

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 ^{99m}Tc-tetrofosmin (^{99m}Tc-TF) scintigraphy were performed before and after therapy. The TcPO₂ was measured while subjects inhaled pure oxygen (maximum TcPO₂). The ^{99m}Tc-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 ^{99m}Tc-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

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than in persons without PAD¹. Although recent advances in revascularization have improved treatment options², PAD still has a poor long-term prognosis and in some cases worsens the quality of life³. 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 PAD⁴⁻⁶. 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 stones⁷ and to relieve orthopedic conditions, such as lateral epicondylitis^{8,9} and plantar fasciitis¹⁰. 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^{12,13} and improve the walking ability of patients with PAD¹⁴. 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 OrthospecTM extracorporeal SW therapy device (Medispec Ltd., Gaithersburg, MD, USA) (**Fig. 1B and C**), as described in previous reports^{15,16}. This device allows an approximate treatment target area of ~70-mm depth and 45-mm local diameter¹⁵. We directly applied an energy flux density of 0.11 to 0.21 mJ/mm² 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

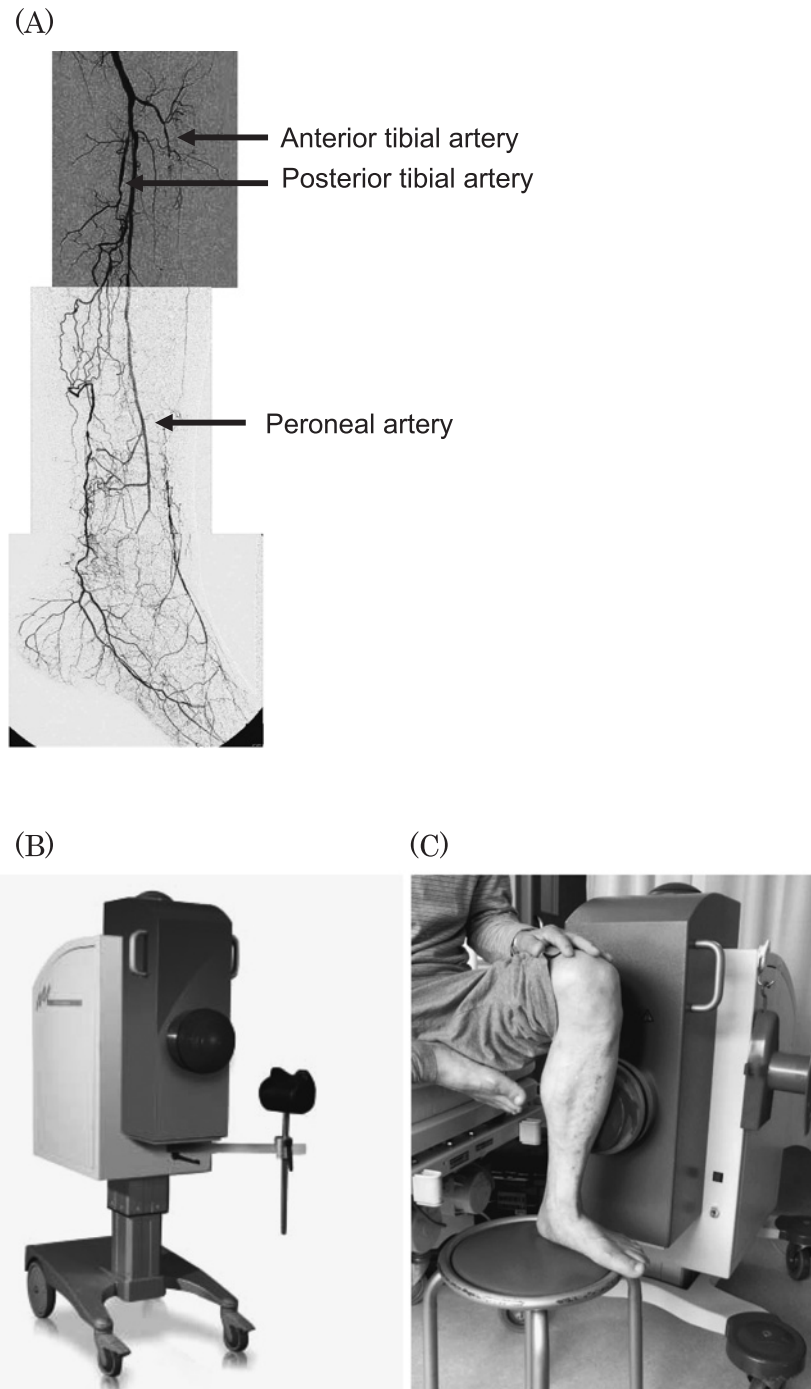


Fig. 1 (A) Representative digital subtraction angiographic image of a below-the-knee artery. The anterior tibial artery and posterior tibial artery are occluded (arrowheads indicate occluded arteries). Collateral arteries have developed around the occluded posterior tibial artery (B) Shockwave generator: Orthospec™ (Medispec Ltd). (C) Extracorporeal shockwave therapy being applied to a patient.

