Successful Stem Cell Therapy Using Umbilical Cord Blood-Derived Multipotent Stem Cells for Buerger’s Disease and Ischemic Limb Disease Animal Model

Sung-Whan Kim,¹ Hoon Han,² Gue-Tae Chae,³ Sung-Hoon Lee,⁴ Sun Bo,⁴ Jung-Hee Yoon,⁴ Yong-Soon Lee,⁵ Kwang-Soo Lee,⁵ Hwon-Kyum Park,⁵ Kyung-Sun Kang

¹Department of Pathology, College of Medicine, The Catholic University, Seoul, Korea; ²The Seoul Cord Bank, Histostem Co., Ltd, Seoul, Korea; ³Laboratory of Stem Cell and Tumor Biology, Department of Veterinary Public Health, ⁴Department of Veterinary Radiology, College of Veterinary Medicine, Seoul National University, Seoul, Korea; ⁵Department of Surgery, College of Medicine, Hanyang University, Seoul, Korea

Key Words. Cord blood • Mesenchymal stem cells • Buerger’s disease • Cell transplantation

ABSTRACT

Buerger’s disease, also known as thromboangiitis obliterans, is a nonatherosclerotic, inflammatory, vasoocclusive disease. It is characterized pathologically as a panangiitis of medium and small blood vessels, including both arteries and adjacent veins, especially the distal extremities (the feet and the hands). There is no curative medication or surgery for this disease. In the present study, we transplanted human leukocyte antigen-matched human umbilical cord blood (UCB)-derived mesenchymal stem cells (MSCs) into four men with Buerger’s disease who had already received medical treatment and surgical therapies. After the stem cell transplantation, ischemic rest pain suddenly disappeared from their affected extremities. The necrotic skin lesions were healed within 4 weeks. In the follow-up angiography, digital capillaries were increased in number and size. In addition, vascular resistance in the affected extremities, compared with the preoperative examination, was markedly decreased due to improvement of the peripheral circulation. Because an animal model of Buerger’s disease is absent and to understand human results, we transplanted human UCB-derived MSCs to athymic nude mice with hind limb ischemia by femoral artery ligation. Up to 60% of the hind limbs were salvaged in the femoral artery-ligated animals. By in situ hybridization, the human UCB-derived MSCs were detected in the arterial walls of the ischemic hind limb in the treated group. Therefore, it is suggested that human UCB-derived MSC transplantation may be a new and useful therapeutic armament for Buerger’s disease and similar ischemic diseases.

STEM CELLS 2006;24:1620–1626

INTRODUCTION

Buerger's disease, also known as thromboangiitis obliterans, is a rare disease characterized by a combination of acute inflammation and thrombosis (clotting) of the arteries and veins in the hands and feet [1, 2]. The obstruction of blood vessels in the hands and feet reduces the availability of blood to the tissues, causes pain, and eventually damages or destroys the tissues. It often leads to skin ulcerations and gangrene of fingers and toes. Rarely, in advanced stages of the disease, it may affect vessels in other parts of the body.

The etiology of Buerger’s disease is unknown, but a strong association with cigarette smoking may contribute to this disorder. Ohta et al. reported that there was a correlation between continued smoking and limb amputation (p = .0070) [5]. Usually, the symptoms start with claudication of the affected limb, and ischemic rest pain develops progressively. It may affect not only toes but also the fingers. Affected digits may show signs of cyanosis or dependent rubor. Ulceration and gangrene are the next step. Finally, the affected limb may become necrotic, and the awful pain requires treatment by amputation [6]. There is no effective medication or surgery for this disease. Recently, many clinical trials have been designed to save the extremities of patients with Buerger’s disease [7–9]. Among these, it was reported that intramedullary K-wire could successfully provide pain relief.

