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Safety and efficacy of granulocyte–colony-stimulating factor administration following autologous intramuscular implantation of bone marrow mononuclear cells: a randomized controlled trial in patients with advanced lower limb ischemia

MOHAMMAD REZA ZAFARGHANDI[1], HASSAN RAVARI[2], NASSER AGHDAMI[3,4], MEHRNAZ NAMIRI[3], KASRA MOAZZAMI[1], EHSAN TAGHIABADI[3], ABDORREZA FAZEL[1], BEHSHAD POURNASR[4], ALI FARROKHI[4], RAMEZAN ALI SHARIFIAN[1], JAVAD SALIMI[1], MAJID MOINI[1] & HOSSEIN BAHARVAND[3,4,5]

[1]*Department of Vascular Surgery, Tehran University of Medical Sciences, Tehran, Iran,* [2]*Department of Vascular Surgery, Mashad University of Medical Sciences, Mashad, Iran,* [3]*Department of Regenerative Medicine, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran,* [4]*Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran, and* [5]*Department of Developmental Biology, University of Science and Culture, ACECR, Tehran, Iran*

Abstract

Background aims. The aim was to investigate the therapeutic effect of granulocyte–colony-stimulating factor (G-CSF) administration following implantation of autologous bone marrow mononuclear cells (BM MNC) for patients with lower limb ischemia. *Methods.* The design was a randomized controlled trial. Fifteen patients with severe chronic limb ischemia were treated with autologous BM MNC [without G-CSF (MNC–G-CSF) or combined with G-CSF administration for 5 days following transplantation (MNC+G-CSF)]. *Results.* All clinical parameters, including ankle brachial index, visual analog scale and pain-free walking distance, showed a mean improvement from baseline, which was measured at 4 and 24 weeks after transplantation in both groups. However, in three (20%) patients, the clinical course did not improve and limb salvage was not achieved. No significant difference was observed among the patients treated in the MNC–G-CSF and MNC+G-CSF groups. No severe adverse reactions were reported during the study period. No relationship was observed between both the numbers of viable MNC or $CD34^+$ cells and the clinical outcome. *Conclusions.* Autologous transplantation of BM MNC into ischemic lower limbs is safe, feasible and efficient for patients with severe peripheral artery disease. However, the administration of G-CSF following cell transplantation does not improve the effect of BM MNC implantation and therefore would not have any beneficial value in clinical applications of such cases.

Key Words: *angiogenesis, bone marrow mononuclear cells, granulocyte–colony-stimulating factor, lower limb ischemia, peripheral artery disease*

Introduction

In patients suffering from lower limb ischemia as a result of peripheral arterial disease (PAD), such as atherosclerosis (AS) and thromboangitis obliterans (TAO, Buerger's disease), revascularization by either bypass or endovascular surgery is the main modality of treatment (1,2). However, in many cases revascularization is not feasible because of the diffuse segmental involvement and distal nature of the disease. As PAD usually has a progressive nature, amputation would be considered the only and final option as a solution to unbearable symptoms in this group of patients (3).

Therapeutic angiogenesis by using cellular therapeutic strategies may be useful in these patients (4,5). Recent publications have described beneficial effects of autologous bone marrow (BM) mononuclear cells (MNC), including endothelial progenitor cells (EPC) (5–7) and autologous peripheral blood MNC (8–11), in the setting of critical limb ischemia (CLI) in humans. Moreover, angiogenic factors, such as granulocyte–colony-stimulating factor (G-CSF), have been reported

Correspondence to: **Hossein Baharvand**, Department of Regenerative Medicine and Department of Stem Cells and Developmental Biology, Royan Institute for Stem Cell Biology and Technology, ACECR, PO Box 19395–4644, Tehran, Iran. E-mail: Baharvand@RoyanInstitute.org

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to mobilize progenitors in the BM compartment to the peripheral blood (12). It has also been shown that the mobilized cells have the capacity to differentiate into EPC and be incorporated into newly forming vessels (12–14). Therefore, G-CSF administration may have a direct effect on collateral vessel growth and perfusion recovery in ischemic tissues (14). However, it is still unclear at present whether G-CSF administration could be useful clinically for enhancing neovascularization following BM MNC transplantation in patients with lower limb ischemia (8). Therefore, the purpose of this study was to investigate the efficacy and safety of autologous transplantation of BM MNC and find out whether administration of G-CSF following BM MNC could augment angiogenesis further and improve clinical outcome in patients with chronic lower limb ischemia.

