Stem cell therapy for peripheral arterial disease: a review of clinical trials
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Abstract

Stem cells have demonstrated significant potential for regeneration in peripheral arterial disease in both animal and human studies. While results of clinical trials have been variable, they have clearly displayed benefit for patients with critical limb ischemia and peripheral arterial disease. Because many of these patients are not eligible for revascularization procedures, there is urgent need for novel therapies. A summary of all clinical trials using stem cell therapy in peripheral arterial disease is described herein with the pertinent findings from each study.

Introduction

The prevalence of peripheral arterial disease (PAD) has been increasing in the United States, with 8 to 12 million people affected.1,2 While catheter-based and surgical therapies continue to improve, 50% of patients with critical limb ischemia or severely disabling claudication are not eligible for revascularization procedures.3 Each year, over 100,000 people undergo limb amputation as a result of PAD.4 Given the high morbidity and mortality associated with this disease and the limited number of treatments available to these patients, there is an immense need for novel therapies.

Preclinical trials have demonstrated the angiogenic and vasculogenic potential of autologous bone marrow-derived stem cells (BM-SC) in the treatment of PAD.5-7 Bone marrow consists of various pluripotent cells. Its angiogenic properties have been attributed to the differentiation of these progenitor cells into endothelial cells, ultimately leading to the formation of new blood vessels.8 In addition, BM-SC release cytokines and growth factors that promote angiogenesis.9,10 There is also evidence to suggest that BM-SC release vascular endothelial growth factor (VEGF) and chorioallantoic membrane in an ischemic environment, leading to increased vasculogenesis.11,12 All of these findings have set the stage for human trials using progenitor cells in PAD.

The main methods of SC delivery tested in human trials include: intramuscular (IM) injection, intraarterial (IA) injection, a combination of IM and IA injections, indirect mobilization of SC using granulocyte-colony stimulating factor (G-CSF) followed by IA injection, and IA administration of fibroblast growth factor (FGF) to the ischemic limb.

Direct delivery of stem cell therapy

Intramuscular injection of bone marrow derived stem cells

Multiple clinical trials have established the safety and feasibility of IM injection of BM SC into ischemic limbs as summarized in Table 1. In the TACT trial, Tateishi-Yuyama et al. studied 25 patients with unilateral critical limb ischemia, injecting BM-SC into the diseased limb and using IM injections of saline into the normal limb as a control. In the same study, 22 patients with bilateral critical limb ischemia received IM BM-SC injections in one leg, with the other limb serving as the control. This study followed patients for six months and demonstrated that IM injection of BM-SC was feasible and safe, with no adverse events related to BM-SC therapy. It also demonstrated that IM injection of BM-SC is efficacious, with improvement in rest pain, increase in ankle-brachial index (ABI), and increase in transcutaneous oxygen levels (TcO₂).9 At 6 weeks in the two BM-SC groups compared to control. This study further demonstrated formation of new collateral vessels by angiography in those subjects receiving IM BM-SC injection.10

Higashi et al. studied the effect of IM BM-SC injection on endothelial cell function. In this non-randomized, non-controlled trial, leg blood flow was measured using a mercury-filled Silastic strain-gauge plethysmograph in 7 patients receiving IM BM-SC injection. Leg blood flow was measured at baseline, during infusion of acetylcholine as a modulator of endothelium-dependent vasodilation, and during administration of sodium nitroprusside as an endothelium-independent vasodilator. The study showed that leg blood flow was increased at baseline after IM BM-SC injection and also in response to acetylcholine infusion. No change in leg blood flow was seen after BM-SC therapy in response to sodium nitroprusside infusion. The authors concluded that the beneficial effects of IM BM-SC injection were likely related to endothelium-dependent vasodilation, not smooth muscle cell function, and that BM-SC therapy improves endothelial cell function.11

A study by Saigawa et al. further helped establish the safety of IM BM-SC injection in seven patients with a follow up time of only one month. In addition to safety, this study showed efficacy with increasing ABIs and TcO₂ in all patients. It was also the first study to report a dose dependent effect on ABI with number of CD34+ cells.12 CD34, a marker of the most primitive population of endothelial progenitor cells, is expressed by a small fraction of bone marrow (1-4%) and peripheral blood mononuclear cells (<1%) and has been shown to enhance vasculogenesis in ischemic tissues in several preclinical studies.13,14 Durdu et al. were the first to examine the long-term effects of IM BM-SC injection with a mean follow-up of 16.6 months. Their study evaluated 28 patients with grade II or III thromboangiitis obliterans as classified by Rutherford et al. In each patient, the more ischemic limb received IM BM-SC injections while the contralateral, less ischemic limb received IM saline injections as control. This study showed improvement in limb ischemia with limb salvage in all patients except one that required a minor amputation. It also documented improvement in Vascular Quality of Life Questionnaire scores and ABIs at 6 months. Similar to the TACT trial, this study showed increasing collateral formation on angiogram for the IM BM-SC treated limbs.15

