

Repeated sauna therapy improves myocardial perfusion in patients with chronically occluded coronary artery-related ischemia

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ABSTRACT

Background: Repeated low-temperature sauna (Waon) therapy relieves ischemic symptoms in patients with peripheral arterial disease. We investigated whether Waon therapy could improve myocardial perfusion in patients with ischemia related to chronic total occlusion (CTO) of coronary arteries.

Methods: Twenty-four patients who had ischemia in the CTO-related area were examined. The severity of ischemia was quantified by thallium-201 myocardial perfusion scintigraphy with adenosine. The Waon group (n = 16) was treated daily for three weeks with a 60 °C far infrared-ray dry sauna bath for 15 min and then kept in a bed covered with blankets for 30 min. The control group (n = 8) underwent myocardial perfusion scintigraphy twice with a three-week interval.

Results: In the control group, neither summed stress score (SSS) nor summed difference score (SDS) of myocardial scintigraphy changed. However, Waon therapy improved both SSS (16 ± 7 to 9 ± 6, p < 0.01) and SDS (7 ± 4 to 3 ± 2, p < 0.01), and the improvement was greater in patients with higher SSS and SDS scores at the baseline. Waon therapy extended treadmill exercise time (430 ± 185 to 511 ± 192 s, p < 0.01) and improved flow-mediated dilation of the brachial artery (4.1 ± 1.3 to 5.9 ± 1.8%, p < 0.05), but tended to decrease the number of circulating CD34-positive bone marrow-derived cells.

Conclusions: Waon therapy improves CTO-related myocardial ischemia in association with improvement of vascular endothelial function. This therapy could be a complementary and alternative tool in patients with severe coronary lesions not suitable for coronary intervention.

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1. Introduction

In recent years, the procedural success and target lesion revascularization rate of percutaneous coronary intervention (PCI) have been improving with the development of novel techniques and devices, including the drug eluting stent [1,2]. However, there are several concerns relating to PCI, and one of the major challenges in interventional cardiology is chronic total occlusion (CTO) of coronary arteries. PCI for CTO is more difficult, and therefore, the duration of irradiation is longer and complications occur more frequently in PCI for CTO compared with PCI for subtotal stenosis [3]. Furthermore, the procedural success rate for CTO has not improved, even in the stent era, particularly in patients with severe calcification and multi-vessel disease [4]. On the other hand, revascularization is associated with a decrease in mortality and improvement of quality of life, because patients with severe coronary lesions often have impaired cardiac function and extensive ischemia [5,6]. Therefore, a novel therapeutic approach for CTO is warranted,

especially in patients with technically complicated CTO lesions or previously unsuccessful PCI for CTO.

Recently, repeated low-temperature sauna (Waon) therapy has begun to draw much attention as a new treatment option for heart failure and peripheral artery disease [7,8]. The precise mechanisms of the beneficial effects of Waon therapy remain unclear, but several contributing factors have been proposed. For instance, Waon therapy is known to improve vascular endothelial function, and increases endothelial nitric oxide synthase (eNOS) activity [9,10]. Moreover, Waon therapy increases capillary density and blood flow in ischemic hindlimbs of mice but not in eNOS-deficient mice [11]. Recently, we have shown that Waon therapy increases capillary densities of non-infarcted myocardium of rat with myocardial infarction in association with increases in myocardial levels of eNOS and VEGF mRNA [12]. Activation of eNOS signaling could accelerate angiogenesis [13]. Therefore, Waon therapy may improve myocardial blood flow in patients with severe coronary artery disease. Accordingly, the purpose of the present study was to investigate whether repeated Waon therapy could improve myocardial perfusion in patients with CTO-related myocardial ischemia.

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2. Materials and methods

2.1. Subjects and study design

The present study was approved by the ethics committee of Toyama University Hospital and written informed consent was obtained from all patients.

Twenty-four consecutive patients who met the following criteria constituted the study group. First, they had CTO vessels detected by coronary angiography and had reversible myocardial ischemia in the CTO-related perfusion area as assessed by stress myocardial perfusion scintigraphy (MPS) with adenosine. Second, they had undergone unsuccessful PCI, had CTO vessels not suitable for PCI, or rejected PCI for CTO. These patients were assigned to the Waon therapy group or control group (without Waon therapy) in a 2:1 distribution. At baseline and three weeks later, stress MPS was performed in all patients. In the Waon group, flow-mediated vasodilation (FMD) and the number of circulating progenitor cells (PCs) were determined, and treadmill exercise test was performed before and after Waon therapy. Medications were not changed throughout the study period in both groups.