Correspondence: Kyung-Sun Kang, Ph.D., Laboratory of Stem Cell and Tumor Biology, Department of Veterinary Public Health, College of Veterinary Medicine, Seoul National University, Seoul, Korea. Telephone: 82-2-880-1246; Fax: 82-2-876-7610; e-mail: kangpub@snu.ac.kr; Hwon-Kyum Park, M.D., Ph.D., Department of Surgery, College of Medicine, Hanyang University, Seoul, Korea. Telephone: 82-31-560-2290; Fax: 82-31-566-4409; e-mail: hkpark@hanyang.ac.kr Received on August 4, 2005; accepted for publication on February 15, 2006; first published online in STEM CELLS EXPRESS February 23, 2006. ©AlphaMed Press 1066-5099/2006/$20.00/0 doi: 10.1634/stemcells.2005-0365

STEM CELLS 2006;24:1620–1626 www.StemCells.com
Therapeutic angiogenesis in patients with ischemic heart and lower limb ischemic disease was also studied by many researchers [11, 12]. Angiogenesis is regulated in a complex balance between pro- and anti-angiogenic mechanisms. Researchers have tried to overcome limitations of the natural angiogenic response by substantially increasing the local concentrations of angiogenic growth factors either by administering recombinant protein for the gene that codes for an angiogenic growth factor or by administering endothelial progenitor cells (EPCs) that will synthesize a cocktail of growth factors in the vicinity of new vessel formation [13, 14]. The EPCs were harvested from peripheral blood, autologous bone marrow, and human umbilical cord blood.

Many clinical trials were done on patients with critical limb ischemia by using vascular endothelial growth factor, gene transfer, and autologous implantation of bone marrow mononuclear cells, including EPCs [15–17].

The treatment of ischemic vascular disease of the limbs remains a significant challenge. Unfortunately, if medical and surgical salvage procedures fail, amputation is an unavoidable result for these patients.

This study is the first report of a clinical trial on patients with Buerger’s disease using MSCs derived from human UCB. Recently we also reported that UCB-derived MSCs could show functional and morphological improvement in a female patient with chronic spinal cord injury [18].

In the present study, we tried clinical applications for the patients with Buerger’s disease to demonstrate the efficacy of UCB-derived MSCs for improvement of peripheral circulation and rest pain. Furthermore, in our animal model, we proved that transplantation of UCB-derived MSCs augmented arteriogenesis in the ischemic limb of immunodeficient nude mice.

### Materials and Methods

#### Patients

Four patients were tried for human UCB-derived MSCs transplantation because they had chronic limb ischemia, including severe rest pain and nonhealing ischemic ulcers, and they had already received currently available medical treatment and surgical interventions such as sympathectomy, bypass surgery, and even amputations. The profile of the patients is as follows: Patient 1, a 53-year-old man who had already received a variety of medical treatments and surgical operations, including a lumbar sympathectomy, bypass surgery, and amputations of digits of the feet and hand; Patient 2, a 44-year-old man with intractable pain in his fingers and an ulcer on his right thumb and for whom a lumbar sympathectomy and other medical treatments had failed; Patient 3, a 39-year-old man who already had a below-knee amputation on his lower left extremity; Patient 4, a 36-year-old man with severe rest pain and an ulcer on his foot who had received a variety of medical treatments. Of particular note, Patient 4 had previously received a bone marrow-derived MSC transplantation at another hospital. Taking his history into account, we waited for 6 months for any sign of possible delayed effects, but there was no change in his rest pain and skin lesions. All patients had to take strong pain killers (for example, three or four injections of pethidine) to sleep at night. The human leukocyte antigen (HLA) typings were done to get a proper match between the patients and preserved umbilical cord blood. These clinical trials were approved by the Korean Food and Drug Administration as emergent cases following informed consent from the patients.

#### Selection of HLA-Matched UCB Units

HLA allele types of cryopreserved human UCB units comparable to those of each patient were identified by polymerase chain reaction (PCR)-sequence-specific oligonucleotide probe (SSOP) and a decrease in major amputation in patients with Buerger’s disease in whom medical and surgical therapy had failed [10]. However, so far, these clinical trials have produced no satisfactory methods to save patients.

