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Neurohumoral Modulation During Waon Therapy in Chronic Heart Failure
— Subanalysis of Waon-CHF Study —

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Background: Heart failure (HF) is a disease of neurohumoral dysfunction and current pharmacological therapies for HF have not improved mortality rates, thus requiring additional new strategies. Waon therapy for HF patients may be a complementary strategy with peripheral vasodilation via nitric oxide. We hypothesized that Waon therapy would improve neurohumoral factors, such as natriuretic peptides (NP) and the renin-angiotensin-aldosterone system (RAAS) in HF.

Methods and Results: Plasma samples were collected from patients enrolled in the WAON-CHF Study (Waon therapy (n=77) or control (n=73)) before and after the treatment. B-type NP (BNP), C-type NP (CNP), and aldosterone (Aldo) levels were measured by respective specific radioimmunoassays. Although clinical parameters significantly improved in the Waon group compared with the control group, BNP, Aldo, and CNP levels were not statistically different between groups. On subanalysis with patient variables, BNP levels were improved in the Waon group treated with angiotensin-converting enzyme inhibitor/angiotensin-receptor blocker or spironolactone. In addition, Aldo levels were improved in the Waon group patients with diabetes mellitus, hypertension, and inotrope use, and CNP levels were improved in Waon group patients with estimated glomerular filtration rate <60 mL/min/1.73 m^2. These changes were not observed in the control group.

Conclusions: Waon therapy may accelerate the favorable actions of RAAS modulators in HF. (WAON-CHF Study: UMIN000006705)

Key Words: Heart failure; Natriuretic peptides; Renin-angiotensin-aldosterone system; Waon therapy

As a cardiovascular disease syndrome, heart failure (HF) continues to grow in importance and is the common outcome of many cardiovascular diseases such as hypertension and coronary atherosclerosis. Invasive and medical therapies are associated with risks of complications and side effects, and do not improve the patient's discomfort that plays a critical role in HF. The NIH National Center for Complementary and Integrative Health has promoted the usefulness and safety of alternative interventions to improve health care. Two decades ago, repeated sauna therapy, now called Waon therapy, was developed for HF patients.[1] Patients are warmed in an evenly heated sauna bath chamber for 15 min at 60°C, resulting in reduced preload and afterload through peripheral vasodilation,[2–4] improvement of the sympathetic nervous system,[5] reduction of oxidative stress,[6] and improved endothelial function through enhancement of the nitric oxide (NO) system.[4,7–10] Indeed, it is important in the treatment of HF that both HF symptoms and quality of life are improved, which is an outcome of Waon therapy.

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In HF, the renin-angiotensin-aldosterone system (RAAS) and the natriuretic peptide (NP) system are activated to regulate sodium and water homeostasis.[11] However, imbalance of these 2 counter-regulatory systems may also cause maladaptive remodeling of the myocardium with further myocardial injury, thereby initiating a vicious cycle known as the "neurohumoral disorder" of HF. The importance of

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Table 1. Baseline Characteristics* of the HF Patients Undergoing Waon Therapy

Variable	Group distribution		P value
	Control (n=73)	Waon (n=76)	
Pre-EF <40, n (%)	15 (22)	15 (20)	0.83
Pre-eGFR, median (Q1, Q3)	62.8 (47.9, 78.9)	57.1 (42.2, 79.9)	0.33
eGFR <60 mL/min, n (%)	33 (45)	42 (57)	0.16
ACEI/ARB, n (%)	59 (81)	61 (80)	0.93
Diuretic, n (%)	72 (99)	74 (97)	0.58
Furosemide	45 (62)	49 (64)	0.72
Spironolactone	45 (62)	42 (55)	0.43
hANP	3 (4)	4 (5)	0.74
Antiarrhythmic therapy, n (%)	34 (47)	32 (42)	0.58
Anticoagulant therapy, n (%)	50 (68)	50 (66)	0.73

*Additional characteristics to the original study.[15] ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker; EF, ejection fraction; eGFR, estimated glomerular filtration rate; hANP, human atrial natriuretic peptide (carperitide); HF, heart failure.

