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A novel bFGF-GH injection therapy for two patients with severe ischemic limb pain

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Abstract
Severe ischemic pain is difficult to treat with a single therapy. Although modern angiogenic therapies have been used in patients with peripheral arterial occlusive diseases, a regimen combining novel angiogenic therapy and classic nerve blocks, including sympathectomy, has not been discussed to date. In this case report, we present two patients with peripheral arterial occlusive disease who were first treated with medication and lumbar sympathectomy, and then with a novel gelatin hydrogel drug-delivery system loaded with basic fibroblast growth factor. The gelatin hydrogel combined with recombinant basic fibroblast growth factor was injected intramuscularly into the ischemic limbs. In the first patient, with arteriosclerosis obliterans, a foot ulcer was healed, and the original score for resting pain (visual analogue scale, 5/10) was decreased to 0/10. In the second patient, with Buerger's disease, a large toe ulcer was healed, and his resting pain (visual analogue scale, 8/10) was decreased to 1/10. Some other parameters, such as skin surface temperature, transcutaneous oxygen partial pressure, and pain-free walking distance, were also improved in both patients after the combined therapy. A multimodal approach is necessary to treat severe ischemic pain. Novel angiogenic therapy combined with nerve blocks seems to be a promising option in patients with severe pain.

Key words Peripheral vascular disorder · Ischemic pain · Angiogenesis · Autonomic nerve block

Introduction

Severe ischemic limb pain, claudication, and tissue necrosis impair daily activities and provoke secondary diseases, such as infection or organ dysfunction [1]. Surgical treatment and/or catheter intervention, or the administration of vasodilative prostaglandins, and antiplatelet and anticoagulant drugs alleviates ischemic symptoms in many patients; however, some patients do not respond to such medical therapies, and limb amputation may become necessary [2]. Mortality is high when such patients have other comorbid diseases, such as diabetes or renal failure [3]. Although their long-term effect is still controversial, nerve blocks, including sympathectomy, are classic but effective therapies for such severe ischemic limb pain [4].

Recently, several novel therapies have been used for peripheral circulation disorders (PAD: peripheral arterial disease). Gene therapies using vascular endothelial growth factor (VEGF) [5,6] or hepatocellular growth factor (HGF) [7]; cytokine therapy with basic fibroblast growth factor (bFGF) [8], and autologous bone marrow transplantation [9] have already been used to treat human patients and were reported to be effective to some extent. Although these therapies were used in patients with severe symptoms, the patients were not necessarily also treated with all of the conventional therapies including nerve blocks, such as continuous epidural block and sympathectomy.

In this case report, we present two patients with peripheral arterial occlusive disease whose ischemic pain was treated by a multimodal approach that included medications, hyperbaric oxygen, lumbar sympathectomy, and finally a novel gelatin hydrogel drug-delivery system (GH) loaded with bFGF (bFGF-GH) [10].

Procedures in bFGF therapy

In order to use bFGF-GH as an experimental therapy, we obtained written informed consent from the patients. Our institutional ethics committee approved the study protocol. In this protocol, patients qualified for the bFGF therapy if they were not candidates for nonsurgical or surgical revascularization. Patients should not have had proliferative retinopathy or evidence of malignant disorder during the previous 5 years. Also,

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the patients should have some symptoms that could not be cured by conventional therapies. The histories of our patients already included conventional drug treatments, such as vasodilative prostaglandins and antiplatelet agents, and several series of hyperbaric oxygen therapies.

bFGF-GH was prepared as described previously [10]. Human recombinant bFGF was purchased from Kaken Pharmaceutical (Tokyo, Japan). Gelatin used for the slow-release drug delivery system was prepared, using an alkaline process, by Nitta Gelatin (Osaka, Japan). Microspheres were designed to slowly release bFGF for approximately 3 weeks. The bFGF-GH microspheres were suspended in saline prior to intramuscular injection. We injected 0.5 ml of bFGF-GH suspension into the ischemic gastrocnemius muscle and foot muscles, using a 3 × 3-cm grid and 26-gauge needle. The total number of injection points was dependent on the size of the ischemic area.

To evaluate the effects of the therapy, we monitored the ankle brachial index (ABI), transcutaneous oxygen partial pressure (tc_{PO_2}), and pain-free walking distance (at 3 $km \cdot h^{-1}$ with no incline). We used the criteria of Rutherford and colleagues to assess limb status [11]. Activities of daily life were assessed by Pomposelli's criteria [12]. A tc_{PO_2} probe (PO-850, Koken-Medical, Tokyo, Japan) was attached 10 cm distal to the tibial tuberosity on the anterior skin surface. The patients were breathing room air or oxygen in a hyperbaric oxygen chamber, where air was pressurized to 2 atm. New collateral vessels on angiographies were assessed as +0 (no improvement), +1 (slight), +2 (moderate), or +3 (greatly improved). Data obtained from air plethysmographs of the digits, thermography, and laser Doppler angiography were assessed according to the above-mentioned +0 to +3 grading. Intensity of pain in the ischemic regions was evaluated by a visual analogue scale (VAS) of 0–10. Values obtained at approximately 1 week before and 4 weeks after bFGF-GH injection were compared to evaluate the effects of the therapy.

