Cytotherapy, 2011; 13: 993–999

informa
healthcare

Application of autologous bone marrow mononuclear cells in six patients with advanced chronic critical limb ischemia as a result of diabetes: our experience

RATHNAVELPANDIAN SUBRAMMANIYAN[1], JOSEPH AMALORPAVANATHAN[1], RAJENDRAN SHANKAR[1], MURUGESAN RAJKUMAR[1], SUBRAMANI BASKAR[2], SADANANDA RAO MANJUNATH[2], RAJAPPA SENTHILKUMAR[2], PALANISAMY MURUGAN[2], VENKABA RAO SRINIVASAN[2] & SAMUEL ABRAHAM[2,3]

[1]*Vascular Surgery Department, Vijaya Hospital, Chennai, India,* [2]*Nichi-In Centre for Regenerative Medicine, Chennai, India, and* [3]*Yamanashi University School of Medicine, Chuo, Japan*

Abstract

Background aims. Previous clinical studies have reported that the injection of bone marrow (BM)-derived mononuclear cells (MNC) results in improvement in symptoms and healing of ulcers in patients with critical limb ischemia (CLI) up to stage IV of Fontaine's classification. However, most patients with Fontaine stage IV CLI limbs had to undergo amputation even after stem cell therapy. We report on six patients, who had poorly controlled diabetes with extensive ulceration and gangrene of limbs because of Fontaine stage IV CLI and had been advised amputation elsewhere, who underwent injection of autologous BM MNC. *Methods*. In all six patients, BM was aspirated and the isolated MNC from the BM were injected intralesionally at various sites of the ulcer and its surroundings after necessary debridement. The patients were followed up at regular intervals for at least 6 months. *Results*. At the end of the 6-month follow-up, the lower limb pain and ulcers had improved significantly in all patients. The mean toe–brachial index had increased from 0.26 to 0.36. One patient died a month after therapy because of causes unrelated to the procedure. Limb salvage was possible in the remaining five patients and they had a pain-free walking distance of 100 m within 6 months. *Conclusions.* Limb salvage was possible in all six diabetic patients with Fontaine stage IV CLI following autologous BM MNC injection. The procedure was safe without any adverse outcomes.

Key Words: *bone marrow mononuclear cells, critical limb ischemia, diabetes, Fontaine stage IV, limb salvage*

Introduction

Diabetes is a chronic disease affecting millions of people world-wide, and one of the most devastating complications of diabetes is peripheral arterial disease (PAD), which includes critical limb ischemia (CLI). It results in significant morbidity and mortality affecting the patient and his/her family members as a whole.

Criteria for diagnosis of CLI include one of the following: more than 2 weeks of recurrent foot pain at rest that requires regular use of analgesics and is associated with an ankle systolic pressure of less than 50 mm Hg; a toe systolic pressure of less than 30 mm Hg; a non-healing wound or gangrene of the foot or toes, with similar hemodynamic measurements (1). Diabetic foot ulcers are estimated to affect 15% of all diabetics during their lifetime and precede almost 85% of all foot amputations (2,3). Diabetes, as a result of its associated complications such as neuropathy and vasculopathy, raises the plantar pressure and makes tissue damage more likely at the weight-bearing sites. This is why most of the skin injuries in diabetics are seen on the plantar surface (4,5).

Current treatment modalities include antiplatelet drugs, vasoactive substances, prostanoids, distal bypass surgery and endovascular interventions. But progressive gangrene, extensive ulcers and continued rest pain can be a significant threat to the limbs and suggest the need for revascularization in patients (4,5). Occlusion of the arterial tree causes tissue hypoxia, which is a strong stimulus for angiogenesis (6,7). The development of collateral vessels occurs physiologically in patients with CLI and is mainly driven by an increased endogenous angiogenic response (8). This process is responsible for increased microvessel density in the muscles of

Correspondence: **Dr Samuel Abraham**, Nichi-In Centre for Regenerative Medicine, C16 &17, Vijaya Health Centre Premises, 175, NSK Salai, Vadapalani, Chennai 600026, Tamil Nadu, India. E-mail: drabrahamsj@ybb.ne.jp, drspp@nichimail.jp

(Received 7 January 2011; accepted 2 April 2011)

ISSN 1465-3249 print/ISSN 1477-2566 online
DOI: 10.3109/14653249.2011.579961

patients with CLI, partially compensating for the occlusion of native arteries, but does not restore normal flow. Various treatment modalities have been tried to augment this angiogenic process in patients with ischemic disease (4,5,6).