Table 2. Circulating Neurohumoral Factors of the HF Patients Undergoing Waon Therapy

Variable	Group distribution		P value
	Control (n=73)	Waon (n=76)	
BNP level (pg/mL)			
Pre	587.00 (439.90, 949.10)	615.15 (420.95, 920.00)	0.89
Post	618.50 (358.10, 1,024.0)	579.00 (361.50, 887.10)	0.73
Change	−28.30 (−143.1, 77.50)	−47.10 (−147.0, 101.10)	0.86
Aldo level (ng/dL)			
Pre	7.10 (3.40, 18.90)	5.95 (2.50, 21.70)	0.47
Post	12.20 (3.70, 31.00)	7.95 (2.50, 26.50)	0.12
Change	0.40 (−1.70, 8.80)	0.00 (−0.70. 6.20)	0.39
CNP level (pg/mL)			
Pre	23.30 (15.30, 42.50)	25.50 (18.30, 36.00)	0.57
Post	27.20 (14.90, 42.10)	25.65 (18.10, 38.60)	0.93
Change	−0.5 (−7.40, 7.00)	0.5 (−5.70, 6.20)	0.64

Data are expressed as median (Q1, Q3). Aldo, aldosterone, BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; HF, heart failure.

these neurohumoral systems became even more critical when it was discovered that modulation of these 2 systems prolongs survival in HF such as with the newly approved drug Entresto (angiotensin-converting enzyme/neprilysin inhibitor), and that monitoring these circulating neurohormones is important in understanding the pathophysiology, diagnosis, and therapeutic strategies of HF.[11]

After confirming the safety of Waon therapy by a first-in-human study,[12] phase I and II clinical trials of Waon therapy in patients with chronic and hospitalized HF were initiated in Japan, which showed safety, and efficacy according to clinical symptoms and B-type NP (BNP) levels.[3,5,6,9,13,14] Most recently, we reported a phase III clinical trial of the safety and efficacy of Waon therapy in a multicenter, randomized study (WAON-CHF Study).[15] NYHA classification, 6-min walk distance, and cardiothoracic ratio were significantly improved in the Waon therapy group compared with the control group and BNP levels improved, but not significantly.[15] In the current study as a subanalysis of WAON-CHF Study, we sought to determine whether neurohumoral factors related to the RAAS and NP systems were modulated by Waon therapy. Specifically, we selected 3 biomarkers: BNP, which is secreted by myocardial stretch and decreases with improvement of HF,[16] aldosterone (Aldo) whose secretion is regulated by RAAS activation,[16] and C-type NP (CNP), which is secreted from endothelial cells in response to inflammatory cytokines, NO, and shear stress.[17] We hypothesized that Waon therapy as a complementary and alternative therapy would improve circulating neurohumoral factors, and that the Waon therapy neurohumoral biomarker responders would give us clues to understanding the mechanisms of action, as well as determining the target patient populations for this therapy.

Methods

WAON-CHF Study

This was a substudy analysis from the previously reported trial (WAON-CHF Study: UMIN000006705) and its study design, patients' characteristics, and key results have been previously described in detail.[15] The ethics committee of each center approved the study protocol. Written informed consent was given by all participants before enrollment.

Table 3. Response of BNP Level in Each Variable of Selected HF Patients Undergoing Waon Therapy

