been shown to exert the therapeutic effects of MSCs in a myocardial infarction model and in a patient with graft-versus-host disease (25–27). Until the crucial components of such "cocktails" have been determined, the administration of cells in many diseasese including PAD may appear more feasible than that of a cocktail of selected cytokines and growth factors.

In the present study, we evaluated the therapeutic effect of a combined intra-arterial and intra-muscular transplantation of autologous BM-MNCs in patients with end-stage PAD with no further option for revascularization.

Methods

Patient selection

Patients were candidates for BM-MNC therapy if they fulfilled the following criteria: Patients with CLI or severe PAD [category 3–6 according to Rutherford classification (28)] with previously invasively diagnosed PAD, no further options for endovascular or surgical revascularization and lack of improvement or deterioration of symptoms under state-of-the-art medical treatment for at least 6 months. CLI was defined by the presence of ischemic rest pain and/or ischemic tissue loss (gangrene or ulceration) objectively attributed to reduced arterial perfusion. Severe PAD was defined by the presence of limiting claudication and the presence of severely reduced perfusion indices, that is, an ankle pressure $<$50 mm Hg, ankle-brachial index (ABI) $<$0.4 or a transcutaneous oxygen pressure ($TcPO_2$) value $<$30 mm Hg. In cases of media sclerosis with uncompressible arteries, a pressure of $<$40 mm Hg of the first toe was required to define severe PAD.

Patients were excluded from BM-MNC therapy if one of the following criteria was present: hematologic disorders such as leukemia, coagulopathies, acute or chronic systemic infectious diseases, severe renal or hepatic failure, patients without exhaustion of best conservative therapy for at least 6 months, revascularization of limb arteries during the last 3 months and any severe comorbidities limiting patients survival to $<$6 months.

The approval of the local ethics committee was obtained, and informed consent of patients was obtained at least 24 h before the procedure. The basic conservative therapeutic management, comprising risk factor modification, walking training and pharmacotherapy, remained unchanged throughout the entire term of the BM-MNC therapy.

Indices of arterial perfusion, tissue oxygenation and clinical parameters

The ABI and $TcPO_2$ are both non-invasive techniques reflecting the arterial perfusion and the