Effect of Autologous Bone-Marrow Cell Transplantation on Ischemic Ulcer in Patients With Buerger’s Disease

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Background Thromboangiitis obliterans, also known as Buerger’s disease, is characterized by peripheral occlusive changes in the arteries of the upper and lower limbs and treatment is often ineffective. Intramuscular transplantation of autologous bone marrow-mononuclear cells (BM-MNC) has been recently reported as improving the symptoms and clinical manifestations in patients with severely ischemic limbs, mostly caused by arteriosclerosis obliterans. The present study focused on the patients with Buerger’s disease presenting with rest pain and/or skin ulcer untreated by conventional treatments.

Methods and Results Fourteen patients with Buerger’s disease (Fontaine III: n=2, Fontaine IV: n=12) underwent transplantation of autologous BM-MNC into ischemic skeletal muscles of either the upper or lower limb. After 4 weeks, rest pain was significantly reduced. In 19 skin ulcers of 9 patients, 8 ulcers were healed and 8 were diminished in the size. These improvements were maintained for 24 weeks without complications.

Conclusions In patients with Buerger’s disease, intramuscular transplantation of autologous BM-MNC improved symptoms and clinical manifestations, especially skin ulcer. (Circ J 2007; 71: 1187–1192)

Key Words: Bone marrow; Buerger’s disease; Cell therapy; Ischemia; Skin ulcer

Thromboangiitis obliterans (Buerger’s disease) is a nonatherosclerotic segmental inflammatory disease, commonly affecting the small and medium-sized arteries, veins, and nerves of the arms and legs including the palmar, plantar, tibial, peroneal, radial, and ulnar arteries and the digital arteries of the fingers and toes. It was first described in 1879, and detailed and accurate pathological findings was further described by Leo Buerger in 1908: Buerger’s disease typically occurs in young male smokers, although there is an increasing prevalence in women; and generally presents as coldness of the fingers and intermit-tent claudication as the initial symptoms. As it progresses, patients develop rest pain and skin ulcers of the extremities and eventually have to undergo limb amputation with subsequent impaired QOL.

Treatment of Buerger’s disease is not well established. Because the of arteries involved are mostly peripheral, diffuse, and segmental, surgical revascularization is generally ineffective. Sympathectomy may prevent amputation and/or reduce rest pain, but its efficacy is limited. Although previous reports show that prostaglandin analogs and/or streptokinase reduce pain at rest and the extent of gangrene of the ischemic limbs, these pharmacological approaches also seem not to be fully effective. The only proven strategy to prevent its progression is complete discontinuance of cigarette smoking or use of tobacco in any form.

We recently reported the efficacy and safety of intramuscular local transplantation of autologous bone marrow-mononuclear cells (BM-MNC) for patients with severely ischemic limbs mostly caused by arteriosclerosis obliterans (ASO). This treatment dramatically reduced their rest pain and the size of the ischemic skin ulcer, probably via increased collateral arteries in the ischemic limbs. However, it is unknown whether this treatment is also effective for Buerger’s disease, so in the present study, we focused on the therapeutic effects, especially on skin ulcers, in patients with Buerger’s disease.

Methods

Patients

In all patients (13 males, 1 female; 42.6±8.0 years), Buerger’s disease was diagnosed based on the diagnostic criteria proposed by the Committee for Buerger’s disease of Japanese Ministry of Health and Welfare (Table I). They had ischemic upper or lower limbs with uncontrolled rest pain (Fontaine III) and/or nonhealing ischemic ulcer/gangrene (Fontaine IV). Before enrolling in this study, they all underwent optimal treatment (Table I). Invasive treatments were performed at least 1 year ago and medical treatments including antiplatelet agents and oral prostaglandin analogs had been continued but without appreciable effect. Exclusion criteria were pregnancy, blood disorder, poorly controlled diabetes mellitus (HbA1c >6.5% and proliferative retinopathy), a malignant disorder during the past 5 years, and/or unwillingness to participate. At 2–3 weeks before admission, we screened them at an outpatient clinic and instructed them not to smoke. After admission, they under-
went routine examinations for 2–3 weeks and then underwent the cell transplantation. Thus, there were 4–6 weeks before cell transplantation during which they abstained from smoking. We confirmed that each patient's clinical status, including ulcer, was unchanged during this period. They abstained from smoking during hospitalization and were instructed not to smoke after discharge. The Ethics Review Board of the Kurume University School of Medicine approved the protocol, and all patients gave written informed consent. Administration of all medical agents was continued.