Variable / Level	Control group			Waon group		
	Patient no.	Adjusted mean post-BNP	P value	Patient no.	Adjusted mean Post-BNP	P value
Diabetes mellitus						
No	40	638.3 (574.6, 709.0)	0.28	54	582.4 (526.2, 644.7)	0.83
Yes	29	584.0 (516.0, 660.9)		19	595.4 (500.7, 708.1)	
Hypertension						
No	40	611.9 (547.3, 684.1)	0.9	41	624.9 (552.8, 706.5)	0.15
Yes	29	618.9 (541.6, 707.4)		32	539.2 (468.0, 621.2)	
Hyperlipidemia						
No	39	676.3 (609.8, 750.1)	0.009	45	614.2 (550.0, 685.9)	0.18
Yes	30	543.2 (482.4, 611.7)		28	542.8 (471.5, 625.0)	
Pre-EF <40						
No	51	589.2 (536.3, 647.4)	0.07	57	608.0 (550.6, 671.3)	0.11
Yes	15	714.7 (597.0, 855.6)		14	502.5 (407.5, 619.6)	
Pre-eGFR <60						
No	37	647.4 (578.8, 724.2)	0.2	29	605.5 (521.7, 702.7)	0.31
Yes	32	579.2 (513.2, 653.8)		42	545.1 (482.9, 615.1)	
ACEI/ARB						
No	13	561.0 (463.8, 678.7)	0.29	15	765.5 (639.2, 916.7)	0.001
Yes	56	628.1 (574.5, 686.7)		58	546.6 (499.2, 598.5)	
Furosemide						
No	25	673.4 (590.4, 768.0)	0.09	25	558.5 (478.6, 651.8)	0.46
Yes	44	583.9 (529.0, 644.5)		48	600.5 (538.4, 669.8)	
Spironolactone						
No	27	671.8 (588.3, 767.2)	0.1	32	643.0 (563.3, 734.0)	0.07
Yes	42	580.8 (523.2, 644.8)		41	544.7 (485.0, 611.7)	
Inotropes						
No	56	609.9 (556.5, 668.4)	0.71	64	588.1 (535.7, 645.6)	0.82
Yes	13	636.6 (518.2, 782.2)		9	569.8 (439.2, 739.2)	

Data are presented as mean (95% CI) after adjustment for age, sex, and pretreatment BNP levels. CI, confidence interval. Other abbreviations as in Tables 1,2.

Data and Sample Collection

On the day before and after the 2-week Waon therapy (or observation in the control group), blood sampling and echocardiography were performed. Blood samples were immediately placed on ice, processed, and stored at −80°C until analysis. Myocardial function and dimensions were assessed by echocardiography using standard 2D and flow Doppler techniques. Estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) was calculated by the Modification of Diet in Renal Disease formula.[18]

Assays

Plasma BNP levels (pg/mL) were measured using EDTA-plasma samples by a 2-site immunoenzymatic sandwich assay on a Beckman Coulter DXI 800 (Beckman Coulter, Inc., USA). Plasma Aldo level (ng/dL) was measured using a competitive radioimmunoassay kit (Coat-a-Count kit, Siemens, Los Angeles, CA, USA). The inter- and intra-assay variability was 16% and 5.8%, respectively. There was no cross-reactivity with other related steroids.[19,20] Plasma CNP22 level (pg/mL) was determined using a nonequilibrium radioimmunoassay (Phoenix Pharmaceutical, Burlingame, CA, USA), using an antibody that detects human CNP22 as previously described.[21] Inter- and intra-assay variability was 11% and 5% respectively. Recovery was 85%. Cross-reactivity was 0% with ANP, BNP, endothelin, and NT-CNP53, and 59% with CNP53.

Statistical Analysis

For continuous parameters, data are summarized as mean±SD or median (Q1, Q3, depending on the distribution). Categorical parameters are summarized as number and percentage. For comparison between groups, 2-sample t-test or nonparametric Wilcoxon rank-sum test was used for continuous parameters and Pearson chi-square test was used for categorical parameters.

For comparison of post-treatment values between subgroups of interest, analysis of covariance was used. In this model, post-treatment values were modeled, after log transformation, as a function of age, sex, and pretreatment value (also log transformed), and a parameter was entered for the subgroup of interest. Using this method, we obtained unbiased estimates of post-treatment values for each subgroup. These post-treatment values were back-transformed from the log scale and are presented with corresponding 95% confidence intervals. All statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC, USA). Statistical tests were 2-tailed, and a P-value <0.05 was considered significant.