Case 1

An 86-year old man (167 cm, 58 kg) with a right foot ulcer (diameter, 2.5 cm) resistant to dermatological treatment was diagnosed, following digital subtraction angiography, as suffering from arteriosclerosis obliterans. He had been healthy and had had no symptoms of diabetes or collagen diseases. Because of aging and lumbar spinal canal stenosis, he had lumbago and slight paresthesia in both legs. Although medical treatments, such as the intravenous infusion of the vasodilative prostaglandin E1, the oral administration of an antiplatelet drug (sarpogrelate) and dermatological treatments had been continued for more than 2 months, the ulcer and intractable pain could not be cured (Fontaine grade IV). Because the pain and coldness in his feet were ameliorated by a lumbar epidural block of 0.25% bupivacaine, chemical sympathectomies were performed at the right second to fourth and the left second to third lumbar vertebral body levels, with 3 ml of 7% phenol at each injection point. His symptoms were ameliorated to some extent (VAS for pain, from 5/10 to 3/10; foot surface temperature, from 27°C to 32°C); however, the pain relief was incomplete, and the ulcer was not cured.

In order to treat the remaining symptoms, bFGF-GH was injected 2 months after the sympathectomies. Twenty-five micrograms of bFGF combined with GH was intramuscularly injected bilaterally into the feet and the gastrocnemius muscles (totally 29 injections). Changes in the patient's symptoms and characteristics are summarized in Table 1. At 4 weeks after the injection, the VAS pain score, and right foot surface temperature were improved. At 2 years after the bFGF-GH injection, there were no symptoms of leg ischemia. The intermittent claudication could not be completely cured, probably because of the lumbar spinal canal stenosis. However, he was able to enjoy riding his bicycle more than 2 km every day without pain. No adverse side effects of the therapy, such as cancer or retinopathy were observed.

Case 2

A 40-year-old man (175 cm, 70 kg) with a toe ulcer (diameter, 3.5 cm) resistant to dermatological treatment was diagnosed, following digital subtraction angiography, as suffering from Buerger's disease. He also had an isolated aortic stenosis at the fourth lumbar vertebral level. He had a long history of smoking, but was without any symptoms of diabetes, collagen diseases, or arteriosclerosis obliterans. Although medical treatments, such as the intravenous infusion of the vasodilative prostaglandin E1 and the oral administration of sarpogrelate were continued for more than 1 month, the ulcer and intractable pain could not be cured (Fontaine grade IV). Because the pain and coldness in his feet were alleviated by a lumbar epidural block of 0.25% bupivacaine, chemical sympathectomies were performed at the bilateral second and third lumbar vertebral body levels, with 3 ml of 7% phenol at each injection point.

His symptoms were alleviated to some extent (pain VAS from 8/10 to 5/10, left foot surface temperature from 30°C to 32°C); however, the ulcer still persisted and he complained of ischemic limb pain. Surgical thrombectomy was applied to the isolated narrowing in his abdominal aorta, at approximately 3 cm above the bifurcation. At 5 months after the surgery, the ulcer had healed, but his resting pain was still at the level of VAS 3/10. In order to treat the remaining symptom, bFGF-

Table 1. Patients' characteristics and symptoms pre- and post-bFGF-GH injection (pre- → post-)

	Case 1	Case 2
Fontaine's criteria [13]	IV → IIa	IV → I
Rutherford's criteria [11]	II4 → I1	II4 → 00
Pomposelli's criteria [12]	2 → 1	1 → 1
Ankle brachial index	0.62 → 0.58	0.39 → 1.2
Pain intensity (VAS-10)	5/10 → 0/10	8/10 → 1/10
Walking distance (m)	15 → 30 (lumbago)	100 → 2000
TC oxygen partial pressure (mmHg)		
21% O2 at 1 atm	64 → 60	63 → 68
95%–99% O2 at 2 atm	626 → 700	530 → 420
Angiography	+0	+0
Plethysmography	+1	+3
Thermography	+2 (26°C → 30°C)	+3 (30°C → 33°C)
Laser Doppler angiography	+0	+2
Side effects	Nil	Nil

Values obtained at approximately 1 week before and 4 weeks after bFGF-GH injection were compared to evaluate the effects of the therapy

GH treatment was administered, 7 months after the sympathectomy. Twenty-five micrograms of bFGF combined with GH was bilaterally injected intramuscularly into his legs and feet (totally 34 injections). At 4 weeks after the injection of bFGF-GH, his pain VAS score, ABI, and surface temperature in the left foot had all improved, as shown in Table 1.