### Table 1. HLA matching of umbilical cord blood-derived multipotent stem cells for Buerger’s disease patients

<table>
<thead>
<tr>
<th>Name of patient</th>
<th>Name of hospital</th>
<th>Date of treatment</th>
<th>Number of stem cell treatment</th>
<th>HLA</th>
<th>HLA mismatch loci</th>
<th>Resolution level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name of patient</td>
<td>Name of hospital</td>
<td>Date of treatment</td>
<td>Number of stem cell treatment</td>
<td>HLA</td>
<td>HLA mismatch loci</td>
<td>Resolution level</td>
</tr>
<tr>
<td>Name of patient</td>
<td>Name of hospital</td>
<td>Date of treatment</td>
<td>Number of stem cell treatment</td>
<td>HLA</td>
<td>HLA mismatch loci</td>
<td>Resolution level</td>
</tr>
<tr>
<td>Name of patient</td>
<td>Name of hospital</td>
<td>Date of treatment</td>
<td>Number of stem cell treatment</td>
<td>HLA</td>
<td>HLA mismatch loci</td>
<td>Resolution level</td>
</tr>
<tr>
<td>Name of patient</td>
<td>Name of hospital</td>
<td>Date of treatment</td>
<td>Number of stem cell treatment</td>
<td>HLA</td>
<td>HLA mismatch loci</td>
<td>Resolution level</td>
</tr>
<tr>
<td>Name of patient</td>
<td>Name of hospital</td>
<td>Date of treatment</td>
<td>Number of stem cell treatment</td>
<td>HLA</td>
<td>HLA mismatch loci</td>
<td>Resolution level</td>
</tr>
<tr>
<td>Name of patient</td>
<td>Name of hospital</td>
<td>Date of treatment</td>
<td>Number of stem cell treatment</td>
<td>HLA</td>
<td>HLA mismatch loci</td>
<td>Resolution level</td>
</tr>
<tr>
<td>Name of patient</td>
<td>Name of hospital</td>
<td>Date of treatment</td>
<td>Number of stem cell treatment</td>
<td>HLA</td>
<td>HLA mismatch loci</td>
<td>Resolution level</td>
</tr>
<tr>
<td>Name of patient</td>
<td>Name of hospital</td>
<td>Date of treatment</td>
<td>Number of stem cell treatment</td>
<td>HLA</td>
<td>HLA mismatch loci</td>
<td>Resolution level</td>
</tr>
<tr>
<td>Name of patient</td>
<td>Name of hospital</td>
<td>Date of treatment</td>
<td>Number of stem cell treatment</td>
<td>HLA</td>
<td>HLA mismatch loci</td>
<td>Resolution level</td>
</tr>
</tbody>
</table>

Abbreviations: HLA, human leukocyte antigen; HUMC, Hanyang University Medical Center at Curi, Seoul, Korea.
and PCR-sequence-specific oligonucleotide (SSO) methods and selected from the inventory. The detailed HLA matching information is described in Table 1.

**MSC Isolation and Expansion**

As described in our previously published papers on UCB-derived MSCs [18], after thawing at 37°C, cord blood cells were separated into a low-density mononuclear fraction (1077 g/ml) by Ficoll-Paque Plus (GE Healthcare AB, Uppsala, Sweden, http://www.amersham.com), and mononuclear cells were washed and suspended in a culture medium (Dulbecco’s modified Eagle’s medium low glucose [Gibco, Grand Island, NY, http://www.invitrogen.com] containing 20% fetal bovine serum [Gibco]) supplemented with 10 ng/ml basal fibroblast growth factor, 100 U of penicillin, 1,000 U of streptomycin, and 2 mM l-glutamine [Gibco]) and then seeded in T-25 flasks at a concentration of 4 × 10^4 to 5 × 10^5 cells per cm^2. Cultures were maintained at 37°C in a humidified atmosphere containing 5% CO2. Three days later, the suspended cells were transferred into new flasks. The medium was changed every 7 days thereafter. Cells were passaged by trypsinization (0.005% trypsin/ethylenediaminetetraacetic acid; Gibco) upon reaching 80%-90% confluence and replated at 5 × 10^4 cells per cm^2. Cells were cultured and expanded for 4 weeks from primary culture and prepared under two passages before being used for clinical trials.