2.2. Waon therapy

Using a far infrared-ray dry sauna, Waon therapy was performed as previously reported [7–9]. Briefly, once a day for three weeks patients underwent sauna therapy at 60 °C for 15 min and were then kept supine on a bed outside the bath room for 30 min with sufficient warmth provided by blankets. Patients were weighed before and after the sauna therapy, and oral hydration with water was used to compensate for the lost weight.

2.3. Myocardial perfusion scintigraphy

All patients underwent thallium-201 (TI)-labeled MPS with adenosine stress. Adenosine was infused intravenously at a rate of 120 µg/kg/min for 6 min. 201TI (92.5–148.0 MBq) at a dose predetermined based on patient's weight was injected at the end of the third minute of adenosine infusion. Planar and single photon emission computed tomography (SPECT) images were acquired at 6 min after 201TI injection (early image) and 3 h later (delayed image) using a three-headed gamma camera (GCA-9300A/DI; Toshiba, Tokyo, Japan) with a high-resolution parallel-hole collimator. After transaxial reconstruction using a filtered back projection algorithm with a Ramp filter, short-axis, vertical and horizontal long-axis tomograms were obtained.

Images were interpreted by two experienced observers who were unaware of patient information. SPECT images were analyzed on the 17 segments of the left ventricle (LV) using a four-point grading scale: 0 = normal uptake; 1 = equivocal reduction of uptake; 2 = moderate reduction of uptake; 3 = severe reduction of uptake; and 4 = no uptake [14]. The global summed stress score (SSS) and summed rest score (SRS) were calculated by adding the scores of each segment on the early and delayed images, respectively. The summed difference score (SDS), an index of defect reversibility, was derived from the difference between SSS and SRS [15].

2.4. Flow-mediated vasodilation

Vascular endothelial function was evaluated by FMD of the brachial artery. Patients were instructed to overnight fast and to abstain from smoking and taking caffeine, vitamins, and medications for at least 12 h prior to FMD testing. Patients rested for at least 10 min in the supine position before the study in a quiet, light- and temperature-controlled (24 °C) room. Vasodilatation responses of the brachial artery were determined by the ultrasound technique using a semi-automatic device (EF18G; UNEX, Nagoya, Japan). Briefly, the diameter of the brachial artery was measured from B-mode ultrasound images using a 10-MHz linear array transducer. Then, a blood pressure cuff was inflated to 50 mm Hg above the systolic blood pressure over the proximal portion of the right forearm for 5 min. The diastolic diameter of the brachial artery was determined semi-automatically using an instrument equipped with software for monitoring the brachial artery diameter. The changes in the diastolic diameter were continuously recorded. Then, FMD was determined as the maximum change in diameter after cuff release normalized to the baseline diameter (% of baseline diameter).

2.5. CD34-positive bone marrow-derived cells

Recently, we showed Waon therapy-induced increases in myocardial VEGF mRNA levels [12]. The angiogenic cytokine of VEGF is a mobilizing factor of endothelial progenitor cells (EPCs) from bone marrow to ongoing neovascularization tissue [16,17]. CD34+ cells are a putative precursor of EPCs [18,19] and, therefore, we determined the number of CD34+ cells in the present study. The number of CD34+ cells in the peripheral blood was quantified using flow cytometry (EPICS-MCL; Beckman Coulter, Brea, CA, USA) in patients treated with Waon therapy at baseline and after the therapy. In six of the 16 patients, serial changes in CD34+ cells were determined during the three-week Waon therapy. White blood cells were dually stained with fluorescein isothiocyanate (FITC)-conjugated CD45 and phycoerythrin (PE)-conjugated CD34 (Stem-One-System; Beckman Coulter). Progenitors were separated by the low levels of CD45 expression and right-angle light scatter properties. Cells expressing CD34 were determined by gating the progenitor population and expressed as the number of cells per microliter. As the negative control, cells were stained with FITC-conjugated CD45 only.

2.6. Treadmill exercise test

In the Waon group, a treadmill exercise test was performed at baseline and after the three-week Waon therapy using the Bruce protocol. If patients seemed not to tolerate the high work load increments of the Bruce protocol, the protocol was modified by starting at 0.5 mph and 0 grade for 3 min before stage 1 of the Bruce protocol. Exercise was terminated when patients developed chest pain, severe fatigue or shortness of breath, or significant ST-segment changes (>1 mm horizontal or downsloping depression).