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

Case	Sex	Age (years)	Previous treatment	HT	DL	DM	CAD	HD	Antiplatelet drugs
1	M	70	CABG/F-P bypass	+	+	+	+	-	ASA, cilostazol
2	M	88	None	-	-	-	-	-	cilostazol
3	M	64	None	+	-	+	-	-	ASA, cilostazol
4	M	81	PCI	-	+	+	+	-	ASA, cilostazol
5	M	62	PCI/EVT	+	+	+	+	-	ASA, clopidogrel
6	M	70	CABG/PCI/EVT	+	+	+	+	-	ASA, cilostazol
7	M	59	None	+	-	+	-	-	ASA, cilostazol
8	M	77	None	+	+	+	-	-	cilostazol
9	F	75	None	+	-	-	-	-	cilostazol
10	M	67	PCI	+	+	+	+	+	clopidogrel, cilostazol

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

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

Abbreviations: PAD, peripheral arterial disease; ASO, arteriosclerosis 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 plantar 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

forefoot¹⁷. The minimum external counter pressure on the underlying skin, elicited by the pressure cuff, was defined as the SPP.

^{99m}Tc-TF scintigraphy was performed as described previously^{5,18}. 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 ^{99m}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 $\pm$ 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 CTAEC, 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, ^{99m}Tc -TF scintigraphy was not performed in 1 patient, and 2 outlier values of the ^{99m}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

^{99m}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/mm²), for orthopedic conditions^{19,20}. 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 ^{99m}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, ^{99m}Tc -TF scintigraphy (^{99m}Tc -TF perfusion index) was also performed as a novel quantitative method to evaluate the muscle microcirculation^{5,18}.

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)¹⁸. 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 ^{99m}Tc -TF perfusion index in the foot, increased significantly,

