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Bone Marrow-derived Mononuclear Stem Cell Implantation in Patients with Buerger's Disease

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Abstract

Background: Patients suffering from Thromboangiitis obliterans (TAO) have endothelial cell dysfunction and the severity of the disease lies in the need for amputation in more than a quarter of all sufferers.
Methods and Findings: We report the safety and feasibility of autologous implantation of circulating mononuclear cells for patients suffering from Buerger's disease following bone-marrow mobilization with granulocyte colony stimulating factor (5 μg/kg/day for 5-7 days). Six patients participated in this study. Mononuclear cells were separated by Cobas Spectra cell separator. MNCs, CD34+ and CD133+ cells were enumerated prior to intramuscular injection into the affected foot/limb muscles at multiple sites on the collection day. Stem cell injection prevented disease progression in all six patients. In this small cohort of patients with critical limb ischemia, quality of life improved significantly over a two year period. Also, pain-free walking distance in all patients showed significant improvement.
Conclusions: Autologous mononuclear cell containing CD34+/CD133+ stem cells collected from peripheral blood following G-CSF mobilization is effective, safe and results in sustained clinical results for patients with severe peripheral occlusive arterial disease.
Keywords: Buerger's disease, Autologous, Bone-Marrow, Stem cell, Endothelial Progenitor, Angiogenesis.

Introduction

Buerger's disease or thromboangiitis obliterans (TAO) is a non-atherosclerotic, imflammatory, vasooclusive disease. Clinical findings show occlusive segmental and more often multiple inflammatory lesions of medium and small blood vessels and superficial vein thrombosis [1,2, 3]. The occlusion of blood vessels in the affected limbs and hands restricts the availability of blood supply to the affected tissues, causing severe pain, and eventually leading to tissue destruction. The disease progresses to ulceration and gangrene and necrosis of affected limb which may lead to amputation [4,5].

The etiology of TAO remains unknown, but previous studies have suggested a strong link with cigarette smoking. Almost all the affected patients are smokers. In spite of strong association indicating that tobacco smoking (amongst other factors) has a role in the pathogenesis of the disease, no sufficient data is available for underlying cellular and molecular mechanism of the disease. Therapeutic options for TAO patients are limited. Their conditions are often refractory to conservative measures and are typically unresponsive to drug therapy [6-11].
In 2002, Tateishi-yayuma et. al [12] published impressive results in patients with critical ischemia treated by the autologous transplantation of bone marrow cells. This study showed some success in pain relief with Buerger's disease. Subsequent experiments by several workers have shown that autologous whole bone-marrow stem cells or bone-marrow derived stem cells from peripheral blood following mobilization with granulocyte colony-stimulating factor have the potential to stimulate angiogenesis and thereby modulate hemodynamic deficit in ischemic limbs in vivo and improve the life quality of the affected patients [13-21].

In the current work, stem cells in the bone marrow were mobilized into the peripheral blood by administration of G-CSF, followed by collection of peripheral blood stem cells using an apheresis technique. The collected cells were then injected into affected limbs.

Patients and Methods

Seven patients with Buerger's disease were enrolled for autologous peripheral blood stem cell therapy. Patient's eligibility was approved by the vascular surgery department bioethics group at the Shahid Beheshti University of Medical Sciences. One patient was excluded from the study due to lower percentages of circulating CD4+ T helper lymphocytes. All patients had severe claudication, rest pain with nonhealing ischemic wounds. Patients had already received currently available medical treatment and surgical interventions such as sympathectomy. The profile of six patients (with mean age 39.5±6.50**)** was as follows: Patient 1, a 36 year-old man with a severe gangrene on his toe and nonhealing wound in his left foot who had undergone medical treatments and lumbar sympathectomy. Patient 2 was a 42 year-old man with intractable pain in his fingers and an ulcer on his second digit and for whom a lumbar sympathectomy and medical treatments had failed. Patient 3 was a 35 year-old man with severe rest pain with an ulcer on his foot who had experienced a variety of medical treatments and interventions. Patients 4 and 5 had ulcers in fourth toe of the left lower extremity. Patient 6, a 42 year-old man had ulcer at the tip of his first toe along with deep ulcer on affected left foot. Pulse oxymetry, O2 Saturation , rest pain, pain-free walking, skin temperature, the size of skin ulceration, color dopler sonography and angiography were done pre- and post-implantation. Patients were screened for HIV, HCV and HBV. Cardiac, hematological, infectious, renal, hepatic, metabolic and clinical parameters were also determined prior to stem cell transplantation. Also, flow cytometric analysis (BD FACSCalibur CA, USA) were carried out for the following marker CD19, CD3, CD4, CD8, CD34, CD13, CD14, and CD45 pre- and post-operatively. Circulating mononuclear cells with CD34+ and CD133+ markers were enumerated on the day of stem cell administration, using flow cytometry. Follow up has been over 24 months.