Table 4. Response of Aldo Level in Each Variable of Selected HF Patients Undergoing Waon Therapy

Variable / Level	Control group			Waon group		
	Patient no.	Adjusted mean post-Aldo	P value	Patient no.	Adjusted mean post-Aldo	P value
Diabetes mellitus						
No	34	12.3 (9.0, 16.9)	0.87	51	9.7 (8.4, 11.1)	0.03
Yes	25	11.9 (8.2, 17.2)		19	7.0 (5.5, 9.0)	
Hypertension						
No	33	12.0 (8.4, 17.0)	0.93	39	10.3 (8.6, 12.3)	0.03
Yes	26	12.3 (8.2, 18.5)		31	7.4 (6.0, 9.1)	
Hyperlipidemia						
No	35	11.3 (8.3, 15.5)	0.5	42	9.1 (7.7, 10.7)	0.61
Yes	24	13.4 (9.2, 19.7)		28	8.5 (6.9, 10.4)	
Pre-EF <40						
No	43	13.9 (10.5, 18.5)	0.06	54	8.2 (7.1, 9.5)	0.3
Yes	14	7.8 (4.7, 13.0)		14	9.8 (7.2, 13.2)	
Pre-eGFR <60						
No	31	13.4 (9.5, 18.8)	0.42	28	8.2 (6.6, 10.3)	0.68
Yes	28	10.9 (7.6, 15.6)		40	8.8 (7.3, 10.6)	
ACEI/ARB						
No	13	13.9 (8.4, 23.3)	0.54	15	11.1 (8.3, 14.8)	0.09
Yes	46	11.7 (8.9, 15.3)		55	8.3 (7.2, 9.6)	
Furosemide						
No	20	9.2 (6.1, 13.9)	0.11	24	8.7 (7.5, 10.1)	0.64
Yes	39	14.0 (10.4, 18.6)		46	9.3 (7.2, 12.1)	
Spironolactone						
No	23	9.6 (6.4, 14.5)	0.17	32	8.0 (6.6, 9.8)	0.2
Yes	36	14.1 (10.2, 19.3)		38	9.6 (8.1, 11.5)	
Inotropes						
No	49	11.5 (8.8, 15.0)	0.37	60	9.4 (8.3, 10.8)	0.03
Yes	10	15.9 (8.3, 30.6)		10	6.1 (4.3, 8.6)	

Data are presented as mean (95% CI) after adjustment for age, sex, and pretreatment Aldo levels. Aabbreviations as in Tables 1–3.

Results

Patients' Characteristics and Circulating Levels of Neurohumoral Factors

Table 1 shows the patients' characteristics which are additionally used for the current analysis from a previous report,[15] including detailed information on medications that possibly affected circulating neurohumoral factors, such as angiotensin-converting enzyme inhibitor (ACEI)/angiotensin-receptor blocker (ARB), diuretics, human recombinant atrial NP (hANP, carperitide), and β-blockers. There was no significant difference between the groups with regard to these medications.

Circulating BNP, CNP, and Aldo levels pre- and post-treatments or changes in both groups are shown in **Table 2**. Pretreatment BNP and CNP levels tended to be higher and Aldo levels tended to be lower, while post-treatment BNP, CNP and Aldo levels were lower in the Waon group compared with the control group, but none were significant after the 2-week Waon therapy. The change in BNP between pre- and post-treatment tended to be greater in the Waon group compared with the controls, but again this was not significant. The change between pre- and post-treatment for both CNP and Aldo were not significantly different between the groups. Therefore, no significant improvements in circulating neurohumoral factors were seen in either group.

Responders According to Circulating BNP Levels

Because all patients remained hospitalized and continued optimal medical therapy during the study, we performed analyses to determine whether Waon therapy may have had differing effects on the circulating levels of neurohumoral factors in specific subgroups. **Tables 3–5** show selected results, including the significant changes, and all analyzed data are shown in **Tables S1–S3**. Data in **Tables 3–5** were adjusted by age, sex, and each pretreatment circulating level. We tried to adjust for ACEI/ARB because the majority of patients in each group took these medications, however, there was no significant change with the adjustment (data not shown).