**Transplantation Procedure**

We delivered our UCB-derived MSCs (1 × 10^6) into just the proximal and adjacent area to the lesions of the patients. The depth of injection was approximately to the level of the subcutaneous tissues and muscles. We used a 23-gauge needle syringe for the effective delivery of the cells. An immunosuppressant was not given to the patients.

**Follow-Up Angiography**

We preformed a pretransplantation angiography of the patients, as well as 1-month and 4-month follow-up angiographies, to evaluate the vascular status in the affected areas.

**Animal Experiments**

**Animal Model.** Nude mice (BALB/cAnNCrjBgi-nu; Charles River Diagnostics) aged 7 weeks were anesthetized with 150 mg/ml Ketamine i.p. for operative resection of left femoral artery and subsequently for angiography imaging. Immediately before sacrifice, the mice were injected with an overdose of Ketamine.

**MSC Transplantation.** Immediately after the resection of one femoral artery, 1.3 × 10^6 culture-expanded MSCs were injected (i.m.) into the ischemic position of the hind limb (n = 8). Control groups were identically injected with medium (n = 3) or saline (n = 7; as control for medium effect).

**Physiological Assessment of Transplanted Animals.** Mice were anesthetized with Ketamine as described previously. The left ventricle was fixed proximally and canulated distally with a 26G polyethylene catheter. Warmed heparinized saline (10 U/ml, 0.4 ml total volume) was injected into the aortic catheter. Iodine was then injected into the aortic catheter. The skin was removed from the mouse hind limbs to avoid imaging the dermal vasculature. Images were acquired by using single-enveloped Kodak X-OMAT TL film at 500 mA, 50 kV, and 0.5-second exposure.

**Probe.** Genomic DNA of human liver cancer cells was extracted with the DNeasy tissue kit (Qiagen, Hilden, Germany, http://www1.qiagen.com). The PCR primers were positioned in the most conserved areas of human Alu sequences and produced a PCR product of 224 base pairs (bp) [19]. The following primers for expanding Alu sequences by PCR were used: Alusense, 5’-ACG CCT GTA ATC CCA GCA CT-3’; Alu-antisense, 5’-TCG CCC AGG CTG GAG TGCA-3’. PCR was carried out under the following conditions: 95°C for 10 minutes; 25 cycles of 95°C for 30 seconds, 58°C for 45 seconds, and 72°C for 45 seconds; and 72°C for 10 minutes. The PCR product was electrophoresed on a 2% agarose gel and stained with ethidium bromide (10 ng/ml). Afterward, a DNA band of 224 bp was eluted with the Qiaquick gel extraction kit (Qiagen). The PCR product was DIG-labeled with the PCR DIG probe synthesis kit (Roche Diagnostics, Basel Switzerland, http://www.roche-applied-science.com). This PCR was performed with 50 ng of eluted DNA by using the same PCR protocol as described above. The labeled probe was purified by ethanol precipitation according to the protocol of the PCR DIG probe synthesis kit.

**H&E Staining and In Situ Hybridization.** At 30 days after the injection, tissue from the lower calf muscles of ischemic and healthy limbs was harvested, fixed with 10% neutrally buffered formalin, and embedded in paraffin. Two serial sections were cut at 4 μm, placed on Superfrost/plus slides (Fisher Scientific International, Hampton, NH, http://www.fisherscientific.com) and stored at room temperature. One section was processed for in situ hybridization as described previously [20] and the other for H&E staining.

Just before application to in situ hybridization, sections were deparaffinized in xylene and rehydrated in PBS. After incubating with PBS containing 0.3% Triton X-100, the slides were then incubated with TE buffer containing 2 mg/ml proteinase K for 30 minutes at 37°C and rinsed again three times for 5 minutes. To reduce nonspecific background staining, the slides were acetylated with TEA buffer containing 0.25% (v/v) acetic anhydride (Sigma-Aldrich, St. Louis, http://www.sigmaaldrich.com) twice for 5 minutes. After prehybridization with hybridization buffer (50% formamide [Sigma-Aldrich] in 5× SSC, 0.1% sodium-lauroylsarcosine [Sigma-Aldrich], 0.02% SDS [Sigma-Aldrich], 2% blocking reagent [Roche]) for 3 hours at 85°C, the slides were incubated with fresh hybridization buffer containing the denatured DIG-labeled DNA probe (10–200 ng/ml) for a further 10 minutes at 94°C. Then, the slides were transferred to ice for 10 minutes and incubated overnight at 42°C. Prehybridization and hybridization steps were performed in a moist chamber containing 50% formamide. After hybridization, the slides were briefly rinsed in 2× SSC at room temperature and three times in 0.1× SSC for 15 minutes at 42°C.