2.7. Statistics

Results are expressed as mean ± SD. Non-parametric pair-wise comparisons were made using the Mann-Whitney *U* test. The differences between baseline and post-treatment values were analyzed using the Wilcoxon signed rank test. Correlation between the changes in SSS or SDS and the baseline scores was determined using linear regression analysis. A value of *p* < 0.05 was considered statistically significant. Statistical analyses were performed with SPSS (SPSS 11.0J; International Business Machines Corporation, Chicago, IL, USA).

3. Results

3.1. Patient characteristics

The characteristics of the study group are summarized in Table 1. In the Waon group, 10 patients had prior myocardial infarction and 13 patients underwent PCI or coronary artery bypass surgery. Also in this group, 9 patients had target CTO in the right coronary artery, 6 patients in the left descending coronary artery, and 3 patients in the left circumflex artery (Table 1). Most patients had coronary risk factors such as hypertension, diabetes mellitus and hyperlipidemia. All patients in the Waon group were treated with statins and 11 patients with angiotensin converting enzyme inhibitors or angiotensin receptor blockers.

Table 1
Patient characteristics.

	Waon therapy (n = 16)	Control (n = 8)	<i>p</i> value
Men n (%)	8 (50)	6 (75)	0.46
Age (years)	71 ± 11	68 ± 6	0.44
Risk factor			
Hypertension	15 (94)	5 (63)	0.09
Diabetes mellitus	9 (56)	3 (38)	0.67
Hyperlipidemia	12 (75)	5 (63)	0.65
Smoking	9 (56)	7 (88)	0.19
Obesity	10 (63)	3 (38)	0.39
Laboratory data			
BUN (mg/dl)	20 ± 11	23 ± 18	0.60
Creatinine (mg/dl)	1.0 ± 0.5	0.9 ± 0.1	0.49
Hemoglobin (g/dl)	12 ± 1	13 ± 2	0.34
BNP (pg/dl)	143 ± 134	183 ± 277	0.66
Treatment			
Statin	16 (100)	7 (88)	0.33
Antiplatelet	15 (94)	8 (100)	1.00
ACE inhibitor/ARB	11 (69)	6 (75)	1.00
β-Blocker	12 (75)	5 (63)	0.65
Ca antagonist	6 (38)	3 (38)	1.00
Nitrate	10 (63)	5 (63)	1.00
CTO vessel			
LAD	6 (38)	4 (50)	0.88
Cx	3 (19)	0 (0)	0.53
RCA	9 (56)	5 (63)	1.00
OMI	10 (63)	4 (50)	0.88
PCI	10 (63)	7 (88)	0.35
CABG	3 (19)	0 (0)	0.52
LVEF (%)	60 ± 10	52 ± 22	0.25
LVEDD (mm)	52 ± 5	54 ± 12	0.59
LVESD (mm)	36 ± 6	40 ± 16	0.34

Figures are mean ± SD or number (%) of patients. ACE indicates angiotensin converting enzyme; ARB, angiotensinII receptor blocker; BNP, B type natriuretic peptide; CTO, chronic total occlusion; LAD, left anterior descending artery; Cx, circumflex artery; RCA, right coronary artery; OMI, old myocardial infarction; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft; LVEF, left ventricular (LV) ejection fraction; and LVEDD and LVESD, LV end-diastolic and end-systolic dimensions, respectively. LVEDD and LVESD were measured by echocardiography, and LVEF were calculated using Teichholtz's formula.