Granulocyte Colony-Stimulating Factor Administration and Stem Cell Collection

The patients received subcutaneous injections of recombinant human G-CSF (Filgrastim, Pooyesh Daru, Iran) daily at 5μg/kg/day for 5-7 days prior to apheresis. Blood samples were collected and leukocytes were measured daily. Once the WBCs reached approximately to 25000-30000/ml, then, approximately 400-500ml suspensions of circulating peripheral blood mononuclear cells were collected using Cobe Spectra instrument (Gambro BCT, CO, USA). The cells were then concentrated to a final volume of 60-70ml, followed by injection of the cell suspension into the gastrocnemius muscle of affected limb under general and/or spinal anesthesia (60-70 sites, 0.5-1ml per site).

Statistical Analysis

Data were analyzed by using Wilcoxon non-parametric test. $P<0.05$ was considered significant.

Results

In all six patients (table 1), normalization of limb temperature in the affected area showed significant improvement after 2-3 weeks post-transplantation as observed by thermograph (an average of 1 ^{0}C). Rest pain and pain-free walking distances were significantly improved 1 to 2 months post-transplantation. O_2 saturation also improved gradually after the treatment in all six patients. Table 2 shows percentage MNCs and CD34+ and CD133+ cells before stem cell therapy. In all patients significant healing of wound was noted. Color Doppler sonographic assessment indicates an increase in blood flow in the affected area possibly of improved collateral circulation, Table 3.

TABLE 1. Characteristics and clinical evaluation of six patients before and after stem cell therapy transplantation.

Case #	Age/ Year Gender/ Male	Smoking History (Years)	Pain Free Walking Distance (m) Before-After (p=0.043)		Prior Therapies	Skin Temperature (0C) Before-After (p<0.05)		Ulcer in Low extremities Before-After	
1	36	>20	150±30	RI	LS	35.0	36.5	yes	WH
2	42	>22	280±50	RI	LS	36.0	37.0	yes	WH
3	35	>15	250±50	RI	LS/AP	35.5	36.0	yes	WH
4	32	>17	156±10	RI	LS	34.5	35.5	yes	WH
5	50	>30	45±2	RI	None	34.5	36.0	yes	WH
6	42	>25	35±2	RI	LS	35.0	36.0	yes	WH

RI = Remarkably Improved, WH = Wound Healed, LS = Lumbar Sympathectomy, AP = Angioplasty

TABLE 2. Total number of MNC and percentages of CD34+/CD133+ Cells Implanted in affected Limb/Foot of Patients (Double Positive Cells).

Case#	MNCs Count X 108/Kg	Percentages of CD34+ and (CD133+/CD34+ Implanted
	Injection Day	Implantation Day
1	8.49	7.8 (2.13)
2	7.98	8.5 (2.19)
3	7.45	4.9 (1.22)
4	5.41	4.8 (1.16)
5	7.56	5.6 (1.61)
6	6.67	4.8 (0.93)

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TABLE 3. Color Doppler Sonographic indices pre- and post-stem cell transplantation.

Case number	Peak systolic volume (cm/s) in Posterior Tibial artery (affected limb) (P<0.05)		Resistance index (%) in Posterior Tibial artery (affected limb) (P<0.05)	
	Before	After	Before	After
Case 1	31.3	44.2	74	62
Case 2	42	48.8	86	60
Case 3	26.3	27.5	59	52
Case 4	36.8	46.2	72	64
Case 5	28.8	36	64	55
Case 6	33.4	41.2	71	58

Discussion

Management of obstructive vascular diseases involving the extremities poses great challenges for physicians and patients. Peripheral arterial disease is often a consequence of obstructive atherosclerosis affecting the ilefemoral circulation but is also a result of arteriosclerotic conditions such as thromboangiitis obliterans. Consequences range from the presence of asymptomatic obstruction to intermittent claudication, development of rest pain, ulceration, gangrene, and amputation [10,13,14,16,17]. A relatively new and promising approach using stem cell has in recent years been developed to treat intractable symptoms related to ischemia in subjects with peripheral arterial disease in whom conventional treatments has failed [1]. It has been postulated that marrow stem cell implantation into ischemic limbs could enhance angiogenesis by supplying endothelial progenitor cells (EPCs) and angiogenic factors and/or cytokines [7].

