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Original article

Waon therapy mobilizes CD34+ cells and improves peripheral arterial disease

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Summary
Background: We previously reported that Waon therapy upregulates endothelial nitric oxide synthase protein, and augments ischemia-induced angiogenesis in mice with hindlimb ischemia, and it improves limb ischemia in patients with peripheral arterial disease (PAD). The aim of this study was to investigate the underlying mechanism of Waon therapy for the treatment of patients with PAD, and to determine whether Waon therapy can mobilize blood-derived progenitor cells.
Methods: 21 consecutive PAD patients received standard medications, and were randomly divided into control ($n = 10$) and Waon therapy groups ($n = 11$). The Waon therapy group received Waon therapy daily for 6 weeks. The control group continued conventional therapy for 6 weeks. Leg pain was scored using a visual analogue scale. The ankle-brachial pressure index (ABPI) and the 6-min walking distance were measured at baseline and 6 weeks after therapy. Frequency of circulating CD34+ progenitor cell numbers was measured by quantitative real-time polymerase chain reaction, and the serum nitrate and nitrite levels were also measured at baseline and 6 weeks after therapy.
Results: The leg pain score, ABPI and the 6-min walking distance improved significantly after 6 weeks in the Waon therapy group, but not in the control group. Frequency of circulating CD34+ cells increased after 6 weeks of Waon therapy [2.0 ± 1.2 ($\times 10^{-4}$) at baseline to 3.9 ± 1.9 ($\times 10^{-4}$), $p = 0.015$], while it remained unchanged in the control group [1.8 ± 1.8 ($\times 10^{-4}$) at

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baseline to 1.2 ± 0.9 ($\times 10^{-4}$)]. Serum nitrate and nitrite levels increased significantly after Waon therapy (29.6 ± 17.6 to 36.0 ± 17.7 μmol/ml, $p < 0.05$), but not in the control group (34.4 ± 9.4 to 38.3 ± 8.8 μmol/ml).
Conclusion: Waon therapy mobilized circulating endothelial progenitor cells and improved limb ischemia in patients with PAD. Waon therapy is a highly promising therapy for patients with PAD.

Introduction

Peripheral arterial disease (PAD) is associated with decreased functional capacity and quality of life. Furthermore, patients with PAD have a higher rate of limb amputation and an increased risk of death compared with the general population. Patients with chronic critical limb ischemia have a 20% mortality rate per year with many patients being poor candidates for revascularization procedures [1]. Therapeutic angiogenesis and vasculogenesis may provide a treatment option for those patients with critical limb ischemia who are not suited for conventional revascularization therapy [2]. Some attempts at therapeutic angiogenesis have induced gene transfer using naked plasmid DNA encoding vascular endothelial growth factor (VEGF), which has demonstrated some promise in improving symptoms of critical limb ischemia [3], although not all studies have shown a benefit [4,5]. In addition, research has examined endothelial progenitor cells, which originate from bone marrow and circulate in the peripheral blood, and are known to participate in postnatal neovascularization [6,7]. There is evidence that autologous implantation of bone marrow mononuclear cells could be effective for achieving therapeutic angiogenesis [8,9]. These therapeutic angiogenesis trials have shown promising early efficacy, but further trials are warranted.

We have developed a form of thermal therapy, termed Waon therapy, which uses a dry sauna maintained at a temperature of 60 °C and differs from a traditional sauna. We previously reported that repeated Waon therapy improves hemodynamics and ameliorates symptoms and ventricular arrhythmias in patients with chronic heart failure (CHF) [10—12]. Furthermore, we reported that repeated Waon therapy improves vascular endothelial dysfunction in patients with CHF, and in patients with coronary risk factors such as hypertension, hyperlipidemia, diabetes mellitus, and smoking [13,14]. We also demonstrated that the molecular mechanism by which repeated Waon therapy improves vascular flow and endothelial function is through increased endothelial nitric oxide synthase (eNOS) expression [15,16].

Nitric oxide (NO) is constitutively produced by eNOS, and is a mediator of angiogenesis. Waon therapy increases eNOS protein expression, blood flow, and capillary density in a mouse model of hindlimb ischemia [17]. Furthermore, Waon therapy does not increase blood flow and capillary density in eNOS-deficient mice, which demonstrates that eNOS is a critical regulator of angiogenesis during Waon therapy. Recently, we reported that Waon therapy is safe and improves leg pain, status, and blood flow in patients with PAD [18,19].

This study was performed to confirm the beneficial effects of Waon therapy using the infrared-ray dry sauna, and to investigate the underlying mechanism of why Waon therapy may be beneficial in the treatment patients with PAD. In particular, we wanted to determine whether Waon therapy could mobilize blood-derived progenitor cells for local vascular regeneration.

Methods

Patients

The present study included patients with PAD with intermittent claudication for a minimum of 4 weeks without evidence of improvement despite conventional therapies. Inclusion criteria were (1) a resting ankle-brachial pressure index (ABPI) <0.75 in the affected limb on 2 consecutive examinations performed at least 1 week apart, (2) lower limb artery lesions were detected with computed tomographic angiography (CTA), magnetic resonance angiography (MRA) or color duplex ultrasound. 21 consecutive PAD patients were enrolled, and were randomly divided into control ($n = 10$) and Waon therapy groups ($n = 11$). They were allowed to continue taking antiplatelet drugs, provided that these therapies had been used for a minimum of 6 months before the enrollment. In the Waon therapy group, they had Waon therapy daily for 6 weeks. In the control group, they had only conventional PAD therapy for 6 weeks. All patients gave their written informed consent. This study design was unanimously approved by the Ethics Committee of Kagoshima University.

Waon therapy

Waon therapy, a form of thermal therapy that uses a far infrared-ray dry sauna at 60 °C and differs from the traditional sauna in that it has no hydration pressure, was performed as previously reported [18]. The patients were placed in a 60 °C dry sauna system for 15 min; after leaving the sauna, they underwent bed rest with a blanket to keep them warm for an additional 30 min. All patients were weighed before and after the therapy, and oral hydration with water was used to compensate for weight lost due to perspiration.

Waon therapy was performed once a day, 5 days a week, for a total of 6 weeks in the Waon therapy group. To rule out an acute effect of Waon therapy, all measurements were performed before the first treatment and on the next day after the last treatment in the Waon therapy group.

Measurements

Leg pain was scored using a visual analogue scale, with a marked 10-cm line extending from ''no pain: 0'' to ''severest pain: 10''. Resting ABPI was calculated by the