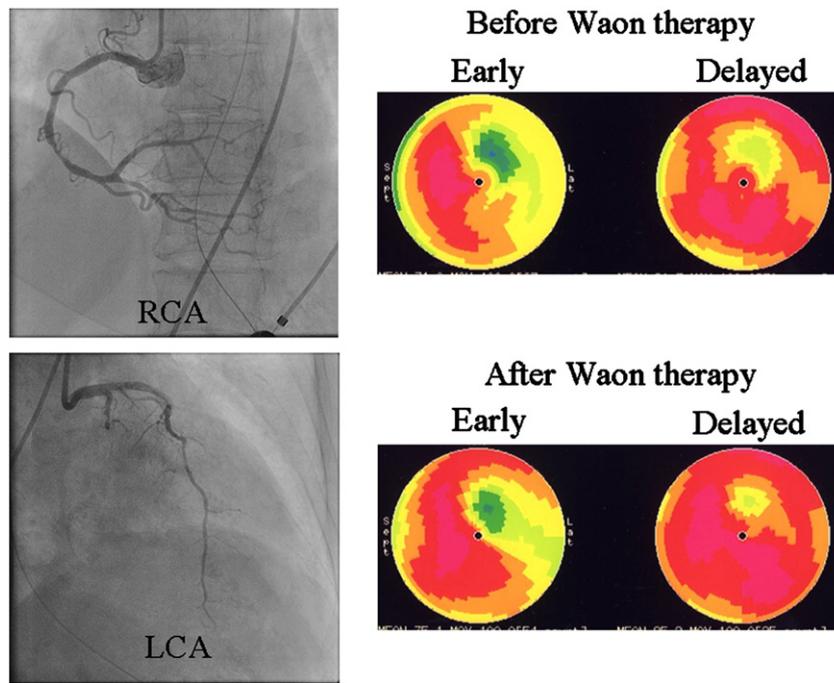


Fig. 1. Coronary angiograms and stress ²⁰¹Tl scintigraphy with adenosine before and after Waon therapy in an 84-year-old woman. She had total occlusion of the left circumflex coronary artery and diagonal branches. Polar maps show an extensive reversible defect in the antero-lateral regions before Waon therapy, but the low perfusion area and its severity were attenuated after Waon therapy.

3.2. Myocardial perfusion scintigraphy

No patient showed worsened clinical symptoms or suffered from complications due to the Waon therapy. Representative examples of myocardial perfusion scintigraphy in a patient treated with Waon therapy are shown in Figs. 1 and 2. Both SSS and SDS were significantly reduced by Waon therapy; whereas in the control group, neither SSS

nor SDS changed during the study period (Fig. 3, Table 2). Consequently, the Waon group had greater improvement of Δ SSS and Δ SDS compared to the control group (Fig. 4).

In the Waon group, the improvement in SSS was greater, as the baseline SSS was greater (Fig. 4). Similarly, the improvement in SDS was greater in patients with greater SDS at baseline (Fig. 4). Thirteen of the 16 patients showed better responses (Δ SDS ≥ 2) to Waon

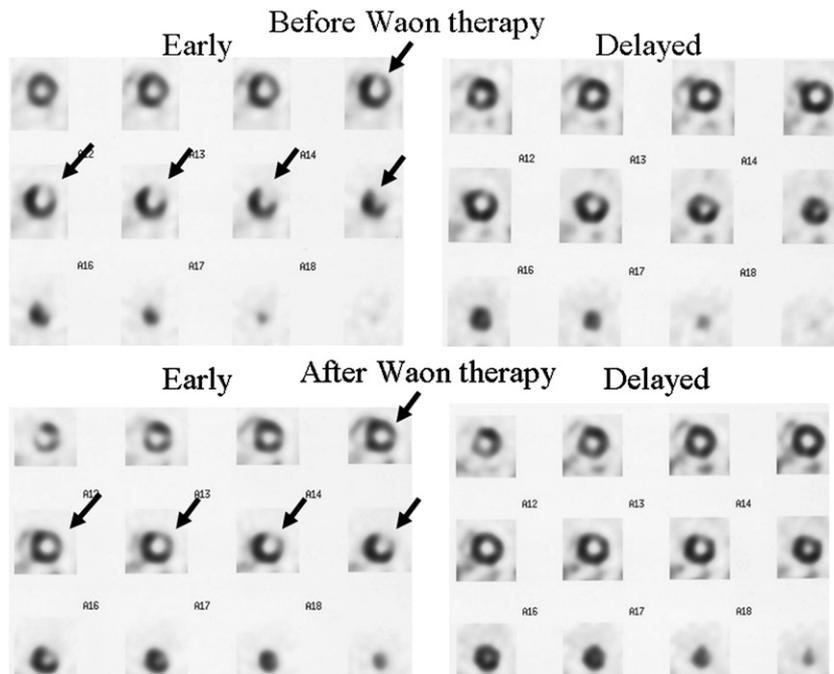


Fig. 2. Short-axis slices of single photon emission computed tomographic imaging before and after Waon therapy in the same patient shown in Fig. 1. Severe perfusion defects (arrows) are seen in the antero-lateral region before Waon therapy, but are improved after Waon therapy.