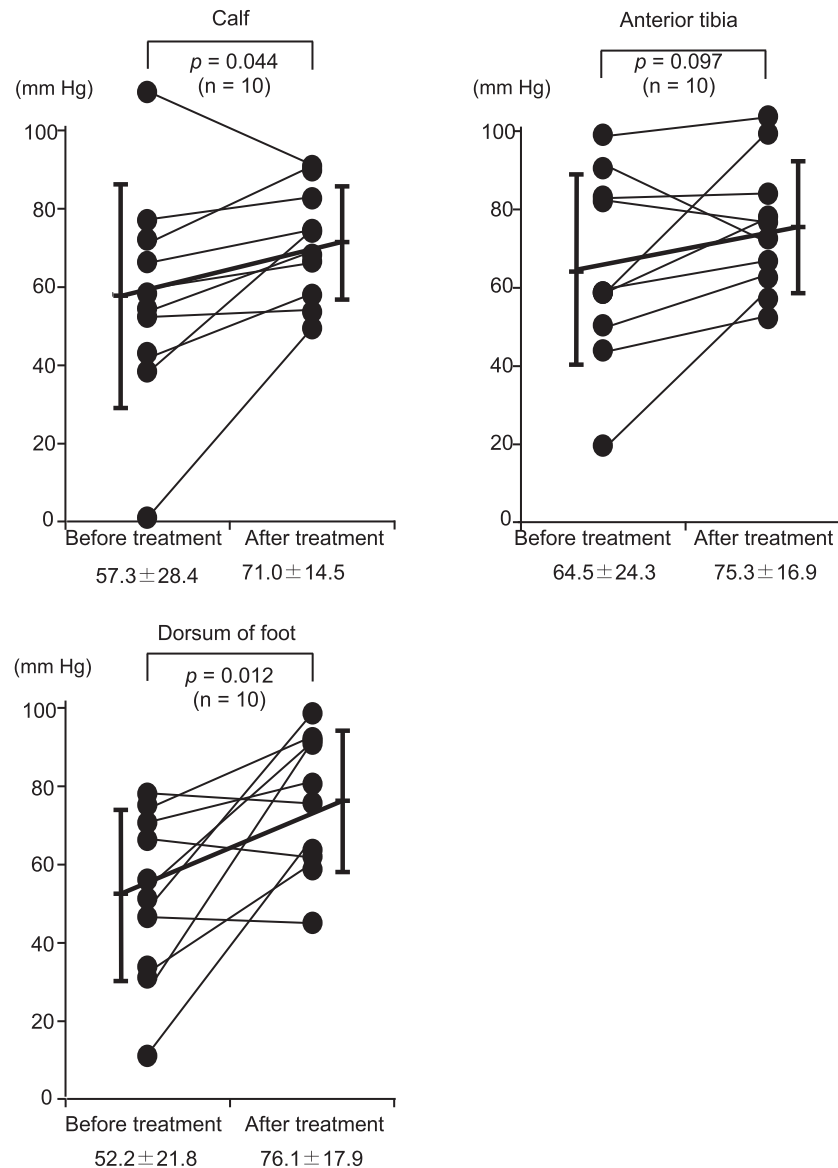


Fig. 2 Maximum transcutaneous oxygen tension

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.

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 angiogenesis¹¹. 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 SW²¹.

Because the physical forces generated by cavitation are highly localized, SW might induce localized stress on cell membranes, as altered shear stress affects endothelial cells²², 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 myocardium¹¹ and ischemic hind-limb myocytes *in vivo*¹².

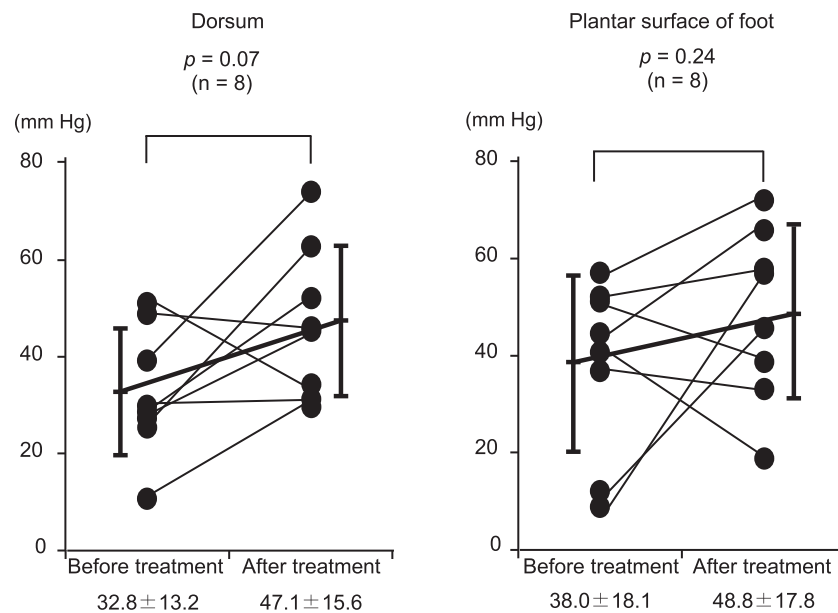
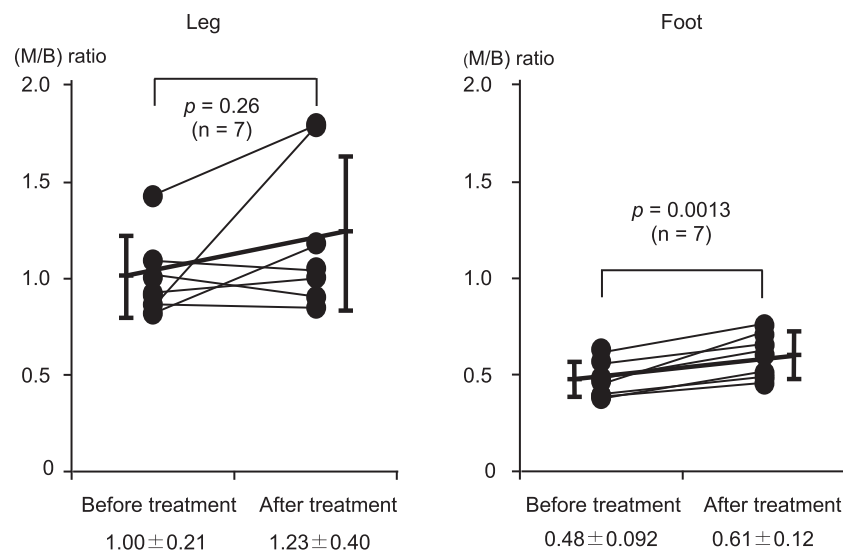


Fig. 3 Skin perfusion pressure

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 ^{99m}Tc -TF scintigraphy, inflammation may produce hot uptake with no relation to blood flow. In fact, there were 2 outlier values of the ^{99m}Tc -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.

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