In the present study, we demonstrated that the transplantation of bone-marrow derived peripheral blood mononuclear cells containing CD34+/CD133+ into lower ischemic limbs of six Buerger's patients was associated with a dramatic improvement in ischemic signs and symptoms possibly of distal circulation improvement as shown in table 1and fig 1. Improvement of rest pain, walking distance limitation, healing of skin ulcer and an increase in distal limb temperature was observed in all six cases. After stem cell injection, limb amputation in all patients has been avoided for 24 months of follow-up. The formation of new collateral vessels was observed in three patients as determined by angiogram and color Doppler sonography. In this regard, the current work supports previous observations by establishing the potential benefits of G-CSF mobilized bone-marrow stem cell transplantation for the treatment of arterial diseases such as TAO. No adverse reaction was observed with G-CSF administration, although some reports suggest that that G-CSF might pose harmful effect. There was no abnormality in the laboratory

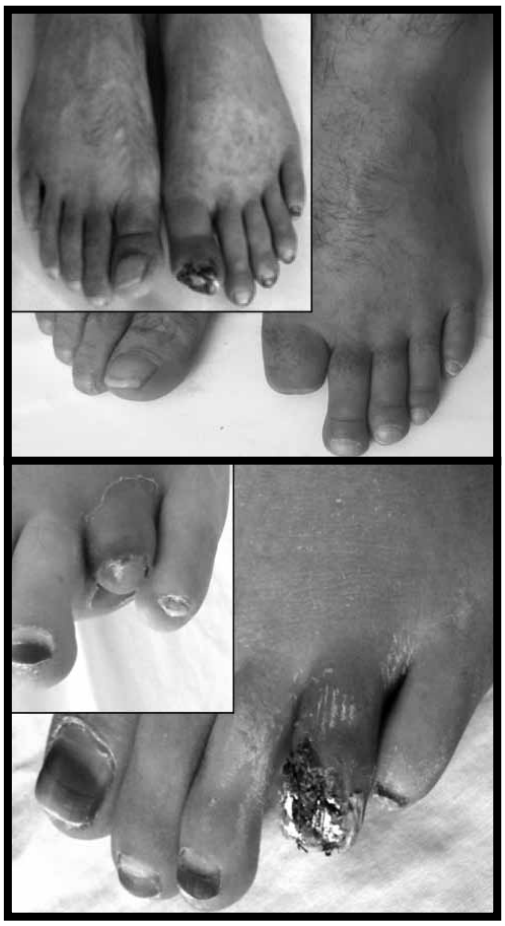

Fig.1. Improvement of wound healing following stem cell transplantation in selected patients

findings of hematology, immunology, kidney, and liver function tests as well as the level of serum proteins in all patients after the treatment, indicating that the injection of EPCs were safe for patients with Buerger's disease. It should be noted that the molecular mechanism and process of angiogenesis in the affected lesions have not been fully understood, and how transplanted CD34+ and/or CD34+/CD133+ stem cells are incorporated into new vessels has not fully been elucidated. Further studies would be required to illustrate the relationship between the numbers of CD34+/CD133+ stem cells injected, angiogenic promoting factors such as cytokines and growth factors. We are currently working on the molecules that are responsible for the homing and the recruitment of endothelial progenitor cells (EPC) into ischemic area.

In conclusion, despite the small number of patients and technical limitations on the assessment of microvascular blood flow, the effectiveness of therapeutic angiogenesis with bone-marrow derived mononuclear cells in patients with Buerger's disease has been established. Therefore, BM-MNC transplantation may provide a promising therapy in such patients in whom conventional medical therapy has been exhausted.

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All authors participated in the design, interpretation of the studies and analysis of the data and review of the manuscript. SS, AF, and HP conducted the experiments. SS and AF wrote the manuscript. The Authors declare no conflict of interest.

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