Angiogenesis Achieved by Granulocyte Colony-Stimulating Factor in Combination With Bypass Surgery in 2 Cases of Critical Limb Ischemia

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Patients demonstrating critical limb ischemia with a long-distance occlusion of the major arteries are sometimes poor candidates for bypass surgery, because tandem occlusion complicates distal anastomoses and poor run-off causes early occlusion of bypass grafts. In order to resolve these problems, angiogenesis therapy was attempted by subcutaneous injection of granulocyte colony-stimulating factor either before or after peripheral bypass surgery in 2 cases. (Circ J 2008; 72: 1385–1387)

Key Words: Angiogenesis; Bypass; Critical limb ischemia

There have been some reports that affirm the efficacy of the combination of surgical revascularization and angiogenic therapy;1–4 however almost all such reports have only addressed coronary artery disease. The angiogenic therapies were intramyocardial injection of a gene, cytokines or autologous cells in combination with coronary bypass grafting. In the present 2 cases angiogenic therapies were performed simply by subcutaneous injection of an ordinary amount of granulocyte colony-stimulating factor (G-CSF) either before or after peripheral bypass surgery for critical limb ischemia (CLI).

Case Reports

Case 1
An 83-year-old non-diabetic woman was referred to hospital because of critical ischemia of her left lower limb with an ischemic ulcer on her second toe. She had also suffered from chronic heart failure and her brachial blood pressure was 70 mmHg. The left ankle blood pressure could not be measured. Her heart rate was 110 beats/min with atrial fibrillation. Angiography revealed a long occlusion between the left Iliac artery and the proximal popliteal artery. The distal popliteal artery and its branches were poorly enhanced by thin collaterals. The deep femoral artery trunk was occluded and the minor branch was enhanced by collaterals from the iliac artery (Fig 1A). Cross-over bypass surgery between the right common femoral artery and the deep femoral artery was carried out with a 6-mm ePTFE graft after profundaplasty. The saphenous vein could not be used because it was very thin. Approximately 3 months later, ischemic ulcers progressed to involve all of the toes of her left foot. Her left ankle blood pressure still could not be measured. Angiography showed poor run-off at the distal anastomosed site (Fig 1B). The popliteal artery was poorly enhanced, even with the additional flow via the bypass graft (Fig 2A). Furthermore, the poor run-off of the bypass flow seemed to be occluding the bypass graft. Cilostazol or prostaglandins could not be used because of the low systemic blood pressure and tachycardia. G-CSF (Lenograstim, Chugai Pharmaceutical Co Ltd, Tokyo, Japan) was used for angiogenesis after the patient and her relative gave their informed consent. Her body weight was 38 kg and she initially received a daily subcutaneous injection of 33 μg G-CSF for 3 days. After 2 weeks, G-CSF injection was repeated. The white blood cell count was 20,000–30,000/μl for several days after each session of G-CSF therapy. Two weeks after the second round of G-CSF therapy, angiography revealed collateral flow from the left iliac artery that had become faster than the bypass flow. The newly developed collateral flow clearly enhanced the popliteal artery without the flow from the bypass graft (Fig 2B). G-CSF therapy was performed a total of 3 times. Although rest pain disappeared and the ankle–brachial blood pressure index reached 0.15, she died of anuria caused by hypotension 2 months after the final G-CSF therapy.

Case 2
A 92-year-old non-diabetic man was referred to hospital because of critical ischemia in his left lower limb with an ischemic ulcer on his 1st, 2nd and 3rd toes. The brachial blood pressure was 98 mmHg and the left ankle blood pressure was 21 mmHg. The left ankle–brachial blood pressure index at rest was 0.21. His heart rate was 102 beats/min with sinus rhythm. Angiography revealed a long occlusion between the left proximal superficial femoral artery and the popliteal artery. A short segment of the popliteal artery was enhanced by tiny collaterals (Fig 3A). There was insufficient saphenous vein to perform a long bypass because of a previous of varicotomy. Neither cilostazol nor prostaglandins could be used because of the low systemic blood pressure and tachycardia. Bypass surgery between the femoral and popliteal arteries with an ePTFE graft was planned. In order to ensure that the bypass graft was anastomosed to the short segment of the popliteal artery, which had poor run-off,
G-CSF was used for angiogenesis after informed consent was given by the patient and his relative. The initial round of 100-µg G-CSF (33 µg/day for 3 days) was followed by a second course after a 2-week interval. Two weeks later, collateral arteries had developed and the popliteal artery had grown (Fig 3B). Finally, a femoropopliteal bypass with 6-mm ePTFE was carried out successfully and 1 month after the surgery the ulcers on his toes had healed. One year after surgery his left ankle–brachial blood pressure index remains over 0.8.

Discussion

In both cases of CLI presented here, the patients had ischemic ulcers on their toes. Management of CLI consists of an interventional approach and medical support that improves the microcirculation; however, many patients with CLI have a poor general status; that is, low systemic blood pressure and tachycardia that do not permit the perioperative use of cilostazol and prostaglandins. In addition, lumbar sympathectomy cannot be attempted when the patient is in poor condition. Thus in the 2 present cases, angiogenesis therapy was attempted using simple subcutaneous injection of G-CSF during the perioperative period of bypass surgery. In comparison with other types of angiogenic therapy, G-CSF therapy is noninvasive, and the safety of an ordinary amount of G-CSF has already been established. Subcutaneously injected G-CSF mobilizes mononuclear cells from the bone marrow to the peripheral blood and these mobilized cells improve peripheral blood flow both by secretion of cytokines and by angiogenesis. Subcutaneously injected G-CSF increased the vascular density in ischemic myocardium of swine and also improved myocardial perfusion of the infarcted myocardium in humans. When 2–5 µg/kg...
of G-CSF was injected subcutaneously daily for 10 days in humans who had peripheral artery disease, the symptoms and ischemic findings improved after 1 month without major adverse effects. However, there have been no reports of the usefulness of combining peripheral bypass surgery with simple G-CSF therapy. In Case 1, cross-over bypass surgery did not improve the ischemia because there was poor run-off. After G-CSF therapy, collaterals developed from the occluded iliac artery beyond the bypass flow. In Case 2, we used G-CSF before bypass surgery because the patent segment was too short for distal anastomosis. After G-CSF therapy, the collaterals fed the distal anastomotic site, resulting in successful bypass surgery. In both cases, the amount of CD34+ stem cells and monocytes were mobilized peaked near 30,000/lL. In previous reports, a considerable amount of CD34+ stem cells and monocytes were mobilized into the peripheral blood when the number of white blood cells reached 30,000–40,000/lL after G-CSF.11,12

When bypass surgery is accompanied by endarterectomy, as described in Case 1, the use of subcutaneous G-CSF has a further beneficial effect, because it prevents neointimal formation of the surgically injured artery.13

**Study Limitations**

Angiography is not completely suitable for proving the efficacy of angiogenesis, because patient factors, such as body temperature, blood pressure and grade of vasospasm, modify the findings. Another reason is that the findings of increased collateral vessels are considered indirect evidence of therapeutic angiogenesis. The vessels that have been developed as the angiogenic response are too small to be evaluated by angiography. In order to decide the benefit of the combination of a relatively small amount of G-CSF with surgical bypass in cases of CLI, we really need a randomized trial with control groups. In addition, the goal of such a beneficial effect should include the major-amputation-free rates.

**References**