Preparation and Transplantation of BM-MNC

While the patients were under general anesthesia, we aspirated whole bone marrow cells (approximately 500 ml) from the ileum into a plastic bag containing heparin and ACD-A solution (Terumo, Tokyo, Japan) as the anticoagulants. Immediately after aspiration, the cells were sorted in a CS3000-Plus blood-cell separator (Baxter, Deerfield, USA) to isolate the BM-MNC, which were finally 95% purified and concentrated to a final volume of approximately 50 ml (4.2±4.0×10⁹ cells). These cells were transplanted within 3 h after marrow aspiration by intramuscular injection into either the ischemic brachial muscle or gastrocnemius muscle (2.9±0.7×10⁹ to 5 upper limbs, 4.5±4.8×10⁹ to 9 lower limbs). We injected 1.0 ml of BM-MNC solution into 50 points (1.0–1.5 cm deep) with a 23-gauge needle.

Assessment of Efficacy After Cell Transplantation

Within 7 days before transplantation and at 4 and 24 weeks after cell transplantation, we performed the following examinations to assess the ischemic status by symptoms (rest pain), physical findings (size of skin ulcer), laser Doppler blood flow (LDBF) analysis, and angiography.

**Visual Analog Scale (VAS)**

To assess the grade of rest pain, we used a VAS as a standard method for quantification and evaluation of rest pain. The patients assessed the severity of their rest pain by depicting the length from 0 to 10 cm, where 0 cm means “pain free” or “no pain”, and 10 cm is “maximum excruciating pain”.

**Digital Subtraction Angiography (DSA)**

The amount of contrast, force of contrast injection and position of the

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**Table 1 Baseline Characteristics of Patients With Buergeis Disease**

<table>
<thead>
<tr>
<th>Case no</th>
<th>Age (years)</th>
<th>Gender</th>
<th>Fontaine classification</th>
<th>Target limb</th>
<th>VSA (cm)</th>
<th>No. injected BM-MNC (×10⁹)</th>
<th>Medical history</th>
<th>Past invasive treatment</th>
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<tr>
<td>1</td>
<td>42</td>
<td>M</td>
<td>III</td>
<td>Upper</td>
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<td>Sympathectomy</td>
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<tr>
<td>2</td>
<td>52</td>
<td>M</td>
<td>III</td>
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<td>IGT,HL</td>
<td>Amputation</td>
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<td>3</td>
<td>48</td>
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<td>Upper</td>
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<td>Sympathectomy</td>
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<tr>
<td>4</td>
<td>48</td>
<td>M</td>
<td>IV</td>
<td>Lower</td>
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<td>5</td>
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<td>None</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
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<td>Amputation</td>
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<tr>
<td>11</td>
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<td>Upper</td>
<td>7</td>
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<td>Sympathectomy</td>
</tr>
<tr>
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<td>16.9</td>
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</tr>
<tr>
<td>14</td>
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<td>F</td>
<td>IV</td>
<td>Lower</td>
<td>0</td>
<td>2.38</td>
<td>None</td>
<td>Bypass operation</td>
</tr>
</tbody>
</table>

*Rest pain well controlled by analgesics.

VAS, visual analog scale; BM-MNC, bone marrow mononuclear cell; IGT, impaired glucose tolerance; HL, hyperlipidemia; HT, hypertension.

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**Fig 1. Changes in the visual analog scale (VAS) and laser Doppler blood flow (LDBF) ratio at 4 weeks and 24 weeks after cell therapy.** Bold line indicates the average. At 24 weeks, 3 patients and 1 patient, respectively, rejected the examinations. VAS was significantly shortened at both 4 (n=14; p=0.001) and 24 (n=11; p=0.002) weeks; LDBF ratio was tended to increase at 4 (n=8) and 24 (n=7) weeks.
catheter tip were fixed to be as objective as possible before and after cell transplantation. Co-investigators who were unaware of the clinical profiles evaluated the findings. When new filling of contrast into the main conducting arteries was observed clearly in a previously avascular area, it was judged to be an increase in the number of visible arteries. Changes in the DSA findings were graded as –1 (decrease), +0 (no change), +1 (slight increase), and +2 (marked increase).

Size of Skin Ulcer  We measured the longest and shortest diameters (mm) of the skin ulcers.

LDBF Analysis  To assess the blood perfusion of ischemic areas, we used a LDBF analyzer (Laser Doppler Perfusion Imager System, moorLDI™, Mark 2, Moor Instruments, Wilmington, Delaware). Blood flow (ie, blood cell movement), mainly under the skin, is displayed as changes in the laser frequency using different color pixels.