At 2 years after the bFGF therapy, he had no ischemic symptoms other than temporary calf pain after 2 km of walking. Also, no adverse side effects, such as cancer or retinopathy were observed.

Discussion

Angiogenesis induced by bFGF or bFGF-GH has been reported in several experimental animal limb-ischemia models [14]. Marui et al. [10] applied accumulated animal data to a human clinical study, and demonstrated that bFGF-GH had the potential to alleviate symptoms of limb ischemia. Ischemic pain was apparently decreased by the novel treatment. Slow release with a gelatin hydrogel drug-delivery system is necessary, because the crude peptide itself is quickly degraded in vivo [15]. This therapy is more convenient than cellular transplantation because there is no necessity for cell preparation. Although there have been no reports of severe side effects to date, possible side effects are similar to those known for other regeneration therapies, such as local inflammation, transient pain at the injection site, and the induction of a neoplasm. Because this therapy has been used in a limited number of patients, further extended monitoring is necessary to evaluate its safety.

The administration of vasodilative prostaglandins, anticoagulants, and antiplatelet agents is now routinely applied to most patients with limb ischemia [1]. When patients have necrotic tissue loss or an ulcer, hyperbaric oxygen therapy is another option for tissue recovery. Sympathectomy or nonsurgical sympathetic nerve blockade is also used in patients with severe symptoms to prevent further deterioration that would be caused by sympathetic vasoconstriction, and to interrupt sympathetically maintained pain [4]. The temporary placement of a continuous epidural infusion catheter is also applied to ameliorate pain and to induce vasodilation. Both patients in the present study had undergone all such therapies, and subsequently they underwent bFGF-GH injection for the treatment of the remaining symptoms, mainly ischemic pain.

The ischemic pain was alleviated in our two patients, and several other parameters changed beneficially after the bFGF-GH injection. These results were in accordance with the first clinical study reported by Marui et al. [10]. Walking distance was also improved in both patients. Possible mechanisms of the pain relief are the alleviation of the ischemia, and anti-nociception induced by vasodilative cytokines. Similar mechanisms of pain relief have also been proposed for the effect of bone marrow mononuclear cell transplantation in patients with peripheral arterial occlusive disease [16]. In our previous study regarding bone marrow transplantation, we also found such pain-relieving effects in patients with thromboangiitis obliterans [17]. Interestingly, for both therapies (i.e., bFGF-GH and bone marrow transplantation), the pain-relieving effect was also observed in patients without ulcer or gangrene. This means that pain unrelated to tissue loss is also relieved by these therapies. Because the bFGF-loaded gelatin hydrogel injection does not require general anesthesia or blood sampling, this regimen, compared to other angiogenic therapies, seems to be more suitable for complicated cases.

The results in our patients suggested that bFGF-GH is effective, and can be safely applied to patients who have undergone sympathectomy. It has been suggested that bFGF has direct vasodilative action [18]. The two therapies used in the present study, sympathectomy and bFGF-GH, could be synergistic or additive. Chemical sympathectomy in advance may contribute to the favorable vasodilative and angiogenic actions of bFGF in the diseased area. Although the long-term effects of sympathectomy itself are still controversial [19], continuous vasodilation following permanent sympathectomy is believed to increase oxygen and nutritional supply, which may promote angiogenesis requiring cell proliferation in the diseased area. The favorable environment induced by sympathectomy seems to be supportive of the required cell migration and tissue reproduction in the ischemic regions. However, the bFGF-GH therapy is undergoing a phase I trial examining the safety of the procedure. Several institutes have started trials by adding this novel therapy to their own pre-existing standard protocol. Further studies will be indispensable to quantitatively evaluate the effects of both the novel therapy alone and the novel therapy combined with some pre-existing protocol. The combined use of sympathectomy and bFGF-GH therapy should be evaluated more precisely through such extended clinical trials.

Although several novel therapies have been proposed for ischemic limb pain, and some of them are undergoing clinical trials [20], ischemic limbs cannot be treated using a single therapy. In addition to the information regarding novel therapies, physicians may be eager to know how to combine classic therapies with novel ones. Nerve blocks, including sympathectomy, prior to the novel therapy seem to be a promising option in patients with severe ischemic limb pain.

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