In another non-randomized, non-controlled trial, Miyamoto et al. treated 11 limbs with IM BM-SC injection in 8 patients. These patients were followed for a mean of 23.5 months and unlike previously mentioned trials, failed to document an improvement in collateral formation or ABIs at one month. This may have been due to the small sample size or the fact that ABIs were measured only one month after BM-SC injection. The study did find long-term improvement in visual analog pain scale (VAS) scores and noted that 6 of 7 patients that were followed long-term had complete healing of ischemic ulcers. There were some adverse events that occurred during follow up, with one sudden death 20 months after BM-SC therapy and one arteriovenous shunt, which could
<table>
<thead>
<tr>
<th>Study</th>
<th>Trial design</th>
<th>Groups (n)</th>
<th>Vascular disease</th>
<th>Cell count (mean)</th>
<th>Follow up (months)</th>
<th>Primary endpoints</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tateishi-Yuyama et al.(^{13})</td>
<td>NRNC</td>
<td>Group A: BM-SC IM injection in ischemic leg of patient with unilateral PAD (25) (IM injection of saline in other leg used as control)</td>
<td>CLI</td>
<td>1.6 x 10^9</td>
<td>6</td>
<td>ABI</td>
<td>Improvement in ABI by 0.11 as compared to control (IM saline injection) Decrease in number of patients with rest pain as compared to control (IM saline injection)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Group B: BM-SC IM injection in ischemic leg of patient with bilateral PAD (20) PB-SC IM injection of in contralateral leg used as control (20)</td>
<td></td>
<td>(3.7 x 10^7 CD34^+)</td>
<td></td>
<td>Rest Pain</td>
<td>Improvement in ABI by 0.09 as compared to control (IM PB injection) Decrease in number of patients with rest pain as compared to control (IM PB injection)</td>
</tr>
<tr>
<td>Higashi et al.(^{14})</td>
<td>NRNC</td>
<td>BM-SC IM injection (7)</td>
<td>PAD</td>
<td>6.0 x 10^9 cells/kg (1.06 x 10^8 CD34^+ cells/kg)</td>
<td>6</td>
<td>ABI</td>
<td>Improvement in mean leg blood flow in response to acetylcholine infusion from 19.3 to 29.6 m/min</td>
</tr>
<tr>
<td>Saigawa et al.(^{15})</td>
<td>NRNC</td>
<td>BM-SC IM injection (8)</td>
<td>CLI</td>
<td>1.6 x 10^9 (3.8 x 10^7 CD34^+)</td>
<td>6</td>
<td>ABI</td>
<td>Improvement in mean ABI from 0.54 to 0.61</td>
</tr>
<tr>
<td>Durdu et al.(^{16})</td>
<td>NRNC</td>
<td>BM-SC IM injection (28)</td>
<td>PAD (non-revascularizable)</td>
<td>1.89 x 10^9 (5.31 x 10^7 CD34^+)</td>
<td>16.6 (mean)</td>
<td>Avoidance of major or minor amputations</td>
<td>Avoidance of major amputation by all patients, one minor amputation (toe) in one patient Improved scores in King’s College Vascular Quality of Life Questionnaire</td>
</tr>
<tr>
<td>Miyamoto et al.(^{17})</td>
<td>NRNC</td>
<td>BM-SC IM injection (8)</td>
<td>CLI with rest pain</td>
<td>3.5 x 10^7 (6.8 x 10^6 CD34^+)</td>
<td>22.5 (mean)</td>
<td>Visual Analog Pain scale</td>
<td>Improvement in Visual Analog Pain scale score from 5.1 to 2.3</td>
</tr>
<tr>
<td>Kajiguchi et al.(^{18})</td>
<td>NRNC</td>
<td>BM-SC IM injection (7)</td>
<td>CLI</td>
<td>4.57 x 10^8 (2.77 x 10^7 CD34^+)</td>
<td>6</td>
<td>Visual Analog Pain scale</td>
<td>Improvement in Visual Analog Pain scale at 4 weeks in patients with Buerger’s disease (3)</td>
</tr>
<tr>
<td>Idei et al.(^{19})</td>
<td>NRC</td>
<td>BM-SC IM injection (51)</td>
<td>CLI</td>
<td>1.8 x 10^7 (3.5 x 10^6 CD34^+)</td>
<td>58 (median)</td>
<td>Amputation free rate</td>
<td>Amputation free rate 48% in patients with PAD (9% in control) and 95% in Buerger disease (6% in controls) No change in ABI for patients with PAD after 3 years, improvement from 0.55 to 0.61 in Buerger’s disease No change in TcO2 for patients with PAD after 3 years, improvement from 15 to 28 in Buerger’s disease</td>
</tr>
</tbody>
</table>