Table 3 presents the adjusted post-BNP levels for subgroups based on patients' characteristics for both the Waon and control groups. In the control group, patients with hyperlipidemia had a significant improvement in post-BNP levels compared with non-hyperlipidemia (P=0.009) patients, which also tended to improve in the Waon group, but not significantly (P=0.18). Patients taking anticoagulant agents had reduced post-BNP levels compared with patients without such treatments in the control group (P=0.02), but there was no difference in post-BNP levels with or without anticoagulants in the Waon group. In the Waon group, post-BNP levels were significantly lower in patients taking ACEI/ARB (P=0.001), and tended to be lower with spironolactone (P=0.07) compared with

Table 5. Response of CNP Level in Each Variable of Selected HF Patients Undergoing Waon Therapy

Variable / Level	Control group			Waon group		
	Patient no.	Adjusted mean post-CNP	P value	Patient no.	Adjusted mean post-CNP	P value
Diabetes mellitus						
No	34	24.8 (20.8, 29.5)	0.2	51	27.0 (24.4, 29.9)	0.41
Yes	25	29.5 (24.1, 36.2)		19	29.3 (24.8, 34.7)	
Hypertension						
No	33	28.3 (23.3. 34.4)	0.43	39	28.7 (25.4, 32.4)	0.39
Yes	26	24.9 (19.9, 31.1)		31	26.3 (22.9, 30.2)	
Hyperlipidemia						
No	35	25.7 (21.5, 30.7)	0.51	42	28.6 (25.6, 32.0)	0.31
Yes	24	28.3 (22.8, 35.1)		28	26.1 (22.7, 30.0)	
Pre-EF <40						
No	43	27.7 (23.6, 32.5)	0.4	54	28.6 (25.6, 32.0)	0.26
Yes	14	24.0 (18.0, 32.0)		14	24.9 (20.3, 30.5)	
Pre-eGFR <60						
No	31	25.2 (20.8, 30.5)	0.4	28	32.4 (28.2, 37.4)	0.004
Yes	28	28.5 (23.3, 34.9)		40	24.2 (21.6, 27.2)	
ACEI/ARB						
No	13	27.0 (20.1, 36.3)	0.93	15	29.2 (24.1, 35.3)	0.52
Yes	46	26.6 (22.9, 31.0)		55	27.2 (24.6, 30.0)	
Furosemide						
No	20	24.1 (19.1, 30.3)	0.27	24	28.1 (24.0, 32.9)	0.17
Yes	39	28.2 (23.9, 33.2)		46	27.3 (24.5, 30.5)	
Spironolactone						
No	23	24.6 (19.6, 31.0)		32	28.4 (25.0, 32.3)	
Yes	36	28.1 (23.5, 33.7)	0.39	38	26.9 (23.9, 30.3)	0.55
Yes	9	25.7 (18.0, 36.6)		10	23.2 (18.2, 29.6)	
Inotropes						
No	49	26.3 (22.6, 30.6)	0.64	60	27.4 (24.9, 30.1)	0.66
Yes	10	29.0 (20.1, 41.8)		10	29.1 (22.7, 37.2)	

Data are presented as mean (95% CI) after adjustment for age, sex, and pretreatment CNP levels. Aabbreviations as in Tables 1–3.

patients not taking these drugs, which was not seen in the control group.

Taken together, the results suggested that hospitalized patients with hyperlipidemia were improved only by optimal medication, although Waon therapy may accelerate the "preferable" effects of RAAS inhibitors, suggesting Waon therapy may have a beneficial effect on the RAAS system to improve HF.

Responders According to Circulating Aldo Levels

Next, we investigated RAAS activation according to circulating Aldo levels in the same subgroups. **Table 4** shows the post-Aldo levels by subgroup based on patients' characteristic for both Waon and control groups. Overall, post-Aldo levels in the Waon group tended to be lower than in the control groups. In the control group, HF patients with reduced ejection fraction (EF) tended to have lower post-Aldo levels than HF patients with preserved EF (P=0.06), and patients taking antiarrhythmic drugs had significantly higher post-Aldo levels than those not taking these drugs (P=0.04), which was not seen in the Waon group. In the Waon group, patients with diabetes mellitus (P=0.03), hypertension (P=0.03), and inotropes (P=0.03) had significantly lower post-Aldo levels than those without these characteristics.

