Therapeutic Outcome of Hyperbaric Oxygen and Basic Fibroblast Growth Factor on Intractable Skin Ulcer in Legs: Preliminary Report

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Background: Beneficial effects of hyperbaric oxygen on ischemic vascular diseases have been noted. Acceleration of wound healing with basic fibroblast growth factor has also been reported. The authors employed combination therapy of hyperbaric oxygen and basic fibroblast growth factor in patients with skin ulcer in legs refractory to conventional therapy.
Methods: Three men and four women were simultaneously treated with hyperbaric oxygen at 2 absolute atmospheric pressures for 90 minutes daily and spray treatment of basic fibroblast growth factor to the ulcer bed daily for an average of 2.6 months. Biopsy specimens obtained from ulcer tissues were divided into two pieces, one for histologic examination and the other for measuring fibrous protein.
Results: Ulcers were completely cured in five of seven patients. Two patients showed shrinkage of ulcer size. This combined therapy induced proliferation of connective tissue of the ulcer tissues, especially collagen and noncollagenous protein.
Conclusions: Combined treatment with hyperbaric oxygen and basic fibroblast growth factor may be useful in patients with intractable skin ulcers in legs, and the shrinkage effect of this therapy is probably related to the proliferation of granulation tissues of the ulcer lesion. (*Plast. Reconstr. Surg.* 117: 646, 2006.)

Hyperbaric oxygen therapy consisting of 100% oxygen inhalation at 2 absolute atmospheric pressures daily has been known to produce beneficial effects in ischemic, necrotic, or radiation-induced tissue injuries by fibroblast proliferation, enhancement of fibroblastic synthesis of collagen, and capillary formation.[1–5] Human basic fibroblastic growth factor (bFGF) appears to participate in inducing granulation tissue formation and potent angiogenesis.[6–9] Although various treatments of intractable skin ulcers of extremities are found in the literature,[10] no definite therapeutic procedures have been proposed. We tried to elucidate the possible effect of hyperbaric oxygen and bFGF on intractable skin ulcers in the legs.

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PATIENTS AND METHODS

A total of seven Japanese patients with ungovernable skin ulcers in the legs participated in this study. All patients had received conventional regional therapy on the ulcer lesions. Two of them were supplemented with bFGF therapy as well. These treatments failed to improve the ulcers and the patients were referred to our hyperbaric oxygenation institute. A clinical profile of the patients is shown in Table 1. Patients with serious tracheobronchial symptoms, persistent otalgia, claustrophobia, cancer, and serious conditions requiring intubation were excluded from this study. Detailed inclusion and exclusion criteria of this therapy were identical to the guideline for hyperbaric oxygenation therapy produced by the Japanese Society for Hyperbaric Medicine.[11] After informed consent had been obtained from each subject, all underwent various cycles of hyperbaric oxygen treatments from 2.2 to 8.9 months (mean, 5.7 ± 1.0 months). Patients received 100% oxygen in a multiplace hyperbaric chamber (Fig. 1). Hyperbaric oxygen therapy consisted of cycles of 90 minutes daily per treatment, 6 days per week, of hyperbaric oxygen at 2 absolute atmospheric pres-

Table 1. Clinical Characteristics in Seven Patients

Case	Sex	Age (yr)	Primary Disease	Previous Therapy before Admission to This Clinic	Therapy in This Clinic: HBO (mo)	Therapy in This Clinic: HBO plus bFGF (mo)
1	M	64	Carbon monoxide poisoning	Conventional therapy (6.8 mo)	8.0	1.3
2	F	72	Diabetes mellitus	Conventional therapy (3.6 mo)	8.1	2.3
3	F	79	Varices of leg	Resection of varices, two times, conventional therapy and then bFGF (14.1 mo)	3.2	4.4
4	M	65	Varices of leg, diabetes mellitus	Resection of varices, once, then conventional therapy (8.1 mo)	6.1	2.1
5	F	74	Diabetes mellitus	Conventional therapy and subsequent bFGF (9.0 mo)	8.9	4.0
6	F	58	Diabetes mellitus	Conventional therapy (7.9 mo)	2.2	2.1
7	M	60	Diabetes mellitus	Conventional therapy and bFGF (3.1 mo)	3.1	1.9
Mean ± SD		67 ± 7 years		7.5 ± 3.4 months	5.7 ± 2.6†	2.6 ± 1.1†