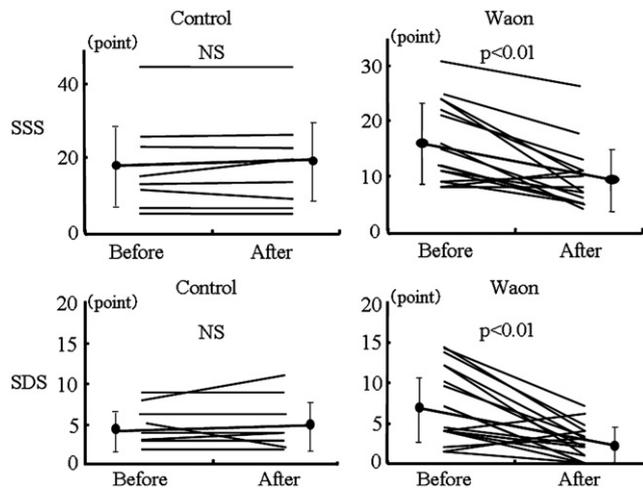


Fig. 3. Myocardial perfusion scores before and after three-week Waon therapy. Upper panels show summed stress scores (SSS), and the lower panels, summed difference scores (SDS). See text for details.

therapy. In these responders, SSS before Waon therapy was greater compared with non-responders ($n=3$) (17.3 ± 6.9 vs. 8.3 ± 0.6), as was SDS (7.5 ± 4.0 vs. 2.7 ± 1.2).

3.3. Flow-mediated dilation and CD34+ bone marrow-derived cells

Waon therapy improved FMD of the brachial artery from 4.1 ± 1.3 to $5.9 \pm 1.8\%$ ($p<0.05$; Fig. 5, left).

Unexpectedly, three week-Waon therapy tended to decrease the number of CD34+ cells ($n=16$, 0.77 ± 0.52 to 0.55 ± 0.29 cells/ μL , $p=0.05$; Fig. 6, left). Serial changes in CD34+ cells during the three-week Waon therapy were determined in six of the 16 patients. Of these 6 patients, 5 patients were responders ($\Delta\text{SDS} \geq 2$) to Waon therapy, and four of these 5 responders showed a transient increase in CD34+ cells during the course of Waon therapy (Fig. 6, right). The one non-responder did not show any such increase in CD34+ cells during the therapy. There was no significant relationship between changes in SDS (ΔSDS) and CD34+ ($\Delta\text{CD34+}$), as shown in Fig. 7.

3.4. Treadmill exercise test

Fourteen of the 16 patients in the Waon group performed the exercise test while the remaining two did not due to orthopedic diseases. In eight of these 14 patients the exercise was terminated due to significant ST segment depression, while in the remaining six patients termination was due to leg fatigue. On the whole, Waon therapy significantly improved exercise time from 430 ± 185 to 511 ± 192 s ($p<0.01$; Fig. 5, right). In three of the 8 patients who terminated the exercise test because of significant ST depression, the exercise-induced ST depression disappeared after Waon therapy. In six of 14 patients who

terminated exercise by leg fatigue, Waon therapy also improved exercise time from 510 ± 236 to 586 ± 231 s ($p<0.01$).

4. Discussion

The present study demonstrates that three-week Waon therapy can improve myocardial perfusion abnormalities in patients with CTO. Waon therapy also improved vascular endothelial function as assessed by FMD of the brachial artery. Waon therapy has previously been shown to be effective for patients with chronic heart failure and peripheral artery disease [7,8]. The present study is the first to show that Waon therapy is effective for treatment of patients with chronic and severe coronary artery disease.

4.1. Waon therapy for CTO-related myocardial ischemia

The precise mechanism by which repeated Waon therapy improves myocardial ischemia due to CTO remains unclear; however, there are several possible explanations for the effectiveness of Waon therapy. First, Waon therapy may increase pre-existing collateral flow. Repeated Waon therapy would improve vascular endothelial function by increasing eNOS activity and up-regulating eNOS expression by increasing shear stress [7,10]. Improvement of endothelial function relates to a decrease in vascular tone and may increase blood flow, including in collateral vessels. Second, Waon therapy may accelerate development of de novo arteries around the ischemic area. Nitric oxide plays an important role in the regulation of angiogenesis in response to tissue ischemia [13,20]. In a study using hindlimb ischemia of mice, Waon therapy enhanced eNOS gene expression and improved blood flow in ischemic hindlimbs by increasing capillary density [11]. Recently, we have shown that Waon therapy increases capillary densities of non-infarcted myocardium of rats with myocardial infarction in association with increases in myocardial levels of eNOS and VEGF mRNA [12]. Statins up-regulate eNOS and promote angiogenesis in response to tissue ischemia [21,22]. All patients in the present study received statins starting from at least three months before the study, and therefore the improvements in MPS scores in the present study were primarily the result of Waon therapy, although the combination therapy with statins may have augmented the NO-mediated angiogenesis induced by the Waon therapy.