Visualization of the DIG-labeled DNA probe was performed according to the protocol of the DIG nucleic acid detection kit (Roche). The slides were blocked for 30 minutes with blocking
buffer (1% blocking reagent [Roche] in maleic acid buffer [0.1 M maleic acid, 0.15 M NaCl, pH 7.5]) and then incubated for 1 hour with an alkaline phosphates/conjugated antibody solution (anti-sheep, 1:2000 in blocking buffer containing 0.1% Triton X-100). Following four washes with maleic acid buffer for 15 minutes, the slides were equilibrated for 5 minutes in Tris buffer, pH 9.5. The color development was carried out with freshly prepared substrate solution (nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate [Roche] in Tris buffer, pH 9.5). After 2–6 hours, enzymatic reaction was terminated with stop buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The slides were washed three times for 15 minutes and counterstained with 0.1% nuclear Fast Red (Sigma-Aldrich) for 2 minutes. Afterward, the slides were again rinsed three times with PBS.

**Angiographic Procedure**

Animals were sedated with Ketamine, and a thoracotomy was performed. The heart was exposed, and the contrast material, iohexol (Omnipaque, 300 mg I/ml; Amersham Health, Cork, Ireland) was administered directly into the left ventricle. The vascular anatomy and anastomosis of the hind limb were evaluated.

**Statistical Analysis**

The data were represented as mean ± SE (standard error) obtained from three separate experiments. The statistical comparison was performed using analysis of variance and Duncan’s t test. A p value less than 0.05 was considered significant.

---

**Figure 1.** Angiographic analysis of patient with Buerger’s disease after transplantation with umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs). Collateral branches and vascularities increased strikingly at the ankle and foot before (upper left), 30 days after (right), and 120 days after (lower left) UCB-MSC implantation. Abbreviation: Tx, transplantation.

**Table 2.** Results and follow-up periods of patients with Buerger’s disease

<table>
<thead>
<tr>
<th>Sex/age</th>
<th>Lesions</th>
<th>Effect on pain</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Male/53</td>
<td>Hands and feet</td>
<td>12 hours</td>
<td>25 months</td>
</tr>
<tr>
<td>2 Male/44</td>
<td>Hands and feet</td>
<td>14 days</td>
<td>21 months</td>
</tr>
<tr>
<td>3 Male/39</td>
<td>Right lower extremity</td>
<td>5 hours</td>
<td>16 months</td>
</tr>
<tr>
<td>4 Male/36</td>
<td>Left lower extremity</td>
<td>12 days</td>
<td>8 months</td>
</tr>
</tbody>
</table>

**RESULTS**

**Clinical Results of Transplantation of UCB-Derived MSCs to Patients**

The improvement of peripheral circulation was proved by angiography, which showed increased capillary formation on the affected lesions and decreased vascular resistance on those areas (Fig. 1). But more strikingly, the pain of the patients was alleviated more rapidly than the formation of the new capillaries. In the early results, pain at rest disappeared between 5 hours and 14 days in all patients (Table 2), and the unhealed skin lesions of the two patients showed skin regeneration within 120 days (Fig. 2). The vascular changes were observed in 120-day follow-up angiography. At this point, the patients were only taking the vasodilatation drug PGE1, and no analgesics. No sort of side effects—fever, rash, or itching sensation—were noted. In addition, symptoms and signs of so-called allograft rejection were not observed without immunosuppression during the follow-up periods of up to 25 months in the case of the first treated patient.