M, male; F, female; HBO, hyperbaric oxygen; bFGF, basic fibroblast growth factor.
*Data values are presented as mean ± SD. Respective values in groups were compared using the paired *t* test.
†$p < 0.05$.

sures. Such hyperbaric oxygen therapy alone failed to improve ulcer lesions completely. Subsequently, we applied simultaneous combined treatments of hyperbaric oxygen and bFGF to these patients for 1.3 to 4.4 months (mean, 2.6 ± 1.1 months). The ulcer and adjacent skin tissues were sterilized with Isodine-involved cotton patches. A dermatologic spray agent, Fiblast, containing a human bFGF, was sprayed three times repeatedly on the surface of the ulcer bed once a day. Then, the ulcer and its surrounding skin tissues were covered with dressings. The size of the ulcer was measured using a tracing picture analysis system (MEGA model MA3P; Meiara Shoji, Tokyo, Japan). Before the initiation and 1.5 months after combined hyperbaric oxygen and bFGF treatments, small specimens in the center portion of the ulcer tissues were removed by means of a biopsy punch (Kai sterile disposable biopsy punch; Kai Europe GmBH, Germany) from each patient. The edge of this biopsy punch consisted mainly of a vacant cylinder (Figs. 2 and 3) that enabled clinicians to grasp and remove a precise size of cylindrical tissue specimens. Each specimen was cut into two pieces. One was used for histologic analysis and another aliquot of remnant tissue was used for determining fibrous protein by the method described previously.[12–14] The specimen for collagen was determined by serial extraction with 5% trichloroacetic acid. The trichloroacetic acid–precipitable protein was extracted in 5% trichloroacetic acid at 90°C for 50 minutes, twice. Then, the extract was washed twice with cold trichloroacetic acid. Remaining precipitates were combined and dialyzed through a semipermeable membrane for 48 hours against distilled water. The dialysate was then lyophilized and weighed. A small amount of collagen was dissolved in 0.1 M phosphate buffer (pH = 7). An aliquot (10 μl) of the buffer was applied for protein assay with bovine serum albumin as the standard. The trichloroacetic acid–treated precipitates were washed twice with 0.1N NaOH and maintained at room temperature for 24 hours. After centrifugation (755 *g* for 15 minutes), the undissolved substance including elastin[14] was washed twice with distilled water. The supernatant comprising noncollagenous protein[13–15] was mixed with 1/10 of the volume of 50% trichloroacetic acid and chilled in an ice bath for 1 hour. The precipitates procured after centrifugation were washed twice with 5% trichloroacetic acid and then heated at 95°C for 50 minutes. The elastin and noncollagenous protein preparations thus obtained were washed with acetone and then with ethanol and dried in an oven at 55°C for 24 hours. An aliquot of each material was dissolved in 0.1 M phosphate buffer and protein concentration was determined.

RESULTS

Table 2 shows the effect of simultaneous application of hyperbaric oxygen and bFGF on ulcer size; the amount of collagen, noncollagenous protein, and elastin in ulcer tissue; and the occlusive status of the wounds. The average size of ulcers treated with this combination therapy was 90.8

percent ($p < 0.001$) smaller than that of the pretreated ones. Five of them (cases 1, 2, 4, 5, and 7) showed complete closure of the wound. Another two showed definite shrinkage of ulcers, 74.7 and 57.2 percent in cases 3 and 6, respectively, following this therapy (Fig. 4). This combination treatment resulted in a 166 percent increase ($p < 0.01$) in the average collagen content of ulcers. A lesser extent of elevation (129 percent increase) ($p < 0.01$) was also noted in the noncollagenous protein content after the same treatment. The average amount of elastin remained unchanged following this combination treatment. Histopathologic study demonstrated numerous fibroblasts and collagen fibers in removed ulcer tissues (Fig. 3).

Fig. 1. The multiplace hyperbaric oxygen chamber (Barotec Hanyuda Co., Tokyo, Japan). Ten patients can be treated simultaneously in this pressure chamber.

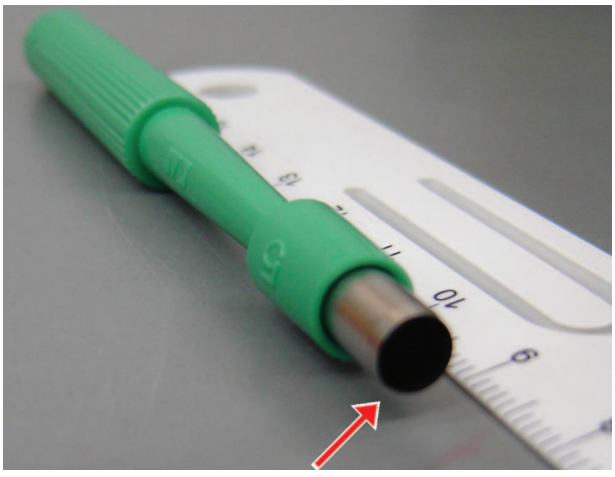

Fig. 2. General appearance of a biopsy punch. Precise quantity of specimen can be obtained by a vacant cylindrical portion (*arrow*).

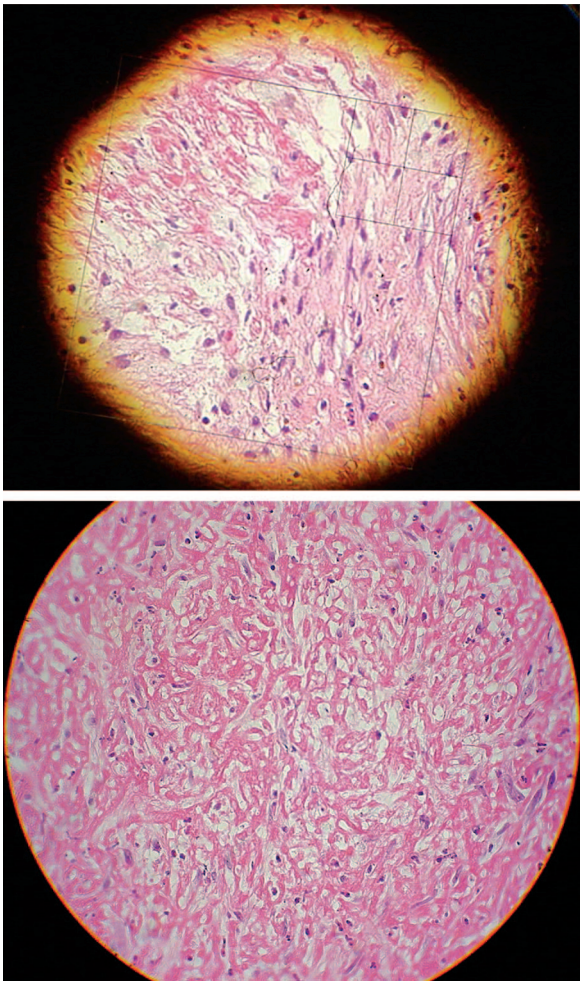

Fig. 3. (*Above*) Histopathologic change of the ulcer in the patient in case 3. The ulcer biopsy specimen shows the existence of a small amount of fibroblasts 0.5 month after hyperbaric oxygen plus bFGF treatment (hematoxylin and eosin; original magnification reduced from ×200). (*Below*) The most peculiar change of the ulcer was the proliferation of the granulation tissue of the ulcer 1.5 months after hyperbaric oxygen plus bFGF treatment (hematoxylin and eosin; original magnification reduced from ×100).

DISCUSSION

It is generally accepted that hyperbaric oxygen treatment exerts a beneficial effect against infection because of the neutrophil-mediated killing of bacteria.[16] Sufficient oxygen tension is also indispensable for the production of collagen matrix, which is essential for angiogenesis.[17] Exogenously administered bFGF has been confirmed to induce potent angiogenesis and granulation tissue formation[7] and to result in stimulation of wound repair in animals.[6–9] It is also well known that macrophages, monocytes, and fibroblasts are visible in the granulation tissue in bFGF-treated di-

Table 2. Changes of Ulcer Appearances before and after Hyperbaric Oxygen and Basic Fibroblast Growth Factor Treatment

Case	Ulcer				Content of Fibrous Protein (mg/g) in Ulcer						Final Histologic Findings of the Ulcer
	Size (mm^2)*		Color		Collagen*		Noncollagenous Protein*		Elastin†		
	Before	After	Before	After	Before	After	Before	After	Before	After	
1	896	0	Red	Skin color	46	236	82	256	23	31	Epithelialization
2	1810	0	Red	Skin color	145	314	220	369	39	45	Epithelialization
3	1981	501	Red	Red	76	215	95	186	50	69	Proliferation of connective tissue
4	1611	0	Red	Skin color	71	280	100	314	46	41	Epithelialization
5	1451	0	Red	Skin color	59	189	110	381	41	51	Epithelialization
6	971	416	Red	Red	201	340	240	416	84	78	Proliferation of connective tissue
7	1202	0	Red	Skin color	97	265	135	327	56	64	Epithelialization
Mean ± SD	1417 ± 383	131 ± 208			99 ± 51	263 ± 50	140 ± 59	321 ± 73	48 ± 17	54 ± 16	

*$p < 0.001$.
†p = not significant.

abetic mice.[9] In our study, numerous fibroblasts and collagen fibers were observed in ulcer tissues in patients treated with hyperbaric oxygen plus bFGF (Fig. 3). A question remains as to the exact mechanism of the increased collagen and noncollagenous protein following the combined treatments. Such change might be attributed to the enhanced synthesis or decreased degradation of these protein fractions. Although the latter possibility cannot be completely ruled out, the accelerated newly synthesized ulcer fibrous protein may be the cause because proliferation of granulation tissue including fibroblasts occupied most of the ulcer lesions (Fig. 3).

However, the exact mechanism that underlies the fibrous dosage alteration is poorly understood. Tissue oxygen tension probably plays an important role in the wound-healing process.[1,18] Tissue oxygen tension of 30 to 40 mmHg appears to be indispensable for fibroblast proliferation, enhancement of fibroblastic synthesis of collagen, and capillary procreation.[1,18] We did not determine tissue oxygen tension in this study, but an early experiment indicates that the tissue oxygen tension showed a satisfactory level following the hyperbaric oxygen treatment.[19]

The question may be raised as to the influence of selection on examined patients. The cause of the wounds included varicose ulcers, diabetic ulcers, and pressure ulcers. In addition, before the start of this combined therapy, some patients had hyperbaric oxygen therapy, some had surgery and hyperbaric oxygen, and some had bFGF. The former question emphasizes the necessity for precise selection of the same primary disease producing ulcers, and such analyses were naturally performed.[20,21] However, a recent review article of hyperbaric oxygen for current therapeutic uses appears to include leg ulcers in nondiabetic patients, chronic diabetic foot lesions, and leg ulcers caused by arterial insufficiency.[22]

CONCLUSIONS

The latter question is quite reasonable for analyzing the combined therapeutic effect. Unfortunately, we could not systematically examine the therapeutic results of the combined therapy because the majority of our patients had problem wounds and had required routine care or routine care plus additional treatments before the initiation of the combined therapy (Table 1). Our results will also be criticized because of the small sample of patients and the paucity of randomized controlled trials. Although randomized controlled clinical trials are the standard for estab-

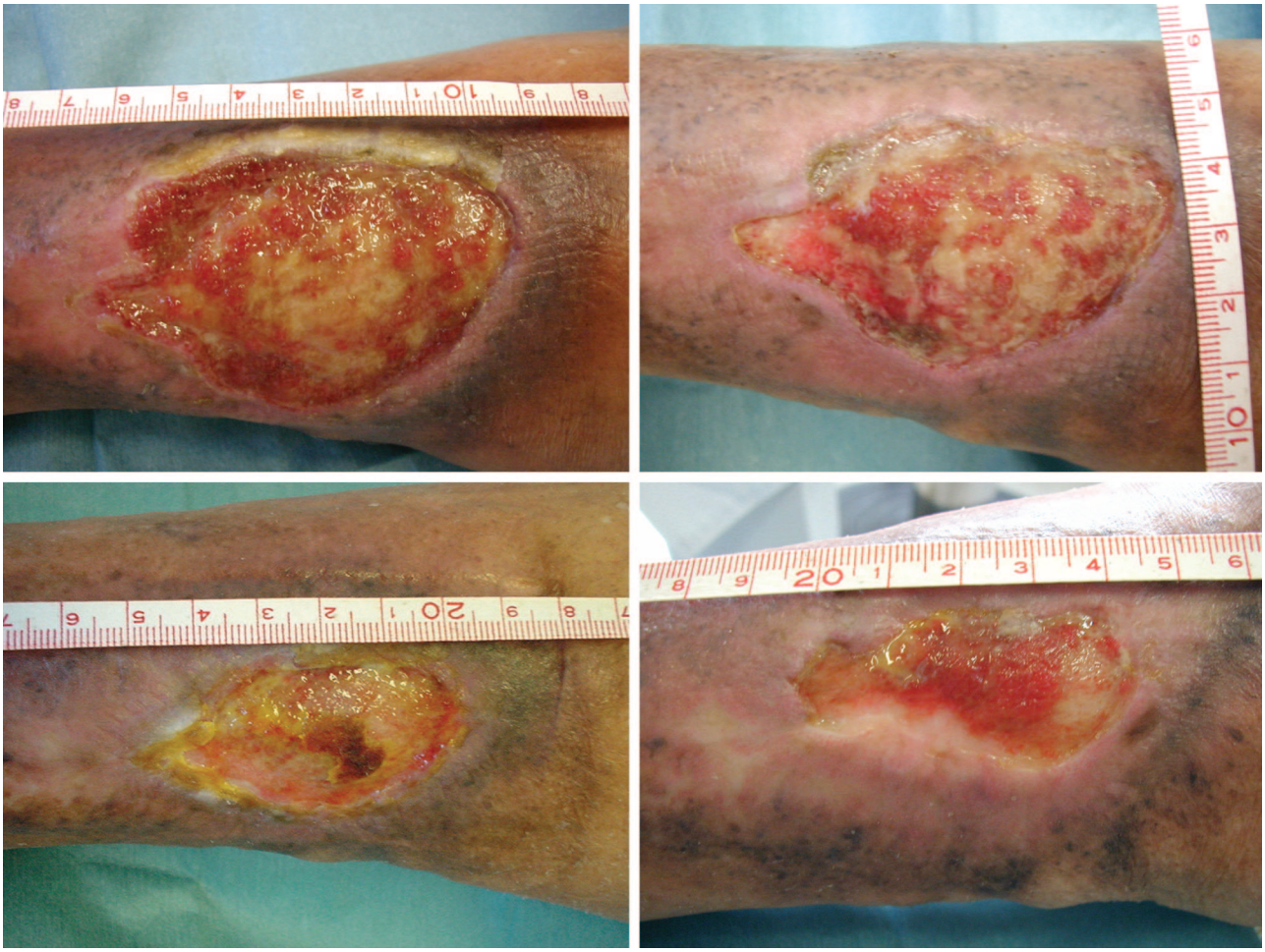

Fig. 4. Macroscopic appearance of the leg ulcer in case 3 shows a large hyperemic deep ulcer before hyperbaric oxygen plus bFGF treatment (*above*, *left*), shrinkage of the ulcer size but still hyperemia at month 1.8 after hyperbaric oxygen plus bFGF treatment (*above*, *right*), further shrinkage of the ulcer with fading redness at 3.1 months (*below*, *left*), and initiation of ulcer epithelialization at 4.4 months (*below*, *right*).

lishing the efficacy of a therapeutic intervention, other evidence including preclinical studies or retrospective case series appears to still have merit.[23] In preparing this article, the authors failed to find any preliminary reports or randomized controlled trials to study the combined therapy of hyperbaric oxygen and bFGF. We believe that the combined therapy should at least be integrated with the usual treatments of this intractable disease.

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