Waon therapy might increase blood flow in the ischemic region by increasing collateral flow due to a reduction in LV end-diastolic pressure. In patients with heart failure, Waon therapy improves heart failure symptoms via a reduction in pulmonary artery wedge pressure [23]. In the present study, no patients were under decompensated conditions of heart failure at baseline, but a reduction in LV end-diastolic pressure induced by Waon therapy might increase blood flow in CTO-related myocardium.

Three of the 16 patients in the Waon group did not show improved MPS scores. As shown in Fig. 4, the scintigraphic improvement by Waon therapy was greater in patients with higher SSS or SDS before the therapy, and all non-responders had lower SSS or SDS before the therapy.

Table 2

Data at baseline and after Waon therapy in control and Waon groups.

	SRS (point)	SSS (point)	SDS (point)	Ex time ($\times 10^2$ s)	FMD (%)	CD34+ (μL)
Control						
Baseline	12.4 ± 14	17.3 ± 13.0	4.9 ± 2.6	–	–	–
After	12.5 ± 14	17.5 ± 13.1	5.0 ± 3.4	–	–	–
Δ	0.1 ± 1.2	0.3 ± 1.8	0.1 ± 1.6	–	–	–
Waon						
Baseline	9.1 ± 7.1	16.1 ± 7.3	7.0 ± 4.4	4.3 ± 1.9	4.1 ± 1.3	0.8 ± 0.5
After	$7.1 \pm 5.7^\dagger$	$9.6 \pm 5.8^{\dagger\dagger}$	$2.5 \pm 2.2^{\dagger\dagger}$	$5.1 \pm 1.9^{\dagger\dagger}$	$5.9 \pm 1.8^\dagger$	0.6 ± 0.3
Δ	-2.1 ± 4.0	$-6.6 \pm 5.4^{##}$	$-4.5 \pm 4.1^{##}$	0.7 ± 0.5	1.8 ± 2.4	-0.2 ± 0.4

Mean \pm SD. SRS indicates summed rest score; SSS, summed stress score; SDS, summed difference score; Ex, treadmill exercise; FMD, flow mediated dilation; Δ , differences between data at baseline and after 3-week Waon therapy. † , † , $p<0.05$, $p<0.01$ vs. baseline, respectively. $^\#$, $^\#\#$, $p<0.05$, $p<0.01$ vs. control, respectively.

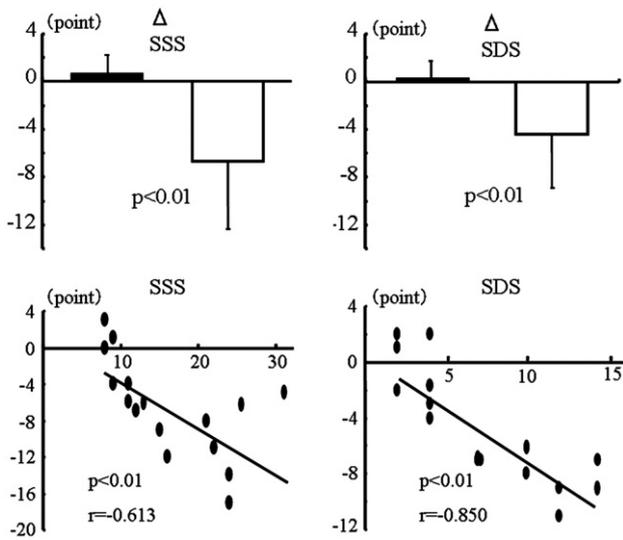


Fig. 4. Comparison of changes in myocardial scintigraphic scores by Waon therapy between the control (black bars) and Waon (white bars) groups. The upper left panel shows changes in summed stress scores (SSS), and the upper right panel, those in summed difference scores (SDS). The lower left panel shows correlation between SSS at baseline (X axis) and changes in SSS by Waon therapy (Y axis), and the lower right panel, correlation between SDS at baseline (X axis) and changes in SDS by Waon therapy (Y axis). Mean \pm SD. See text for details.