To avoid data variations because of ambient light and temperature, we determined the ischemic/nonischemic limb blood flow ratio.

Assessment of Safety After Cell Transplantation  At 4 and 24 weeks after cell transplantation, we checked for the following complications by blood tests, ECG, X-rays, fundoscopy, and computed tomography: proliferative retinopathy, malignant tumor, myocardial infarction and stroke, hemangioma, and ectopic formation of bone- or adipose-tissues.

Statistical Analysis  Continuous variables are presented as mean ± SD. Statistical comparisons were performed by nonparametric paired t-test. Statistical significance was assumed at a value of p<0.05.
Fig 3. Changes in the angiographic findings at 4 weeks and 24 weeks after the cell therapy. The changes were graded as –1 (decrease), +0 (no change), +1 (slight increase), +2 (marked increase).

**Results**

Although a total of 14 subjects were initially enrolled, 3 patients rejected re-evaluation at 24 weeks after cell transplantation. No complications occurred at the time of aspiration or at transplantation of marrow cells. Unexpected complications as described before were not observed at 4 and 24 weeks after cell treatment.

**VAS**

We assessed pain in 14 patients at 4 weeks using the VAS. Pain had significantly improved and the VAS was shortened from 5.4±3.3 at baseline to 2.5±2.7 cm (p=0.001, Fig 1). At 24 weeks we were able to evaluate pain in only 11 patients, but the improvement had been maintained (1.1±2.2 cm; p=0.002 vs baseline).

**LDBF Analysis**

We did not calculate the LDBF ratio in patients with history of large amputation in the target or nontarget limb.

Thus we assessed the ratio in 8 patients at 4 weeks. Although the ratio tended to increase from 0.66±0.16 at baseline to 0.75±0.15 cm (Fig 1) at 4 weeks to 0.8±0.15 cm (Fig 1) at 24 weeks, it was not statistically significant. Fig 2B shows a representative LDBF.

**Angiography (DSA)**

The DSA findings are summarized in Fig 3. At 4 weeks, angiographic improvement (increase number of visible arteries) was observed in 10 of 14 patients. Although we were able to evaluate only 9 patient at 24 weeks, the improvement was maintained in 7 patients.

**Size of Skin Ulcer**

Skin ulcers were initially present in 12 patients, but Case numbers 9, 11, and 12 (Table 1) rejected re-evaluation at 24 weeks. The changes in 19 ulcers in 9 patients are shown in Table 2. At 4 weeks, 8 ulcers were completely healed and 8 ulcers were diminished in size. Although 12 ulcers were completely healed and 3 ulcers had diminished at 24 weeks, 4 ulcers had become larger.

**Case Number 6** A 33-year-old male had a history of sympathectomy and amputation of the right and left lower legs, and the right 4th finger and left 2nd and 3rd fingers during past 10 years. Ulcers developed on the stump of the 3rd finger and the dorsal surface of the left hand with uncontrollable rest pain. On angiography, the left ulnar and radial arteries were not visible and poor cork-screw like collateral arteries were present. After injection of BM-MNC (3.64×10^6 cells) into the muscles of the left lower arm and hand, the size of both ulcers gradually diminished, with less rest pain at 4 weeks. The ulcers were completely healed at 24 weeks (Fig 2A).

**Case Number 8** A 43-year-old male had intermittent claudication with 200-m walking and coldness in the left lower limb for 3 years. Later, skin ulcers developed on the 3rd, 4th, and 5th toes of the left foot. He had severe rest pain despite sympathectomy and hyperbaric oxygen therapy. Amputation of the 5th toe was performed and the 3rd and 4th toes auto-amputated without healing. DSA showed total

<table>
<thead>
<tr>
<th>Table 2 Changes in Ulcer Characteristics</th>
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<tbody>
<tr>
<td><strong>Case no.</strong></td>
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</tbody>
</table>
occlusion of the left popliteal artery and distal dwindling small arteries, which were filled by poor collateral vessels originating from the deep femoral artery. We injected BM-MNC (4.14×10⁹ cells) into the left lower leg and sole after debridement of the 3rd and 4th toes. At 4 weeks the ulcers were smaller, associated with less rest pain, and were completely healed at 24 weeks (Fig 4).