NRC, nonrandomized controlled; NRNC, nonrandomized noncontrolled; BM, bone marrow; PB, peripheral blood; PL, placebo; IM, intramuscular; CLI, critical limb ischemia; PAD, peripheral arterial disease; ABI, Ankle-Brachial Index; TcO2, transcutaneous oxygen measurements.
have been a direct consequence of the IM BM-SC injection.\textsuperscript{17}

In a continuation of the TACT trial, Kajiguchi \textit{et al}. set out to determine if there was a relationship in post-procedural changes versus the number of transplanted total BM-SC, CD34+ BM-SC, and CD133+ BM-SC. The CD133 surface marker is present on a small number of CD34+ cells and the subset of CD34+/CD133+ cells is thought to be the most primitive type of vasculogenic cell.\textsuperscript{25-27} This was a relatively small study with 6 patients receiving IM BM-SC injection and 1 receiving IM PB-SC injections. All patients were assessed for symptomatic improvement by VAS score and also with objective tests such as ABI, TcO2, and angiography. Responders were defined as patients who showed improvement in subjective symptoms and objective findings, and this was observed in only 3 patients. All 3 responders had Buerger’s disease, leading the authors to conclude that IM BM-SC is effective for patients with Buerger’s disease. There was no statistically significant relationship between effect and number of total BM-SC, CD34+, and CD133+ cells administered, but responders tended to have higher numbers of each.\textsuperscript{19}

Idei \textit{et al}. carried out the largest clinical trial to date involving IM BM-SC injection and, like the TACT trial, showed greater benefit in patients with Buerger’s disease. This trial studied 97 patients in non-randomized, controlled fashion with 51 receiving BM-SC therapy and the others receiving no treatment. All patients had critical limb ischemia, with 46 having Buerger’s disease and 51 having atherosclerotic PAD. The patients were followed long-term with a median follow up of 58 months. Over this time period, patients receiving BM-SC therapy had higher survival compared to control. Both patients with atherosclerotic PAD and Buerger’s disease had a short-term increase in ABI, TcO2, and VAS scores, but only patients with Buerger’s disease sustained these findings long-term, and patients with atherosclerotic PAD had all of these parameters return to pre-therapy levels.\textsuperscript{19}

### Intraarterial injection of bone marrow derived stem cells

Few studies have tested IA injection alone of progenitor cells in patients with ischemic limbs as shown in Table 2.

Cobellis \textit{et al}. carried out a non-randomized, controlled trial that was the first to compare IA injection of BM-SC alone to a control group.\textsuperscript{28} Patients in this trial had phase III or phase IV of the Leriche-Fontaine classification.\textsuperscript{30} The study included 10 patients who received BM-SC therapy, and a control group of 9 patients who did not receive cell therapy. The patients that received BM-SC therapy underwent IA injections at two time points with the second injection occurring 45 days after the first injection. The authors observed improvement in ABI and pain-free walking distance in 8 of 10 treated patients at one year, while in the control group, only 4 patients had 12-month follow-up because surgical intervention became necessary in the rest and none of those followed up displayed any improvement in ABI or pain-free walking distance. The treated group also had higher blood flow at rest by Doppler and increased capillary density by videocapillaroscopy compared to the control group. This study, however, did not examine a dose dependent response in BM-SC therapy.\textsuperscript{28}