**Tissue Salvage Achieved by MSC Transplantation**

Enhanced neovascularization in mice transplanted with MSCs led to important biological consequences. Because hind limb neovascularization is inherently impaired in athymic nude mice, these mice typically develop extensive limb necrosis, often leading to auto-amputation of the ischemic limb; rarely does the limb survive the entire 28-day study period intact (Fig. 3). Indeed, among the mice in which induction of hind limb

---

**Figure 2.** Necrotic lesion on right thumb of Patient 2. Patient showed necrotic lesion of right thumb before treatment (left). Umbilical cord blood-derived mesenchymal stem cell treatment can cure the necrotic lesion on day 120 after injecting (right).

**Figure 3.** Human umbilical cord blood-derived multipotent stem cells can salvage limbs in ischemic hind limb mouse model. Representative photographs of control medium (left) showed autoamputation within a week after ligation. Umbilical cord blood-derived mesenchymal stem cell treated ischemic limb showed limb necrosis (middle) and limb salvage (right) on day 28 after ligation.
Table 3. Amputation rate and onset of symptoms after implantation of human umbilical cord blood-derived multipotent stem cells in nude mice

<table>
<thead>
<tr>
<th></th>
<th>Amputated individual numbers (amputation rate)</th>
<th>Symptom days ± SD (n)</th>
<th>Amputation days ± SD (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7 of 7 (100%)</td>
<td>1.86 ± 0.38 (7)</td>
<td>6.57 ± 2.64 (7)</td>
</tr>
<tr>
<td>Immediate injection of MSCs</td>
<td>5 of 8 (62.5%)**</td>
<td>4.00 ± 1.77 (8)**</td>
<td>7.80 ± 3.56 (5)</td>
</tr>
<tr>
<td>Medium control</td>
<td>3 of 3 (100%)</td>
<td>1.00 ± 0 (3)</td>
<td>3.33 ± 0.58 (3)</td>
</tr>
</tbody>
</table>

\* Significantly different from control (p < .05).
Abbreviation: MSC, mesenchymal stem cell.

ischemia was followed by medium control and uninjected control, limb salvage was not observed in either the medium control (three animals) or the uninjected control of seven animals. Additionally, grossly extensive forefoot necrosis developed at 1 day after femoral artery ligation (n = 10), leading to a rate of spontaneous amputation of 7 of 10 (70%) within 7 days in both controls. Likewise, a preserved limb was observed in only three of seven (43%) mice in the uninjected control.

In contrast, UCB-derived MSCs transplantation was associated with successful limb salvage in five of eight (63%) mice (Table 3). Foot necrosis was limited to four of eight (50%) animals, and only three (38%) showed spontaneous limb amputation (Table 3). The difference in outcome between the MSC-treated mice and both medium-injected and uninjected groups was statistically significant (p < .05) (Table 3).

Histological Assessment of MSC Transplantation
To confirm the homing and incorporation of administered MSCs to the sites of vessels, in situ hybridization for the human-specific *Alu* gene was performed. Tissue sections were extracted from the thigh muscle of the hind limb and examined. The mouse vessels were visualized by regular histopathological findings (Fig. 4). The serial tissue section was hybridized by human-specific *Alu* gene probe, and human MSCs were identified in the arterial walls of mouse ischemic hind limb tissue. Human-specific cells were found in the endothelial area of vessels (Fig. 4).

Angiography of the Hind Limb of mice
Angiography of the hind limb mouse model on day 28 after femoral artery ligation clearly showed the artery in the UCB-derived MSC-treated hind limb (Fig. 5).

**DISCUSSION**
Recently, some experimental and clinical studies have also supported the view that angiogenesis might be stimulated by PGE1 treatment, corticotomy, and distraction osteogenesis [21–23]. However, it has not been shown that Buerger’s disease can be satisfactorily cured by any given treatment. On the basis of clinical experience, Buerger’s disease tends to progress gravely over the years in spite of treatment. In this study, we were able to show the possibility of treating Buerger’s disease patients using multipotent mesenchymal stem cells from human umbilical cord blood.