Discussion

Although we previously reported the efficacy and safety of intramuscular autologous BM-MNC transplantation into the ischemic limbs of patients with ASO, this is the first report for patients with Buerger’s disease with rest pain and/or skin ulcer that were uncontrolled with optimal treatment. As was the case for ASO, this treatment was not only effective for ameliorating the subjective symptoms, but also for objective improvements such as healing of ulcers and angiographic findings. Moreover, the efficacies lasted for at least 24 weeks with no complications. Thus, this treatment may be considered an option for severe Buerger’s disease.

Methodological Consideration

We enrolled only those patients who had suffered from rest pain and/or skin ulcer for many years after conventional therapies, including smoking cessation. As shown in Table 1, most patients had already undergone invasive treatments such as amputation, sympathectomy and, if possible, bypass grafting. Thus, the patients received our cell therapy as the last choice.

Although we observed subjective improvements in rest pain, it may have been a placebo effect. Therefore, we assessed efficacy by measuring the size of the ulcers. As shown in Table 2, ulcers healed in many patients in whom no other treatment had been effective. It is unlikely that only the present treatment induced the placebo effects; however, we can not be certain because we did not have a control group.

We further evaluated the objective efficacy using DSA. In order to be objective, the DSA method was fixed and comparable before and after treatment. Moreover, radiologists who were not the investigators of this study and who were unaware of the clinical information, assessed the angiographic findings. Even with this strict methodology, reading of DSA is subjective in the absence of the use of vasodilators such as Lipo-PGE1 and thus the improvement was judged semi-quantitatively, as described in the Methods.

Efficacy and Safety

In our previous study for ASO, there were no appreciable side-effects or complications associated with this treatment, such as proliferative retinopathy, malignant tumor, myocardial infarction and stroke, hemangioma, and ectopic formation of bone- or adipose-tissues, for 24 weeks. In the present study, there were no complications either. Thus, the use of autologous BM-MNC is probably safer than other strategies such as gene encoding viruses or recombinant proteins. However, we can not deny the possibility of complications during a longer follow-up.

Clinical reports describing therapeutic efficacy for ischemic ulcers in Buerger’s disease have been very few. Intramuscular injection of naked HGF plasmid DNA partially improved 2 ulcers in 3 patients after 3 months. Isner et al reported that intramuscular administration of VEGF165 gene in 7 hind limbs improved 3 gangrenes after 3 months. As apparent from Table 2, our treatment was very effective for ulcer healing; many large ulcers were completely healed. Only 4 (case no. 5, 10, and 13) of 19 ulcers became larger after 24 weeks compared with before treatment. At present, we have no explanation for this enlargement of the ulcers. Moreover, we were sorry that we lost some patients to follow-up and that we did not obtain data for every patient at 24 weeks. Nonetheless, this treatment seems to be effective for at least 24 weeks and we are now collecting data for a longer period.
Mechanism of Action

It has been shown that endothelial progenitor cells (EPC) derived from BM-MNC contribute to postnatal neovascularisation.13-17 We previously reported in animals that BM-MNC contain many EPC and that intramuscular transplantation of autologous BM-MNC into ischemic limbs induces neovascularization and increases blood flow.18 Because we did not examine the histology in the present study, we do not know whether the mechanism was angiogenesis, vasculogenesis or arteriogenesis.19 Although it was considered that these favorable results were induced by transdifferentiation and subsequent proliferation of the transplanted BM-MNC enabling EPC to regenerate microvasculature, there are other possibilities; it has been reported that bone marrow cells secrete many angiogenic cytokines such as VEGF, FGF-2,20 angiopoietin-1,21 and interleukin-1.22 which play an important role in the maturation and maintenance of the vascular system.20,23

Study Limitations

One limitation was the lack of a control (placebo) group. However, it is unlikely that our patients had a spontaneous decrease of rest pain and healing of ulcers, because they had already undergone the optimal therapies (Table 1) on many occasions without success. The second limitation is the lack of monitoring the smoking status. Although our patients stopped smoking at 2-3 weeks before admission and during hospitalization and were instructed not to smoke after discharge, we were not sure about their smoking status after discharge because we did not check the serum level of nicotine or cotinine. The third limitation is that the efficacy was done at 4 weeks, which could lead to overestimation of nicotine or cotinine. The third limitation is that the efficacy was done at 4 weeks, which could lead to overestimation of nicotine or cotinine. The third limitation is that the efficacy was done at 4 weeks, which could lead to overestimation of nicotine or cotinine. The third limitation is that the efficacy was done at 4 weeks, which could lead to overestimation of nicotine or cotinine. The third limitation is that the efficacy was done at 4 weeks, which could lead to overestimation of nicotine or cotinine.

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References