The PROVASA trial\textsuperscript{29} is the most recent and largest trial to date using IA BM-SC injection, with a total of 40 patients enrolled in a double blind, randomized fashion, and assigned to receive BM-SC treatment or placebo, and followed for 3 months. After the 3-month time period, those patients that originally received placebo were given IA injection of BM-SC while those that received BM-SC at baseline received a second BM-SC treatment and the patients reevaluated at 6 months. Patients with ulcers or delayed wound healing received up to 3 additional IA BM-SC injections. A statistically significant increase in ABI was not appreciated in this study; however, BM-SC therapy did improve ulcer healing. Patients that received IA BM-SC therapy at baseline had decreased ulcer area than that of the control group and these patients continued to have decreased ulcer area at 6 months after a second BM-SC treatment. Furthermore, the group that received placebo at baseline and IA BM-SC therapy at 3 months had a significant reduction in wound area after treatment with BM-SC. In the 12 patients that were part of the extended study, 10 had complete healing of wounds. In both TcO2 and pain symptoms, the IA BM-SC group displayed improvement at 3 months and continued to improve with the second treatment. The placebo group did not show improvement in either of these categories but after receiving the IA BM-SC therapy at 3 months, began to show improvement. The total number of cells administered and the characterization of these cells were closely examined in this study and the total number of BM-SC administered was found to be an independent predictor for complete ulcer healing. In addition, the number of CD45+/CD34+ cells was significantly higher in patients with healed wounds compared to those that had persistent ulcers. CD45 is a common leukocyte antigen, and similar to CD34+ and CD133+ cells, they are thought to be capable of enhanced vasculogenesis.\textsuperscript{31,32}

### Table 2: Summary of clinical trials using intraarterial injection of bone marrow derived stem cells for peripheral arterial disease

<table>
<thead>
<tr>
<th>Study</th>
<th>Trial design</th>
<th>Groups (n)</th>
<th>Vascular disease</th>
<th>Cell count (mean)</th>
<th>Follow up (months)</th>
<th>Primary end points</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cobellis \textit{et al}.\textsuperscript{21}</td>
<td>NRC</td>
<td>BM-SC IA injection (10)</td>
<td>PAD</td>
<td>Not specified</td>
<td>12</td>
<td>ABI</td>
<td>Improvement in ABI seen in 8 of 10 (80%) of experimental group (no improvement in control)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No stem cell therapy (9)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cobellis \textit{et al}.\textsuperscript{21}</td>
<td>RC</td>
<td>BM-SC IA at baseline and at 3 months (19) PL at baseline, BM-SC IA at 3 months (21) Additional BM-SC IA treatments at 3 month intervals (12)</td>
<td>PAD</td>
<td>Variable (30.2)</td>
<td>30.2 (mean)</td>
<td>ABI</td>
<td>Improved ulcer healing associated with number of IA BM-SC treatments</td>
</tr>
</tbody>
</table>

RC, randomized controlled; NRC, nonrandomized controlled; BM, bone marrow; PL, placebo; IA, intraarterial; PAD, peripheral arterial disease; ABI, Ankle-Brachial Index.
Combination of intramuscular and intra-arterial injection of bone marrow derived stem cells

Several studies have been performed combining IM and IA injection as summarized in Table 3.

The TAM-PAD trial was a non-randomized, controlled trial with 13 patients receiving both IA and IM injection of BM-SC and 12 receiving no cell therapy as control. Of note, there was no cell characterization in this study and therefore progenitor composition was unknown. All patients in this trial had extensive occlusion of the superficial femoral artery. The strategy for delivery of SC involved generation of an ischemic environment in the affected limb just prior to delivery of progenitor cells, based on data showing that ischemic stimuli is the primary factor that leads to SC homing. The patients were instructed to perform bicycle ergometry until they had ischemic pain in the leg, when a blood pressure cuff was inflated to suprasystolic pressures. After a few minutes, the cuff was deflated and the SC injected into the femoral artery. The blood pressure cuff was then reinflated to stop blood flow for a few minutes and the cuff deflated a second time before IM injection of BM-SC followed by repeat bicycle ergometry. This method resulted in a 3.4 fold increase in maximal walking distance at one year with no change in the control group. ABI was also significantly improved from 0.66 to 0.80 after one year, while there was no change in the control group. Other parameters such as venous occlusion plethysmography and arterial blood flow at rest also improved at one year. This was the only study to create an ischemic environment prior to SC administration, and the improved clinical indices infer a need to study this strategy more extensively.