Therefore, the magnitude of ischemic stimuli might also contribute to the improvement of myocardial perfusion with Waon therapy. However, these 3 non-responders suffered from diabetes mellitus and one of them had severely impaired renal function (plasma creatinine level of 2.7 mg/dl); no patient, except this patient in the Waon group, had plasma levels of creatinine \geq 1.5. In animal studies [11,12], Waon therapy-induced increases in vascular densities were associated with increasing eNOS levels. Enhanced reactive oxygen species, detected in patients with diabetes mellitus, renal failure or smoking, might inhibit eNOS activity and NO bioavailability. It has reported that diabetes mellitus and renal failure inhibit angiogenesis [24,25], a finding consistent with the present results, in which all non-responders were accompanied by these diseases. We were unable to predict any parameters about the effectiveness of Waon therapy before the completion of the 3-week therapy. There were no differences in responses of clinical indices to Waon therapy between the responders and non-responders. In the

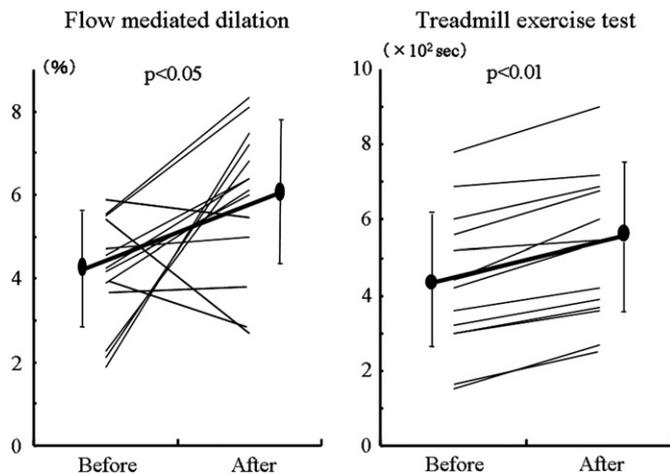


Fig. 5. Flow-mediated dilation of the brachial artery (left) and treadmill exercise time (right) before and after Waon therapy. These two variables increased significantly after Waon therapy.

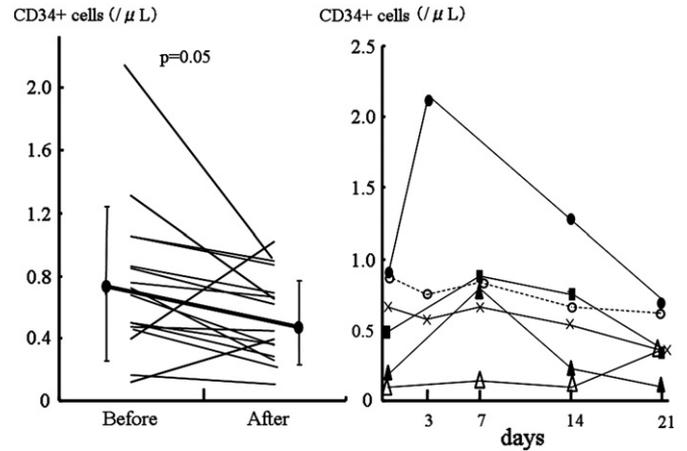


Fig. 6. The number of circulating CD34+ cells before and after Waon therapy. Left panel: the number of circulating CD34+ cells tended to decrease after Waon therapy. Right panel: serial changes in CD34+ cells during Waon therapy in six patients are shown. Solid lines indicate the five patients with improved myocardial perfusion after Waon therapy, and the dashed line shows the one patient without improvement after Waon therapy. See text for details.

present study, only 3 patients were non-responders, but a further study performed in more patients may clarify predictors about the effectiveness during the course of Waon therapy.

4.2. Effects of Waon therapy on exercise tolerance

Waon therapy extended the treadmill exercise time. In 8 patients, the exercise time to significant ST depression was lengthened by Waon therapy, and the exercise-induced significant ST depression disappeared after Waon therapy in three of these 8 patients. Thus, improvement in exercise time in these 8 patients was due to Waon therapy-induced improvement in myocardial ischemia. In the other 6 patients, however, exercise was not terminated by myocardial ischemia but terminated by leg fatigue, and their exercise time was significantly lengthened by Waon therapy. In patients with heart failure, the release of endothelial NO is impaired and vascular smooth muscle responsiveness to NO is also reduced, resulting in impaired exercise tolerance [26–28]. Previous studies have shown that Waon therapy increases