Methods

Patients

Fifteen patients were enrolled in the present study. All patients included had advanced severe chronic limb ischemia (rest pain), were not candidates for open or endovascular revascularization and did not show any evidence of improvement in response to standard therapy for the previous 6 weeks.

The exclusion criteria included poorly controlled diabetes mellitus (hemoglobin A1C>8%), severe heart failure (ejection fraction <30%), previous malignancy or history of chemotherapy or radiation affecting the BM, renal insufficiency (creatinine > 2.5), history of organ transplantation, current serious infectious disease and life expectancy less than 1 year. Patients with acute myocardial infarction, angina pectoris and a cerebrovascular accident within 1 month were also excluded, as the administration of G-CSF may adversely affect these diseases.

The protocol of the study was approved by the ethics committees of Tehran University of Medical Sciences and Royan Institute (Tehran, Iran). Informed consent was obtained from all patients before enrollment into the study. The trial was registered at NIH clinical trials (www.clinicaltrials.gov, last accessed May, 2008) with the identifier NCT00677404.

Procedures

From the 15 patients, approximately 400 mL autologous BM were aspirated from the iliac crest under epidural anesthesia and collected into plastic bags containing citrate phosphate dextrose anticoagulant (CPDA; Beasat, Tehran, Iran) the day before the BM MNC implantation. MNC were isolated under good manufacturing practice conditions by Ficoll–Hypaque (Lymphodex, Inno Train, H9L6114, Kronberg, Germany) density separation within 90–120 min. At the end of the separation, the cells were washed twice with normal saline and then counted and assessed for viability using trypan blue dye exclusion. The cells were suspended in 40 mL normal saline including 2% of the patient's own serum and kept at 4°C before the procedure. The cells were injected intramuscularly into the calf and interosseous foot muscles of the ischemic leg under epidural anesthesia. We implanted approximately 1 mL BM MNC suspension into each injection site using a 26-gauge injection needle (40 sites, 1.5 cm deep) at 3 × 3-cm grid intersects as injection site markers. The patients were divided randomly into two groups receiving autologous BM MNC alone (seven patients, MNC–G-CSF) or combined with G-CSF adjuvant (eight patients, MNC+G-CSF; B1055, Neupogen, Roche, Basel, Switzerland). G-CSF (10 μg/day) was administered by subcutaneous injection for 5 days, starting from the day of BM MNC injection. Blood samples were obtained from the patients each day and G-CSF injection was discontinued if adverse reactions, including severe bone pain, headache or leukocytosis (White Blood Cell >30 000), developed. All patients were treated with aspirin (100 mg/day) and were allowed to continue previous medications.

Clinical assessment

On admission, laboratory tests and serologic profiles were obtained from all patients, including complete blood count with differential, fasting blood glucose, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, creatinine, urea, uric acid, erythrocyte sedimentation rate, C-reactive protein, lipid profile and troponin T.

The limb condition of patients was evaluated with the Rutherford classification for ischemic limbs (15). Ischemic pain was assessed with a visual analog pain scale (VAS) of 10 levels, where 0 is no pain at all and 10 is the most severe pain experienced for ischemic and control limbs. Ischemic ulcers were assessed with the Wagner classification (16). Assessment of pain-free walking distance (PFWD) was performed using a standard treadmill test. A resting ankle–brachial pressure index (ABI) was calculated as the quotient of absolute ankle pressure and brachial pressure. According to international standards, an increase of at least 0.1 is recognized as a significant improvement and a value >0.9 is regarded as normal (15). Angiographic assessment was performed with digital subtraction angiography for all patients before transplantation to exclude any option for open or endovascular revascularization.