Van Tongeren et al. were the first to compare IM injection of BM-SC alone in 15 patients to that of combined IM and IA injection of BM-SC in 12 patients in a randomized controlled fashion. A greater proportion of patients receiving IM injection alone required amputation, although this was not statistically significant. While BM-SC therapy in both groups cumulatively resulted in improvement in ABI from 0.52 to 0.66 at one year, there was no difference between the two modes of delivery. There was also a cumulative increase in pain-free walking distance with no additional benefit seen with either form of delivery. In addition, the authors did not observe a dose dependent benefit by total number of cells or total number of CD34+ cells.

Franz et al. carried out a non-randomized, non-controlled study in 9 patients in whom amputation was considered the only viable option. Follow-up was short-term at 3 months and all patients received a combination of IA and IM injection of BM-SC. While the improvements seen in ABI 3 months after the procedure were non-significant, six patients avoided major amputation and had symptomatic improvement with complete healing of all ischemic ulcers within the 3-month follow-up. Progenitor cell numbers and composition were not specified.

Indirect recruitment of stem cells with growth factors

Mobilization of stem cells with granulocyte-colony stimulating factor

A summary of trials utilizing mobilization of SC with G-CSF for treatment of PAD is listed in Table 4. Huang et al. helped establish indirect mobilization of SC into peripheral blood using G-CSF and subsequent IM injection of PB-SC as a safe procedure. In their first study, 5 patients were studied for 3 months after receiving pretreatment with G-CSF for 5 days, followed by collection, concentration, and IM injection of PB-SC. While follow-up was short, they observed a significant increase in ABI from 0.52 to 0.67, which helped establish this strategy as a viable and safe method of SC delivery. Their next study was a randomized, controlled trial with 14 patients receiving the same G-CSF and PB-SC therapy. A second treatment of IM PB-SC was given to the experimental group 40 days after the first transplant, while the control group received injections of prostaglandin E1 and did not receive G-CSF or cell therapy. There were statistically significant decreases in rest pain symptoms and increases in ABI in the G-CSF arm compared to the control group. No patients in the experimental group required amputation while 7 of 18 limbs in the control group required amputation.

In a larger but non-randomized, non-controlled trial, Kawamura et al. used the same method of delivery to demonstrate limb salvage in 22 of 30 patients and improvement in limb temperature in 21 of 30 patients by thermogra-

Table 3. Summary of clinical trials using combined intramuscular and intraarterial injection of bone marrow derived stem cells for peripheral arterial disease.

<table>
<thead>
<tr>
<th>Study</th>
<th>Trial Design</th>
<th>Groups (n)</th>
<th>Vascular Disease</th>
<th>Cell Count (mean)</th>
<th>Follow Up (months)</th>
<th>Primary End Points</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartsch et al.</td>
<td>NRC</td>
<td>BM-SC IA and IM injection (13)</td>
<td>PAD</td>
<td>Not specified</td>
<td>13</td>
<td>Pain free walking distance</td>
<td>3.4 fold increase in pain free walking distance (no improvement in controls)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No stem cell therapy (12)</td>
<td></td>
<td></td>
<td></td>
<td>ABI</td>
<td>Improvement in ABI from 0.66 to 0.80 (ABI worsened in control group)</td>
</tr>
<tr>
<td>Van Tongeren et al.</td>
<td>RC</td>
<td>BM-SC IM injection only (15)</td>
<td>PAD</td>
<td>1.23x10^6 (3.07x10^6 CD34+)</td>
<td>12</td>
<td>Limb salvage</td>
<td>No statistically significant difference in limb salvage between two groups</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BM-SC IA and IM injection (12)</td>
<td></td>
<td></td>
<td></td>
<td>ABI</td>
<td>Improvement in ABI from 0.52 to 0.66 (no difference between two groups)</td>
</tr>
<tr>
<td>Franz et al.</td>
<td>NRNC</td>
<td>BM-SC IA and IM injection (9)</td>
<td>PAD</td>
<td>Not specified</td>
<td>3</td>
<td>ABI</td>
<td>Improvement in ABI seen in 4 of 9 (44%) patients</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rest pain</td>
<td></td>
<td></td>
<td></td>
<td>Rest pain</td>
<td>Improvement in rest pain symptoms in 5 of 6 (83%) patients</td>
</tr>
</tbody>
</table>

RC, randomized controlled; NRC, nonrandomized controlled; NRNC, nonrandomized noncontrolled; BM, bone marrow; IM, intramuscular; IA, intraarterial; PAD, peripheral arterial disease; ABI, Ankle-Brachial Index.
Table 4. Summary of clinical trials using g-csf to mobilize stem cells in the treatment of peripheral arterial disease.