Taken together, the results suggested that Waon therapy may play a role in suppressing the RAAS system in patients with a background of endothelial damage or who are under inotrope therapy.

Responders According to Circulating CNP Levels

Finally, we examined an endothelial marker, CNP, in the subgroup analyses. **Table 5** presents the post-treatment CNP levels for each of the subgroups of interest. In the control group, there were no significant differences between any of the subgroups. Interestingly, patients with eGFR <60 mL/min/1.73 m^2 had significantly lower post-CNP levels in the Waon group (P=0.004) compared with eGFR ≥60 mL/min/1.73 m^2, suggesting that Waon therapy may be useful for patients with renal impairment to improve endothelial injury/function.

Discussion

This study was a subanalysis of a phase III clinical trial with Waon therapy to demonstrate its actions on the neurohumoral factors, BNP, Aldo and CNP. Although Waon therapy for 2 weeks tended to lower the circulating levels of these neurohumoral factors, significance was not achieved compared with the control group. However, Waon therapy did have significant beneficial actions on the NP-RAAS system in subgroups of patients.

Several neurohumoral actions of a complementary medicine strategy with sauna treatment have been reported. Neurohumoral activation, including increased catecholamines, renin, and Aldo levels by exercise-induced dehydration, was not observed with a single heat-induced dehydration.[22] Other reports studying a single Finnish sauna (92°C) ≤60 min therapy showed conflicting neurohumoral activation with no change in healthy volunteers,[23] and decreased ANP levels in children,[24] but increased Aldo, renin, and ANP levels in male healthy volunteers.[25] For the treatment of HF, the temperature and duration of therapy are thought to be important in the activation of these systems. In 1991, we reported the first pilot chronic sauna study, which is now called Waon therapy, for HF patients. With Waon therapy, the sauna temperature is set at 60°C for 15 min, and performed 3 times per week for 4 weeks, which has resulted in decreased noradrenaline and a trend to decreasing ANP levels.[12] After confirmation of the safety of the treatment in terms of neurohumoral action, phase I and II clinical trials of Waon therapy for 2–4 weeks in patients with chronic, hospitalized HF were initiated in Japan, which showed safety, and efficacy according to the clinical symptoms and reduced BNP levels.[3,5,6,9,13,14]

With regard to pathophysiological mechanisms, Waon therapy may play an important role in endothelial function. In animal models, repeated sauna therapy increased eNOS expression and NO synthesis from the aorta of HF hamsters,[4] upregulated the heat shock protein 90/Akt/eNOS pathway and induced angiogenesis in mice with hindlimb ischemia,[7] and decreased cardiac hypertrophy and fibrosis with reduction of ANP and BNP mRNA levels and activation of the eNOS system in salt-induced hypertensive rats.[8] In addition, Waon therapy improved the exercise tolerance and endothelial function according to flow-mediated dilation tests, and improved BNP and norepinephrine levels in patients with HF,[9] and increased NO production in patients with peripheral arterial disease.[10]

Therefore, we anticipated Waon therapy would also reduce circulating BNP levels through improvement of endothelial function in this phase III trial. However, after 2 weeks of Waon therapy, there were no significant differences in BNP levels compared with the control group, although BNP levels in the Waon group tended to improve, and other clinical parameters were significantly improved.[15] Also, because there is cross-talk between the NO and RAAS systems, and RAAS is upregulated by endothelial dysfunction, and anti-RAAS drugs enhance the eNOS system,[26] we hypothesized that both the NP and RAAS systems would be improved by Waon therapy. However, the results did not show significant changes in either the NP or RAAS systems. Notably, the current HF population in this phase III trial had the highest pre-BNP levels (≈600 pg/mL in each group) compared with previously published clinical trials of Waon therapy,[3,5,6,9,13–15] suggesting a relatively severe group was investigated. Further, successful Waon therapy clinical trials that recorded pre-BNP >500 pg/mL were performed for 3 or 4 weeks of treatment, so more severe HF patients as in the current phase III trial may require longer treatment periods. We also speculate that possible explanations for the current observations include: (1) the enrolled population was insufficient in number to reach significance, and (2) the targeted population again was not optimal. Therefore, we performed subgroup analyses to define responders according to the neurohumoral biomarkers.