It has been published that neovascularization was observed in the ischemic limb disease model using EPCs [11]. However, that study has limitations. Newly formed vessels defined post-operatively may not be visible preoperatively by angiography because of arterial obstruction (Fig. 1), and this is probably not neangiogenesis. This could be the result of arteriogenesis, the conversion of preexistent collateral arterioles into large conductance arteries. In our present study, we were able to show that human-specific *Alu* gene-expressed cells were found in the arterioles of the hind limb in nude mice, as shown in Figure 4. This result strongly supports the theory that angiogenesis is one of the therapeutic mechanisms used by UCB-derived MSCs in our Buerger’s disease patients. Because Buerger’s disease is a nonatherosclerotic vasoocclusive disease in which the inflammatory component plays a major role, this study was not able to find a suitable animal model for humans. However, a nude mouse model with femoral artery ligation has been used for angiogenesis and cell therapies in several studies [22] and is well-established, even though this animal model is somewhat different from human diseases. Therefore, using this animal model, we have some evidence for explaining how stem cells contribute to Buerger’s disease patients.

Once a skin ulcer occurs in ischemic limb disease, it is important to prevent major amputation and prolonged hospitalization, which markedly influence the quality of the patient’s life. Although successful bypass surgery dramatically improves symptoms of ischemia, it has poor long-term results and is not frequently applicable in the case of Buerger’s disease. The
results of arterial reconstruction using UCB-derived MSCs were better than others reported by Ohta et al. and Sasajima et al. [5, 24].

This study showed that arterial reconstruction or prevention of arterial obstruction using UCB-derived MSCs completely controls the rest pain and shortens the healing process of ischemic ulcers. The specific mechanism and the reason for the rapid improvement in the patient still remain to be fully explained. The possible explanations for these dramatic effects are as follows: 1) UCB-derived MSCs are able to produce a large amount of cytokines and growth factors (unpublished data); 2) UCB-derived MSCs can directly reconstitute arterioles, as shown in the animal experiment (Fig. 4), because we also demonstrated that UCB-derived MSCs were capable of differentiating into endothelial cells in vitro (unpublished data). Interestingly, the quick reduction of rest pain before vessel formation in all treated patients was observed. At this moment, we still do not understand the underlying mechanistic details related to pain release in the patients. Therefore, an additional study is needed to examine this. However, we can assume that there are some possible mechanisms underlying pain reduction that might be related to growth factors or pain releasers from injected stem cells before formation of vessels in ischemic lesions. Currently, we are continuing to monitor these patients for any potential long-term negative effects. Our data lead us to conclude that our UCB-derived MSCs retain endothelial differentiation potential that is suitable for basic and clinical studies aimed at development of vasculature-directed regenerative medicine (unpublished data). In clinical trials, we are still investigating the appropriate doses for human at this moment. Therefore, we need more clinical trials. In this animal study, we just used the maximum cell number (1,000,000) from one unit from one donor. Now, we are trying more cells from dual donors in patients.

UCB-derived MSCs have many advantages because of 1) the immaturity of newborn cells compared with adult cells, and 2) the fact that immune reaction causing dysfunctional grafts can be avoided. Therefore, we established a new paradigm for stem cell therapy without immunosuppressants, because cord blood-derived stem/progenitor cells are less likely to attack a recipient’s body than bone marrow-derived cells are.

Based on their large ex vivo expansion capacity, as well as their differentiation potential, UCB-derived MSCs can be visualized as an attractive source for cellular or gene transfer therapy for incurable vasocclusive and neuro-degenerative diseases, such as Alzheimer’s disease, Parkinson’s disease, and Niemann–Pick’s disease, and so on. In further studies, we still need to grow and expand the UCB-derived MSCs in a serum-free medium to ameliorate any potential future animal-borne diseases via bovine fetal serum in the medium for clinical trials.

ACKNOWLEDGMENTS
S.K. and H.H. contributed equally to this work. This study was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (01-PJ10-PG-01E01-0015) and a grant from Seoul City. This work was also supported by a grant from the Korea Science & Engineering Foundation (R01-2005-000-10190-0).

DISCLOSURES
The authors indicate no potential conflicts of interest.

REFERENCES