<table>
<thead>
<tr>
<th>Study</th>
<th>Trial design</th>
<th>Groups (n)</th>
<th>Vascular disease</th>
<th>Cell count (mean)</th>
<th>Follow up (months)</th>
<th>Primary end points</th>
<th>Results</th>
</tr>
</thead>
</table>
| Huang et al.  
2011 | NRNC         | Pretreatment with G-CSF followed by IM PB-SC injection (5) | PAD | Not specified | 3 | ABI | Increase in ABI from 0.52 to 0.67 |
| Huang et al.  
2011 | RC           | Pretreatment with G-CSF followed by IM PB-SC injection (14) | CLI | 1.9×10^6 PB-SC (7.8×10^6 CD34^+) | 3 | ABI | Increase in ABI from 0.50 to 0.63 (Improved 0.49 to 0.51 in control) |
| Huang et al.  
2011 | NRNC         | Pretreatment with prostaglandin E1 without cell injection (14) | CLI | 1.9×10^6 PB-SC (4.2×10^6 CD34^+) | 6.2 (mean) | Limb Salvage | Limb salvage in 22 of 30 patients |
| Kawamura et al.  
2011 | NRNC         | Pretreatment with G-CSF followed by IM PB-SC injection (30) | CLI | 3.9×10^7 PB-SC (1.2×10^7 CD34^+) (9.0×10^6 CD133^+) | 3 | ABI | Improvement of pain free walking distance from 6 to 195 meters |
| Lenk et al.  
2011 | NRNC         | Pretreatment with G-CSF followed by IA PB-SC injection (7) | PAD | 3.9×10^8 PB-SC (2.01×10^7 CD34^+) | 6 | ABI | No improvement in ABI at 6 months |
| Ishida et al.  
2011 | NRNC         | Pretreatment with G-CSF followed by IM PB-SC injection (6) | PAD | 3.98×10^10 PB-SC | 9.3 | | Improvement of subjective symptoms in 86% of limbs |
| Kawamura et al.  
2011 | NRNC         | Pretreatment with G-CSF followed by IM PB-SC injection (92) | CLI | 3.98×10^10 PB-SC (4.0×10^6 CD34^+) | 3 | | Limb Salvage | Rescue from amputation in 91% of limbs without necrosis before cell transplantation |
| Huang et al.  
2011 | RC           | Pretreatment with G-CSF followed by IM PB-SC injection (72) | PAD | 7.2×10^5 PB-SC (2.3×10^5 CD34^+) (1.2×10^5 CD133^+) (3.9×10^5 BM-SC (1.2×10^5 CD34^+) (9.0×10^5 CD133^+) | 3 | ABI | Improvement in ABI by 0.17 in G-CSF + IM PB-SC group |
| Burt et al.  
2011 | NRNC         | Pretreatment with G-CSF followed by IM PB-SC injection (9) | CLI | Total PB-SC not specified | 12 | ABI | Limb salvage in 9 of 12 patients |

RC, randomized controlled; NRNC, nonrandomized noncontrolled; BM-SC: bone marrow-stem cells; PB-SC: peripheral blood-stem cells; G-CSF: granulocyte stimulating factor; CLI: critical limb ischemia; IM: intramuscular; IA, intraarterial; PAD, peripheral arterial disease; ABI, Ankle-Brachial Index.
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Review

BM-SC therapy and the other group of 72 patients received pre-treatment with G-CSF followed by IM injection of PB-SC. Significant improvement in clinical indices was observed in both groups; however, the improvement in ABI, skin temperature, and rest pain was better in the group receiving combination of G-CSF and PB-SC. There was no difference in pain-free walking distance, ToC2, ulcer healing, or rate of limb amputation. The number of cells administered was not controlled for, and the G-CSF group received a second injection of cells 40 days after the first therapy. The G-CSF group was administered a significantly higher number of mononuclear cells and this may have been the reason for the extra clinical benefit seen in this group. Given the invasive nature of a bone marrow biopsy and the superior results seen by G-CSF administration and use of PB-SC, the authors concluded that this method is more favorable.

Intraarterial fibroblast growth factor

Preclinical trials have shown that FGF improves collateral development in animal models via proliferation of progenitor cells. Human trials have shown it to be a safe and efficacious treatment for peripheral vascular disease as summarized in Table 5. Lazarous et al. completed a double blind, placebo-controlled, dose-escalation trial in patients with ABI less than 0.8 with 13 patients receiving one or two doses of IA FGF, 6 receiving placebo. Treatment with FGF was safe and resulted in increased calf blood flow at 6 months. There appeared to be a dose dependent response but it was not statistically significant. The TRAFFIC study was a randomized, controlled, placebo trial with 190 patients distributed to three groups: one group received placebo, another received a single dose of FGF, and the third received 2 doses of FGF. Treatment with FGF resulted in an improvement of peak walking time as compared to placebo. However, a dose dependent response was not recognized.