Recent years have seen an array of treatment attempts using angiogenic cytokines, such as vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and bone marrow (BM) mononuclear cells (MNC), in basic and clinical investigations. Studies have reported that, in patients with diabetes, the circulating endothelial progenitor cells (EPC) exhibit impaired proliferation, adhesion and incorporation into vascular structures (9,10). In response to tissue injury and remodeling, neovascularization usually occurs via the proliferation and migration of endothelial cells from pre-existing vasculature (7) or those cells residing within the BM and circulating in the peripheral blood (4,5,8).

There have been numerous clinical trials in the past few years that have demonstrated the safety and efficacy of injection of BM MNC or peripheral blood MNC rich in $CD34^+$ cells in inducing angiogenesis and improving the functional activity of ischemic limbs, permitting limb salvage (11–26). The largest clinical trial by Kawamura *et al.* in 2006 (27) concluded that therapeutic angiogenesis produces a good outcome in critically ischemic limbs caused by diabetes up to Fontaine stage III, but 60% of stage IV limbs are amputated. The Fontaine classification, first described in 1954 in *Surgical Treatment of Peripheral circulation disorders* (28), is still one of the most well-known classifications. It classifies vascular insufficiency into four stages: I, asymptomatic; II, intermittent claudication; III, rest pain; IV, ulcer/gangrene. Therefore, the efficacy of therapeutic angiogenesis is currently limited for stage IV patients.

We report on six patients who had poorly controlled diabetes with extensive ulceration and gangrene as a result of Fontaine Stage IV CLI and were indicated for amputation. All of them had successful limb salvage and pain-free walking for 100 m within 6 months of injection of autologous BM MNC rich in $CD34^+$ cell content.

Methods

Patients

Six patients (five males and one female) with a mean age of 60.67 years were involved in the study. All the patients had poorly controlled diabetes mellitus (mean diabetic age 15.6 years, with an average fasting blood glucose level of 131.50 mg/dL and post-prandial 177.83 mg/dL at the time of admission, despite routine medication for control of diabetes). They all presented with Fontaine stage IV CLI and had extensive necrotic ulcers with pain at rest.

The first patient was a 68-year-old female who had CLI of the left lower limb with diffuse multiple critical stenosis of the only patent tibial artery, and a large ischemic, infected leg ulcer. The ulcer measured 30×12 mm at the posterior aspect of the leg, exposing the gastrocnemius and Achilles tendon and also extended to the medial aspect of the foot, where it measured 14×10 mm. A lateral extension of the wound was present at two places just above the ankle joint, each measuring 4.5×4.0 mm (Fig IA).

The second patient was a 64-year-old male who showed extensive ulcer and gangrene of the right lower foot, measuring 12×7 mm. His left foot had already been amputated as a result of CLI (Fig IG).

The third patient was a 69-year-old male, who had a deep ulcer extending to the bone in the heel of the left leg, measuring 2×2 mm. The fourth patient was a 61-year-old male who had a gangrenous ulcer in the foot measuring 7.5×5 mm, with all the toes previously amputated because of CLI. The fifth patient was a 45-year-old male, who had an extensive wound measuring 6×3 mm with infection.

The sixth patient was a 60-year-old male who had an ulcer extending to the medial aspect of the foot measuring 9×6 mm, with loss of three toes as a result of necrosis (Fig IE). His other two toes were amputated during debridement of the wound during the stem cell therapy because of infection. The characteristics of the patients included in the study are given in Table I.

BM aspiration, isolation and injection procedure

The ethics committee of Vijaya Hospital (Chennai, India) approved this study and informed consent was obtained from all the patients. Under short general anesthesia, 110 mL BM was aspirated each time, transported in 110 mL acid citrate dextrose under cold chain, and immediately underwent processing. In the laboratory, 5 mL BM from each sample were sent for immunophenotyping (IPT) for $CD34^+$ analysis by flow cytometry (Table II). The samples were processed under a Current Good Manufacturing Practices (cGMP) Standard Operating Protocol (SOP) class 10 000 clean room and class 100 biosafety cabinet. The samples were subjected to a Ficoll gradient centrifugation procedure and the BM MNC were collected by removing the buffy coat. The viability of the cells was checked using trypan blue and a cell count was carried out using a Neubaur's hemacytometer (Table II). Finally, the cell pellet was suspended in 15 mL sterile saline and given as an intralesional injection in the area of the lesion in each case. The MNC concentrate was injected at various