In the subgroup analyses, we found "responders" for the neurohumoral biomarkers based on additional patient variables. First, according to BNP levels, patients treated with anti-RAAS drugs such as ACEI, ARB or spironolactone experienced more favorable effects with Waon therapy, suggesting Waon therapy may enhance ACEI/ARB or spironolactone's actions in HF. Notably, there has not been a clinical trial that has shown the 2 weeks of ACEI/ARB treatment improved hospitalized HF. Secondly, RAAS activation according to Aldo levels was more improved in patients with diabetes mellitus, hypertension, and inotrope usage, suggesting Waon therapy could be effective in the setting of "endothelial injury" or neurohumoral activation by inotropes. Finally, endothelial impairment according to CNP levels was more improved in patients with renal impairment. CNP production is stimulated by endothelial damage by pro-inflammatory cytokines or shear stress,[17] so a high circulating levels can predict the risk of myocardial infarction,[21] and CNP plays a role in coronary relaxation via NO system,[27] suggesting a potential link to NO and endothelial injury in which Waon therapy is also involved. Therefore, we speculate patients with renal impairment would be a potential target for Waon therapy.

However, there are some discrepancies between the BNP and Aldo data in the subanalyses that should be discussed. Aldo levels in the ACEI/ARB groups showed a trend to be lower but not significant, whereas BNP levels decreased. This lack of change in the Aldo levels may be explained by "Aldo escape", the phenomenon of increased plasma levels of Aldo after chronic administration of ACEI[28,29] or ARB.[30] We speculate that Aldo escape in the ACEI/ARB group may have contributed to the greater variability in the Aldo data. In terms of diabetes or hypertension, BNP did not change but Aldo decreased. BNP levels trended to be lower in hypertension but there was no trend in diabetes. We have reported that the power of BNP to predict the risk of death in 7 years is weakened by additional adjustment for diabetes and hypertension,[31] suggesting that BNP level in diabetes and hypertension may not be a sensitive predictor with these comorbidities. Although there is no study focusing on the diagnostic power of BNP in diabetes or hypertension, we speculate it is less responsive to the RAAS in these comorbidities. Because this study was not designed to clarify the pathophysiological mechanism of Waon therapy, we can only speculate on the mechanism behind the data, so further study is warranted.

Why did the current phase III not show the "significant" improvement in BNP levels that phase I and II clinical trials demonstrated? We speculate that longer Waon therapy for up to 4 weeks may be needed to improve BNP levels in all patients, as was done in the phase I and II trials. There has not been a successful clinical trial of short-term treatment for HF. To perform a longer than 4 week clinical trial with Waon therapy, we would need to consider the feasibility of a multicenter worldwide trial, and the possibility of performing an outpatient trial, or developing a more compact sauna system.

Study Limitations

Specifically, the sample size for the subgroup analysis was relatively small, and we need further study with larger populations to confirm our theory. Also, we may add bio-

markers related to the sympathetic nervous system, which Waon therapy may improve[5] with relationship to NPs and RAAS in the future study.

Conclusions

Waon therapy is a noninvasive, complementary, and alternative therapy that may contribute to vascular/endothelial relaxation. Although Waon therapy for 2 weeks did not improve circulating neurohumoral factors directly, it may improve endothelial injury and the RAAS and thus accelerate the favorable actions of RAAS inhibitors in HF patients, especially those with comorbidities. Further investigation is warranted, focusing on pathophysiology and the subgroups of "responders".

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Relationship With Industry

None.

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Appendix

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Supplementary Files

Supplementary File 1

Table S1. Response of BNP level in each variable of HF patients undergoing Waon therapy

Table S2. Response of aldo level in each variable of HF patients undergoing Waon therapy

Table S3. Response of CNP level in each variable of HF patients undergoing Waon therapy

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