Discussion

Animal models have demonstrated clinical benefit in the treatment of PAD with SC therapy. Clinical trials have also displayed a benefit in humans using BM-SC and PB-SC after mobilization with G-CSF; however, few randomized, controlled trials exist. The interpretation of the few trials that are available is further complicated by the multiple variables that exist between trials.

Method of delivery

Methods of delivery include IM injection or IA injection of BM-SC or PB-SC after SC mobilization by administration of G-CSF. Combinations of IM and IA injection have also been examined. The results of clinical trials using IM injection of BM-SC are conflicting and this represents variation in study design and a lack of randomized, controlled trials. All studies show symptomatic improvement in patients receiving IM BM-SC therapy and it appears that BM-SC treatment results in greater limb salvage and increased blood flow to the ischemic limb. However, ABI in studies with the longest follow-up periods show no long-term improvement except for a subset of patients with Buerger’s disease. There is no consensus as to the best mode of delivery and further randomized, controlled trials are needed with long-term follow-up to determine if one method or a combination of methods is more efficient than the others. IA injection alone and in combination with IM injection appears to be safe and efficacious. In the only trial to compare the combination therapy to IM injection alone, both methods appeared to be equally successful. However, this was a small study and randomized, controlled trials are needed to determine if there is an extra benefit in receiving both modes of SC delivery. The PROVASA trial was the largest study to date and showed IA injection of BM-SC and there appeared to be a significant response in the treatment group with the healing of ischemic ulcers. The administration of G-CSF to mobilize

Table 5. Summary of clinical trials using intraarterial injection of fibroblast growth factor for peripheral arterial disease.

<table>
<thead>
<tr>
<th>Study</th>
<th>Trial design</th>
<th>Groups (n)</th>
<th>Vascular disease</th>
<th>Follow up (months)</th>
<th>Primary end points</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lazarous et al.</td>
<td>RC</td>
<td>IA injection of varying doses of FGF (13) PL (6)</td>
<td>PAD</td>
<td>6</td>
<td>Calf blood flow</td>
<td>Dose dependent increase in blood flow for FGF treated (no change in PL)</td>
</tr>
<tr>
<td>Lederman et al.</td>
<td>RC</td>
<td>IA injection of single dose of FGF (66)</td>
<td>PAD</td>
<td>6</td>
<td>Peak walking time</td>
<td>Increased peak walking time in FGF treated compared to PL (not dose dependent)</td>
</tr>
</tbody>
</table>

| IA injection of two doses of FGF (61) PL (63) | ABI | No significant improvement in ABI at 6 months |

RC, randomized controlled; PL, placebo; BM, bone marrow; PB, peripheral blood; FGF, fibroblast growth factor; IA, intraarterial; PAD, peripheral arterial disease; ABI, Ankle-Brachial Index.
SC followed by intramuscular or intrarterial injection of PB-MNCs has had varying results, again likely due to variation in study design. Overall, this strategy of SC therapy appears to provide clinical benefit to patients with peripheral vascular disease. More randomized, controlled trials are needed as only one has been done to date. While this study showed improvement in ABIs and enhanced ulcer healing, it followed patients for only 3 months. More studies with long-term follow-up are needed as the longest follow-up time to date has been only one year. In addition, no studies have been carried out comparing IA injection to IM injection of PB-MNC after pretreatment with G-CSF. Given that progenitor cells are being mobilized to the peripheral blood with administration of G-CSF (Figure 1), a study treating patients with G-CSF alone without administration of concentrated PB-MNCs intramuscularly or intraarterially is needed. An ischemic environment alone in a diseased limb may lead SC in the peripheral blood to home to the affected tissue resulting in angiogenesis and vasculogenesis. Therefore it is possible that PB-SC mobilized with G-CSF therapy alone will home to the diseased limb without need of extra procedures. A large randomized, controlled trial comparing IM BM-SC therapy to pretreatment with G-CSF followed by IM injection of PB-SC showed improvement in both groups, but the group receiving G-CSF and IM PB-SC did so to a greater degree. Using G-CSF and PB-MNC resulted in a greater yield of mononuclear cells, CD34+ cells, and CD133+ cells. While this study had a relatively large sample size and was randomized, long-term results were not studied as all findings were made at 3 months. More long-term and randomized, controlled trials are needed to determine the most efficient method of SC delivery.