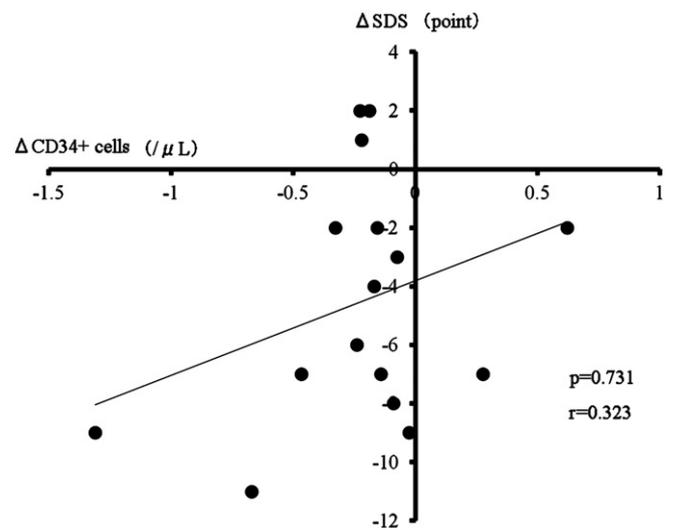


Fig. 7. Relationship between Waon therapy-induced changes in CD34+ cells (Δ CD34+) and summed difference scores (Δ SDS). There was no significant relation between these variables.

eNOS activity and improves FMD of the peripheral artery [7,10], a finding suggestive of augmenting NO bioavailability. Moreover, Waon therapy in patients with heart failure improved exercise tolerance in association with improvement of endothelial function [29]. In the present patients treated with Waon therapy, basal levels of FMD were considerably lower compared to healthy subjects [30], and Waon therapy improved FMD significantly. Thus, Waon therapy might have contributed to the improvement in exercise tolerance in the present patients who terminated exercise by leg fatigue.

4.3. Influences of Waon therapy on progenitor cells in peripheral blood

Recently, vasculogenesis, a process of in situ formation of blood vessels from endothelial progenitor cells (EPCs), was shown to occur not only in the embryo but also in the adult [31]. Neovascularization in the adult is not restricted to angiogenesis, a phenomenon of extension of already formed primitive vasculature [31]. We have showed that Waon therapy increased myocardial VEGF levels in rats [12]. The angiogenic cytokine of VEGF is a mobilizing factor of EPCs from bone marrow to ongoing neovascularization tissue [16,17]. However, in the present study, CD34+ cells, a putative precursor of EPCs, unexpectedly tended to decrease at the end of three-week Waon therapy. We therefore determined serial changes in CD34+ cells during Waon therapy in 6 patients and found a transient increase in the number of CD34+ cells during the therapy (Fig. 6). The improvement in myocardial perfusion abnormality by Waon therapy, i.e., a decrease in ischemic stimuli, may result in reduction in circulating CD34+ cells because tissue ischemia is the primary stimuli for recruitment of progenitor cells from bone marrow into peripheral blood [18]. However, there was no significant relationship between Waon therapy induced changes in CD34+ cells and scintigraphic improvement, i.e., Δ SDS (Fig. 7), although scintigraphic improvement was associated with a reduction in CD34+ cells in many patients. Thus, the precise mechanism of Waon therapy-induced reduction in CD34+ cells remains to be elucidated.

4.4. Limitations

There are several limitations in interpreting the present results. First, the number of participants in this study was small and was not sufficient to understand the precise mechanisms of Waon therapy in patients with coronary artery disease. Further studies are mandatory to investigate the influences of coronary anatomy, atherosclerotic risk factors and medications on Waon therapy-induced improvement of myocardial perfusion, and to clarify the duration of the beneficial effects of Waon therapy after discontinuation. Second, we determined circulating CD34+ mononuclear cells as a putative precursor of EPCs derived from bone marrow. CD34 is a membrane surface marker for bone marrow-derived stem cells and CD34+ cells give rise to hematopoietic progenitors and to EPCs [18,19]. Shintani et al. [32] showed a transient increase in CD34+ cells during an acute coronary event in humans. However, it remains unclear whether an increase in circulating CD34+ cells or EPCs occurs before the improvement in CTO-related myocardial ischemia by Waon therapy. Third, the treadmill exercise test and determination of FMD and CD34+ cells were not performed in the control group. Training effects cannot be excluded from a contribution to the improvement in exercise tolerance in the Waon group.

In conclusion, repeated Waon therapy improved CTO-related myocardial perfusion. This therapy is substantially cheaper compared to other revascularization therapies, including catheter-based myocardial gene transfection [33]. Furthermore, Waon therapy is applicable for patients who are unable to receive exercise training and patients of advanced age. Therefore, Waon therapy would be a useful complementary and alternative tool in patients with severe coronary lesions not suitable for coronary intervention.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology.

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