Low-energy Extracorporeal Shock Wave Therapy Improves Microcirculation Blood Flow of Ischemic Limbs in Patients with Peripheral Arterial Disease: Pilot Study

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Abstract

Background: Because direct application of low-energy shock waves induces angiogenesis, we investigated the safety and efficacy of this new therapy to develop a noninvasive method of repeatable therapeutic angiogenesis for treating peripheral arterial disease (PAD).

Subjects and Methods: The subjects were 10 patients who had symptomatic PAD and limited ischemia in a below-the-knee artery. Low-energy shock waves were directly applied to the calf muscles 6 times every other day. Intracorporeal changes were evaluated with ultrasonography to determine adverse effects of therapy. To assess blood flow of the microcirculation, transcutaneous oxygen tension (TcPO₂), skin perfusion pressure (SPP), and technetium-tetrofosmin (⁹⁹mTc-TF) scintigraphy were performed before and after therapy. The TcPO₂ was measured while subjects inhaled pure oxygen (maximum TcPO₂). The ⁹⁹mTc-TF perfusion index was determined as a ratio of uptake in muscle to that in the brain (control) for quantitative analysis.

Results: No adverse effects were noted in any patient. Maximum TcPO₂ values increased significantly on the calf (57.3±28.4 to 71.0±14.5 mm Hg, p=0.044) and the dorsum of the foot (52.2±21.8 to 76.1±17.9 mm Hg, p=0.012). The SPP tended to increase after therapy on the dorsum and plantar surfaces of the foot, but the differences were not significant. The ⁹⁹mTc-TF perfusion index in the foot significantly increased (0.48±0.09 to 0.61±0.12, p=0.0013), but that in the leg did not change.

Conclusion: We have demonstrated that low-energy shock wave therapy is safe and can restore blood flow in the microcirculation in patients with symptomatic PAD.

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Key words: peripheral arterial disease, shock waves, angiogenesis, microcirculation

Introduction

Peripheral arterial disease (PAD) is a progressive illness that is an important manifestation of systemic atherosclerosis. In persons with PAD the risk of heart attack is 5 times greater and the risks of stroke and total mortality are 2 to 3 times greater.
than in persons without PAD\(^3\). Although recent advances in revascularization have improved treatment options\(^3\), PAD still has a poor long-term prognosis and in some cases worsens the quality of life\(^2\). The methods of revascularization, including endovascular therapy and bypass surgery, are only for medium-sized or large arteries and do not always improve blood flow in the microcirculation. Therapeutic angiogenesis has been used to improve blood flow in the microcirculation at the ischemic site, and, evidence suggests, is safe and effective for PAD\(^4\). However, these treatments cannot be performed repeatedly, because they involve invasive procedures. Thus, additional noninvasive and repeatable methods of therapeutic angiogenesis are required.

Extracorporeal shock wave (SW) therapy is commonly used to shatter kidney stones\(^7\) and to relieve orthopedic conditions, such as lateral epicondylitis\(^6\) and plantar fasciitis\(^9\). Recently, Nishida et al. have shown that low-energy SW enhances angiogenic factors in cultured human endothelial cells in vitro and improves cardiac function in a porcine model of chronic myocardial ischemia in vivo\(^11\). Furthermore, low-energy SW induces angiogenesis and improved microcirculation in animal models of hindlimb ischemia\(^22,23\) and improve the walking ability of patients with PAD\(^4\). However, few studies have evaluated the effect of low-energy SW on blood flow in patients with PAD.

The objectives of this study were to provide a repeatable method of therapeutic angiogenesis for PAD and to investigate the safety and efficacy of this technique in improving blood flow in the microcirculation in ischemic tissues.

**Subjects and Methods**

**Study Population and Design**

From February 2009 through September 2011, we performed a nonrandomized, single-arm pilot study in 10 patients with PAD. The subjects met the following criteria: a) symptomatic PAD, in which the occlusive or stenotic lesion was limited to a below-the-knee artery and was diagnosed with digital subtraction angiography (Fig. 1A); and b) continuous ischemic PAD with symptoms lasting more than 2 months despite conventional treatments. Patients requiring additional therapy for an ulcer or skin defect were excluded.

All patients (9 men and 1 woman; mean age, 71.3±9.0 years) were hospitalized for SW therapy during the 2-week treatment period. The characteristics of the patients are summarized in Table 1. As conventional therapy for PAD, cilostazol or other antiplatelet agents or both had been administered for all patients for at least 2 months before the start of SW therapy and were continued throughout the study. Medication and exercise therapy were not changed during the study period. The clinical conditions and diagnosis related to PAD in each patient are shown in Table 2.

**Endpoints**

The primary endpoint of this study was the occurrence of an adverse event as defined by the Common Terminology Criteria for Adverse Events (CTCAE) Version 4 supplied by the United States Department of Health and Human Services. Symptoms were assessed through gross inspection of the skin and through patient interviews. Intracorporeal changes were evaluated with ultrasonography (SonoSite Japan, Tokyo, Japan) after therapy. The secondary endpoint was an increase in local blood flow of the microcirculation at the ischemic site.

**Extracorporeal SW Therapy and Protocol**

To generate SWs, we used an Orthospec™ extracorporeal SW therapy device (Medispec Ltd., Gaithersburg, MD, USA) (Fig. 1B and C), as described in previous reports\(^25,26\). This device allows an approximate treatment target area of ~70-mm depth and 45-mm local diameter\(^3\). We directly applied an energy flux density of 0.11 to 0.21 mJ/mm\(^2\) to 6 spots on the ischemic leg (300 shots/spot) with a frequency of 2 pulses per second, 3 times a week for 2 weeks.

**Evaluation of Microcirculation Blood Flow**

To evaluate the blood flow of the microcirculation before and after SW therapy, transcutaneous oxygen
tension (TcPO₂) and skin perfusion pressure (SPP) were measured, and ⁹⁹ᵐ-technetium-tetrofosmin (⁹⁹ᵐ-Tc-TF) scintigraphy was performed. Posttreatment examinations were performed within 1 week after therapy.

The TcPO₂ was measured with a transcutaneous monitor (TCM 400, Radiometer K.K., Tokyo, Japan). The sampling sites (calf, anterior tibia, and dorsum of the foot) were carefully selected so as not to overlie a bony prominence, superficial vessel, or
Table 1 Clinical Characteristics

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Previous treatment</th>
<th>HT</th>
<th>DL</th>
<th>DM</th>
<th>CAD</th>
<th>HD</th>
<th>Antiplatelet drugs</th>
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<td>1</td>
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<td>70</td>
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<td>+</td>
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<td>-</td>
<td>-</td>
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<td>5</td>
<td>M</td>
<td>62</td>
<td>PCI/EVT</td>
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<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>ASA, clopidogrel</td>
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<tr>
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<td>M</td>
<td>70</td>
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<td>+</td>
<td>-</td>
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<td>+</td>
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<td>clopidogrel, cilostazol</td>
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</tbody>
</table>

Abbreviations: M, male; F, female; CABG, coronary artery bypass graft surgery; F-P bypass, femoropopliteal artery bypass; PCI, percutaneous coronary intervention; EVT, endovascular therapy; HT, hypertension; DL, dyslipidemia; DM, diabetes mellitus; CAD, coronary artery disease; HD, hemodialysis; ASA, aspirin

Table 2 Clinical condition related to peripheral arterial disease and outcome

<table>
<thead>
<tr>
<th>Case</th>
<th>Cause of PAD</th>
<th>Rutherford classification</th>
<th>Stenosis of below-knee arteries (%)</th>
<th>Symptoms</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ASO</td>
<td>II-4</td>
<td>75 90 100</td>
<td>Resting numbness of sole</td>
<td>Slight improvement</td>
</tr>
<tr>
<td>2</td>
<td>ASO</td>
<td>I-3</td>
<td>100 100 100</td>
<td>Severe IC</td>
<td>No change</td>
</tr>
<tr>
<td>3</td>
<td>ASO</td>
<td>III-5</td>
<td>100 100 100</td>
<td>Minor tissue loss on toe</td>
<td>Recovery</td>
</tr>
<tr>
<td>4</td>
<td>ASO</td>
<td>I-3</td>
<td>100 100 100</td>
<td>Severe IC</td>
<td>Slight improvement</td>
</tr>
<tr>
<td>5</td>
<td>ALI (chronic stage)</td>
<td>II-4</td>
<td>25 100 100</td>
<td>Resting foot pain</td>
<td>Marked improvement</td>
</tr>
<tr>
<td>6</td>
<td>ASO</td>
<td>III-5</td>
<td>100 100 90</td>
<td>Minor tissue loss on toe</td>
<td>No change</td>
</tr>
<tr>
<td>7</td>
<td>ASO</td>
<td>I-2</td>
<td>100 100 100</td>
<td>Moderate IC</td>
<td>No change</td>
</tr>
<tr>
<td>8</td>
<td>ASO</td>
<td>I-3</td>
<td>100 100 100</td>
<td>Severe IC</td>
<td>Slight improvement</td>
</tr>
<tr>
<td>9</td>
<td>ASO</td>
<td>I-2</td>
<td>100 100 100</td>
<td>Moderate IC</td>
<td>Marked improvement</td>
</tr>
<tr>
<td>10</td>
<td>ASO</td>
<td>I-2</td>
<td>100 100 0</td>
<td>Moderate IC</td>
<td>No change</td>
</tr>
</tbody>
</table>

Abbreviations: PAD, peripheral arterial disease; ASO, atherosclerosis obliterans; ALI, acute limb ischemia; ATA, anterior tibial artery; PTA, posterior tibial artery; PA, peroneal artery; N/A, not available; IC, intermittent claudication

pulse site. The transducers were warmed to 43.5°C to increase the permeability of the skin to oxygen molecules. With the subject resting in the supine position, baseline TcPO2 was acquired at ambient conditions for about 20 minutes. The TcPO2 was acquired again while the subject inhaled 100% oxygen at 5 L/min for 5 minutes to determine TcPO2 in response to oxygen (maximum TcPO2).

The SPP was measured at room temperature with the SensiLase PAD3000 flowmeter (Kaneka Medix Corp., Osaka, Japan) and a laser Doppler probe on the dorsum and planter surface of the foot. With the subject in a supine position, the probe was enclosed within the bladder of a cuff and wrapped around the forefoot. The minimum external counter pressure on the underlying skin, elicited by the pressure cuff, was defined as the SPP.

18F-Tc-TF scintigraphy was performed as described previously. Both the anterior and posterior views were used for quantitative analysis. Regions of interest of equal size were drawn around the leg region (from knee to ankle) and the foot region (from ankle to toes) in the anterior and posterior projections (muscle uptake; M). Additionally, intracranial uptake (brain uptake; B) was obtained and used as the control. The 18F-Tc-TF perfusion index was defined as the muscle-to-brain (M/B) ratio of average counts per pixel.
**Statistical Analysis**

All data are presented as mean±SD. Data before and after therapy were compared by means of paired t-tests. A value of *p*<0.05 indicated statistical significance. The Smirnov-Grubb outlier test was used for outlier rejection.

**Ethics Statement**

This study was approved by the medical ethics committee of Nippon Medical School in 2009 (no. 20-07-21) and was registered with the University Hospital Medical Information Network-Clinical Trial Registry, which is accepted by the International Committee of Medical Journal Editors (no. UMIN 000004265, 20590844). All patients provided written, informed consent for participation in this trial.

**Results**

**Adverse Events and Outcome**

No patients had an adverse event categorized by the CTCAE, including pain, pruritus, erosion or redness of the skin, hematoma, or edema or bleeding in muscle or connective tissue. Symptoms improved in 6 patients but were unchanged in 4 patients (Table 2). No patient showed a worsening of condition during the study period.

**Blood Flow Examinations**

The SPP could not be assessed in 2 patients because of pain due to cuff pressure or involuntary movement of the patient. Furthermore, **99**Tc-TF scintigraphy was not performed in 1 patient, and 2 outlier values of the **99**Tc-TF perfusion index were rejected according to the Smirnov-Grubb outlier test.

Maximum TcPO$_2$ increased significantly at the calf ($57.3±28.4$ to $71.0±14.5$ mm Hg, *p*=0.044) and the dorsum of the foot (dorsum of the foot: $52.2±21.8$ to $76.1±17.9$ mm Hg, *p*=0.012) but showed no significant change at the anterior tibia ($64.5±24.3$ to $75.3±16.9$ mm Hg, *p*=0.10; Fig. 2). The SPP tended to increase after therapy at both the dorsum ($32.8±13.2$ to $47.1±15.6$ mm Hg, *p*=0.07) and the plantar surface of the foot ($38.0±18.1$ to $48.8±17.8$ mm Hg, *p*=0.24; Fig. 3), but the increases were not significant. Although the **99**Tc-TF perfusion index after therapy did not change in the leg region ($1.00±0.21$ to $1.23±0.40$, *p*=0.15), the value in the foot increased significantly ($0.48±0.092$ to $0.61±0.12$, *p*=0.0013; Fig. 4).

**Discussion**

The primary goal of SW therapy in patients with symptomatic PAD is to safely increase blood flow in the microcirculation of the lower extremities. Hematomas and petechial bleeding have been reported after SW therapy, particularly with high-energy pulses (>0.32 mJ/mm$^2$), for orthopedic conditions**99**Tc. Because all patients in the present study had an increased risk of bleeding owing to their being treated with at least 1 antiplatelet agent, we carefully observed them for bleeding complications. The absence of adverse events indicates the safety of SW therapy, even for patients receiving antiplatelet therapy.

In the present study, blood flow in the microcirculation was evaluated before and after therapy with 3 noninvasive methods: assessments of TcPO$_2$ and SPP and **99**Tc-TF scintigraphy. Assessments of TcPO$_2$ and SPP are commonly used as noninvasive examinations reflecting local skin arterial blood flow. In addition to these conventional methods, **99**Tc-TF scintigraphy (**99**Tc-TF perfusion index) was also performed as a novel quantitative method to evaluate the muscle microcirculation**99**Tc.

The TcPO$_2$ is affected by physiological, methodological, and technical factors. Thus, we have used the value of maximum TcPO$_2$ (in response to inhalation of 100% oxygen) to minimize these effects. In fact, we have previously demonstrated that the maximum TcPO$_2$ more accurately reflects blood flow than does baseline measurement (while the subjects breathes room air)**9. Although SPP was less affected by conditions, such as arterial calcification, skin damage, and edema at the measurement site, no report has compared the accuracy of ischemia assessment by means of maximum TcPO$_2$ and SPP.

In the present study, local blood flow at ischemic sites, indicated by maximum TcPO$_2$ at the calf and the dorsum of the foot and by the **99**Tc-TF perfusion index in the foot, increased significantly.
but SPP only tended to increase. These results suggest that low-energy SWs improve blood flow in the regional microcirculation. Although the precise mechanism by which low-energy SWs improve blood flow in the microcirculation remains unclear, the following successive mechanism is presumed to be involved in SW-induced angiogenesis. When an SW hits tissue, cavitation is first induced by compression by the positive-pressure component, and then expansion is induced by the tensile part of the SW.

Because the physical forces generated by cavitation are highly localized, SW might induce localized stress on cell membranes, as altered shear stress affects endothelial cells, which secrete angiogenic factors. In fact, recent reports have demonstrated that low-energy SWs up-regulate vascular endothelial growth factor, as well as its receptor fms-like tyrosine kinase (Flt-1), in endothelial cells in vitro and in the ischemic porcine myocardium and ischemic hind-limb myocytes in vivo.

Fig. 2 Maximum transcutaneous oxygen tension
The calf, anterior tibia, and dorsum of the foot were selected as sampling sites. With the patient resting in the supine position and inhaling 100% oxygen at 5 L/min, maximum transcutaneous oxygen tension (TcPO2) was acquired to determine TcPO2 in response to oxygen. Maximum TcPO2 at the calf and the dorsum of the foot increased significantly, although there was no significant change at the anterior tibia.
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Fig. 3  Skin perfusion pressure
Skin perfusion pressure (SPP) was measured on the dorsum and plantar surface of the foot. SPP at both the dorsum and plantar surface of the foot tended to increase after therapy, but the increase was not significant.

Fig. 4  $^{99m}$Tc-TF perfusion index
Muscle blood flow was estimated with $^{99m}$Tc-TF scintigraphy (muscle to brain [M/B] ratio of mean counts per pixel). Although the $^{99m}$Tc-TF perfusion index in the leg region did not change after therapy, the value in the foot increased significantly.

Therefore, considering the results of the present study and the possible mechanism of blood flow recovery by SWs described above, the best candidates for SW therapy are patients with PAD who have microvessel ischemia. Then, the combination of SW therapy and conventional revascularization may have a synergistic effect by improving blood flow from large vessels to the microcirculation.

The present study had several limitations. First, this was a prospective but nonrandomized, short-term study with a small sample size. Thus, factors other than medical treatment, such as rest and dietary control, may have influenced the result. We
will continue to follow up the subjects of this study and evaluate the long-term safety and efficacy. Second, the diagnosis of PAD might be incorrect in some cases despite of our careful examinations, because the ankle/brachial index of some patients was higher than the commonly used threshold of 0.9 for diagnosing PAD. Third, each method of blood flow examination has disadvantages. The main limitation of SPP is that errors can be caused by pain due to cuff inflation or involuntary movements of patients. Regarding ⁹⁹mTc-TF scintigraphy, inflammation may produce hot uptake with no relation to blood flow. In fact, there were 2 outlier values of the ⁹⁹mTc-TF perfusion index in the present study. Because these patients had inflammation at the measurement site due to ulcer or osteomyelitis, hot uptake might have caused incorrect values of blood flow. Fourth, because the severity of disease in our subjects was diverse (Rutherford I-2 to III-5), it was difficult to categorize symptoms by subjective methods. Therefore, we just stated the clinical symptoms and outcomes in Table 2.

In conclusion, we have demonstrated that low-energy SW therapy is safe and achieves noninvasive restoration of blood flow in the microcirculation in patients with symptomatic PAD.

Conflict of Interest: None.

References

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