**Mechanism of action**

It is evident that treatment with SC therapy, whether by injection of BM-SC or indirect mobilization with G-CSF and injection of PB-SC, provides clinical benefit to patients with PAD. However, the mechanism of action remains unknown. Multiple theories have been proposed and supported by animal models and the mechanism of action may be multifactorial. Transdifferentiation of progenitor cells into endothelial cells that form new blood vessels or the release of cytokines and angiogenic factors such as VEGF or FGF which result in the proliferation of surrounding tissue remain possible mechanisms of action (Figure 2). FGF administration resulted in increased calf blood flow in one study and increased peak walking time in another. More clinical trials are needed to assess the effect of FGF therapy on ischemic wound healing. These theories have been challenging to confirm in humans since tracking of cellular differentiation remains difficult.

**Patient selection**

All clinical trials have been carried out in patients with critical ischemic limbs that are ineligible for revascularization. Further studies are needed to determine if patients receiving revascularization procedures, whether with open surgery or endovascular intervention, can gain additional benefit from SC therapy. Also, studies are needed to determine if SC therapy in early stages of PAD can prevent patients from developing critical limb ischemia.

**Stem cell lineage**

SC composition can vary greatly based on cell-surface markers and method of isolation. It is possible that cells with certain cell markers have greater capacity for angiogenesis and vasculogenesis and these cells must be identified to maximize therapeutic benefit. Studies have shown that CD34+, CD45+, and CD133+ cells contribute to improved blood flow and wound healing in peripheral vascular disease. The PROVASA trial provided additional evidence that CD45+ and CD34+ cells may have increased capacity for angiogenesis. Further studies with larger sample size are needed to determine if a subgroup of progenitor cells have a greater potential for clinical improvement of PAD.

**Cell numbers**

Saigawa et al. showed a dose dependent relationship with number of CD34+ cells. However, in the TACT trial, the authors did not observe improved results with increasing numbers of mononuclear cells, CD34+ cells, or CD133+ cells, although they stated that patients who responded to the therapy seemed to have higher numbers of cells administered. The PROVASA trial also demonstrated a dose dependent effect with absolute number of mononuclear cells and clinical improvement. Due to the great variability in cell numbers and mode of delivery in each study, the optimal number of SC needed for therapeutic benefit remains unclear. More studies with larger sample sizes are needed to determine the optimal number of cells.

**Follow-up**

The timing of follow-up in clinical trials has been extremely variable with the longest follow-up being a median of 58 months. Several studies have occurred within the past 5 years, making long-term data accumulation difficult at this time. Additional information regarding the prolonged effects of SC therapy in PAD will become available as these trials continue to be followed over time.

**Tracking stem cell engraftment**

Human SC trials have been limited by the inability to track administered progenitor cells. Because of this limitation, investigators have had difficulty in determining the most effective method of delivery and confirming the fate of the administered cells. Cell imaging strategies are limited to labeling cells with specific markers in vitro prior to transplantation or indirect labeling of cells with imaging reporter genes transduced into the progenitor cell prior to engraftment.
to transplantation. However, these methods have their limitations as neither provide quantitative or qualitative data about transplanted cells. Direct labeling methods using imaging modalities such as PET and MRI are limited by the short half-lives of their tracers and are not sensitive enough to image cells in vivo in larger animal models or humans. Recently, nanoparticles have been utilized as a promising platform due to advantages of large absorption cross-section, slow photo-bleaching, and low cytotoxicity, thereby applicable to gene delivery, non-invasive imaging, and differentiation manipulation of SC. However, the selectivity and specificity of nanoparticle targeting are still under investigation.

Future direction

The use of SC for the treatment of PAD has yet to reach its potential as few randomized, controlled trials have been completed. Current data suggests that SC therapy results in increased blood flow to the ischemic limb and improved wound healing of ischemic ulcers. Among the future clinical trials to be undertaken, many questions remain to be answered: optimal technique of delivery, type of cells, method of preparation, dosage of progenitors, its efficacy in patients with early, revascularizable, or end-stage disease, and long-term follow-up data.

References


56. George J, Hoit B, Fuster V, et al. Imaging Stem Cells: Can we Track Their Fate? In:
