Circulation Journal
Official Journal of the Japanese Circulation Society
http://www.j-circ.or.jp

Effect of Waon Therapy on Oxidative Stress in Chronic Heart Failure

Shoji Fujita, MD; Yoshiyuki Ikeda, MD; Masaaki Miyata, MD; Takuro Shinsato, MD; Takuro Kubozono, MD; So Kuwahata, MD; Narisato Hamada, MD; Takahiro Miyauchi, MD; Tsuyoshi Yamaguchi, MD; Hiroyuki Torii, MD; Shuichi Hamasaki, MD; Chuwa Tei, MD

Background: A previous report by our team showed that Waon therapy, using a far infrared-ray dry sauna at 60°C, improves cardiac and vascular function in patients with chronic heart failure (CHF). The purpose of the present study was to clarify the effect of Waon therapy on oxidative stress in CHF patients and investigate its mechanism by animal experiments.

Methods and Results: Forty patients with CHF were divided into control (n=20) and Waon therapy (n=20) groups. All patients received standard optimal medications for CHF. Waon therapy group was treated with Waon therapy daily for 4 weeks. After 4 weeks of Waon therapy, concentrations of hydroperoxide and brain natriuretic peptide (BNP) decreased significantly (hydroperoxide, 422±116 to 327±88 U.CARR, P<0.001; BNP, 402±221 to 225±137 pg/ml, P<0.001), and the nitric oxide metabolites increased (71.2±35.4 to 92.0±40.5 mmol/L, P<0.05). In contrast, none of these variables changed over the 4-week interval in the control group. Furthermore, animal experiments were performed using TO-2 cardiomyopathic hamsters. On immunohistochemistry, cardiac expression of 4-hydroxy-2-nonenal, a marker of oxidative stress, was decreased in the 4-week Waon therapy compared to untreated hamsters. On Western blotting, cardiac expressions of heat shock protein (HSP) 27, manganese superoxide dismutase and HSP32, which reduce oxidative stress, were significantly upregulated in the 4-week Waon therapy compared to untreated hamsters.

Conclusions: Waon therapy decreases oxidative stress in patients and hamsters with heart failure. (*Circ J* 2011; **75:** 348–356)

Key Words: Heart failure; Heat shock protein; Oxidative stress; Waon therapy

We developed a form of thermal therapy for heart failure in 1989; it uses a dry sauna with a temperature maintained at 60°C, which differs from the traditional sauna. In 2007, we changed the name to Waon therapy[1] ("*Wa*" means soothing, and "*On*" means warmth); hence Waon infers a warmth that comfortably refreshes the mind and body. Waon therapy is defined as warming the entire body in a uniformly heated chamber for 15 min at a temperature that relaxes the mind and body. After the core temperature has increased by 1.0–1.2°C, the patient rests outside the sauna for a further 30 min to maintain the soothing effect, and fluids corresponding to perspiration are supplied to protect against dehydration at the end of the therapy.

We discovered that this new thermal therapy is very beneficial for patients with cardiovascular diseases, including chronic heart failure (CHF)[2–9] and peripheral artery disease,[10,11] as well as lifestyle-related diseases.[12,13] Waon therapy improves cardiac function, neurohormonal factors, ventricular arrhythmias, prognosis, and vascular endothelial function in patients with CHF. We later demonstrated that the molecular mechanism by which Waon therapy improves vascular endothelial function involves increased endothelial nitric oxide (NO) synthase (eNOS) expression.[14]

Oxidative stress is elevated in CHF as a result of increased production of free radical species capable of attacking lipids, proteins, and nucleic acids. Chronic increases in oxygen radical production in the mitochondria can lead to a catastrophic cycle of mitochondrial DNA damage, as well as functional decline, further oxygen radical generation, and cellular injury in heart failure.[15] The degree of oxidative stress is linked to

Received June 29, 2010; revised manuscript received October 5, 2010; accepted October 6, 2010; released online December 14, 2010
Time for primary review: 6 days

Department of Cardiovascular, Respiratory and Metabolic Medicine, Graduate School of Medicine, Kagoshima University, Kagoshima (S.F., Y.I., M.M., T.S., T.K., S.K., N.H., T.M., S.H., C.T.); Kagoshima City Medical Association Hospital, Kagoshima (T.Y., H.T.), Japan

The first two authors contributed equally to this work (S.F., Y.I.).

Mailing address: Chuwa Tei, MD, Department of Cardiovascular, Respiratory and Metabolic Medicine, Graduate School of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan. E-mail: tei@m.kufm.kagoshima-u.ac.jp

ISSN-1346-9843 doi:10.1253/circj.CJ-10-0630

the severity of heart failure.[16–18] Administration of angiotensin II receptor blockers (ARB), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins), vitamin C, β-blockers, allopurinol and growth hormone-releasing peptide reduces oxidative stress and improves cardiac and vascular function in CHF.[19–23] Furthermore, exercise training decreases oxidative stress in CHF.[24]

We have reported that 2 weeks of Waon therapy decreased urinary concentrations of 8-epi-PGF$_{2\alpha}$, a marker of oxidative stress, in patients with at least one atherosclerotic risk factor.[13] However, the effect of Waon therapy on oxidative stress in CHF has not been elucidated. The purpose of the present study was to clarify the effect of Waon therapy on oxidative stress in CHF patients and address its mechanism using cardiomyopathic hamsters with heart failure.

Methods

Clinical Study

Participants and Study Design The study participants included 40 CHF patients who were admitted to Kagoshima University Hospital or Kagoshima City Medical Association Hospital between 2006 and 2009.

The inclusion criteria were the presence of symptomatic CHF, left ventricular ejection fraction (LVEF) <50% on echocardiography, and New York Heart Association (NYHA) functional classes II or III. Exclusion criteria were the presence of severe aortic stenosis, severe obstruction with hypertrophic obstructive cardiomyopathy, and NYHA functional class IV. Also excluded were patients who changed medications, such as angiotensin-converting enzyme inhibitors, ARB, β-blockers, statins, and allopurinol, because changes in medications might affect the oxidative stress.

All patients were treated with standard optimal therapy for at least 1 week after admission, and then were randomized to the Waon therapy group (n=20) or the control group (n=20). All patients continued optimal treatment for heart failure for an additional 4 weeks. The patients in the Waon therapy group received Waon therapy once a day, 5 days a week, for 4 weeks. The patients in the control group continued conventional treatment for 4 weeks.

All examinations were performed at baseline and on the next day after the last treatment of 4 weeks.

Informed consent was obtained from all patients prior to participation, and the protocol was approved by the Ethics Committee of the Faculty of Medicine, Kagoshima University.

Waon Therapy Waon therapy uses a far infrared-ray dry sauna, which is evenly maintained at 60°C and differs from the traditional sauna. Waon therapy has no hydration pressure, and was performed as previously reported.[2] The patients were placed in a 60°C sauna system for 15 min and then, 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 they drank water at the end of the Waon therapy to compensate for weight lost due to perspiration.

Physical Examination and Cardiac Function The body weight, heart rate (HR), systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at baseline and 4 weeks after treatment. The cardiothoracic ratio (CTR) determined by chest radiography and LVEF evaluated by echocardiography were measured at baseline and 4 weeks after treatment.

Laboratory Examination A fasting plasma blood sample was taken in the morning to measure plasma concentrations of brain natriuretic peptide (BNP), uric acid, hydroperoxide, nitrate, and nitrite at baseline and 4 weeks after treatment. Plasma BNP concentrations were measured with chemiluminescent enzyme immunoassay using a commercially available kit (PATHFAST, Mitsubishi Chemical Medience Co, Ltd, Tokyo, Japan). Serum uric acid concentrations were measured using an automated technique based on the uricase/pod method (Roche Diagnostics Co, Ltd, Basel, Switzerland), implemented in an autoanalyzer (Modular Analytics, Roche Diagnostics Co, Ltd). Concentrations of plasma hydroperoxide, which is an index of oxidative stress, were measured using the Free Radical Analytical System (FRAS, Diacron, Grosseto, Italy).[25,26] Plasma concentrations of nitrate and nitrite were measured using a high performance liquid chromatography-Griess system.[27]

Animal Experiments

Animals Male TO-2 cardiomyopathic hamsters (Bio Breeders, Fitchburg, MA, USA) were used as a model of clinical dilated cardiomyopathy. This hamster develops heart failure, which is characterized by symptoms such as general edema and pleural effusion, at around 30 weeks of age and dies within a year.[28,29] All hamsters were allowed access to food and water ad libitum and were maintained under controlled environmental conditions (24°C, 12-h light/dark cycles). This study was performed in accordance with the Guide for Animal Experimentation at the Faculty of Medicine at Kagoshima University.

Experimental Protocol Thirty-week-old TO-2 hamsters were divided into Waon therapy and untreated groups (n=11 per group). Hamsters in the Waon therapy group underwent Waon therapy once a day, 5 days per week, for 4 weeks, whereas TO-2 hamsters in the untreated group did not receive any treatments. At 34 weeks of age, hemodynamic parameters were measured, and the TO-2 hamsters in both groups were sacrificed for immunohistochemistry (n=3 per group), enzyme-linked immunosorbent assay (ELISA) and Western blotting (n=8 per group). The hearts of the TO-2 hamsters were excised, rapidly frozen, and stored at –80°C. All examinations were performed on the next day after the last treatment of 4 weeks.

Waon Therapy for TO-2 Hamsters Waon therapy was performed using an experimental far infrared-ray dry sauna system at 39°C for 15 min followed by 30°C for 20 min as reported previously.[14] In this setting, their core temperatures were increased by 1°C and remained elevated for 20 min as shown in the clinical setting.[2]

Measurements of Cardiac Function In order to estimate the effect of Waon therapy on cardiac function in TO-2 hamsters, left ventricular (LV) +dP/dt and % fractional shortening (%FS) were measured at the age of 34 weeks as described previously.[14,30] Millar catheter pressure transducers (Millar Instruments, Houston, TX, USA), which were cannulated into the right carotid artery, and echocardiography (Toshiba SSH-380A Power Vision, Toshiba Medical System, Tokyo, Japan) were used for the measurements.

Immunohistochemistry The labeled streptavidin biotin method was performed using a Histfine kit (Nichirei, Tokyo, Japan) for immunohistochemistry as previously described.[14] Briefly, cross-sections of hearts were incubated overnight with mouse monoclonal antibodies specific for 4-hydroxy-2-nonenal (4-HNE; Oxis, Foster City, CA, USA), which is a marker of oxidative stress.[20,29,31] The specimens were then incubated with biotinylated anti-mouse IgG. They were developed with

Table 1. Patient Characteristics

	Waon therapy (n=20)	Control (n=20)	P value
Mean age, years	64±14	65±13	0.77
Male, %	85	80	0.68
NYHA functional class (II/III)	17/3	18/2	0.63
Diagnosis, %			
Dilated cardiomyopathy	50	45	0.75
Ischemic cardiomyopathy	30	35	0.74
Valvular heart disease	10	5	0.55
Others	10	15	0.63
AF, %			
Chronic AF	15	20	0.68
Paroxymal AF	30	10	0.11
Medication, %			
ACE inhibitor/ARB	100	95	0.31
β-blocker	90	95	0.55
Statin	20	30	0.47
Allopurinol	25	20	0.70
Calcium channel blocker	15	15	1.00

NYHA, New York Heart Association; AF, atrial fibrillation; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

diaminobenzidine and counterstained with hematoxylin.

ELISA We assayed the concentration of cardiac 4-HNE in TO-2 hamsters using OxiSelect HNE-His Adduct ELISA Kit (Cell Biolabs, Inc, San Diego, CA, USA) according to the protocol supplied with the kit.[32] In brief, 100 μl of the 10 μg/ml protein samples from whole hearts of TO-2 hamsters were probed with an anti-4-HNE antibody and absorbed on a microplate reader using 450 nm as the primary wavelength.

Western Blotting Western blotting was performed as described previously.[14] In brief, 10 μg of protein samples from whole hearts of TO-2 hamsters were detected with the NuPAGE Electrophoresis System (NOVEX, San Diego, CA, USA) using rabbit polyclonal heat shock protein (HSP) 27, HSP32, manganese superoxide dismutase (Mn-SOD), copper/zinc-SOD (Cu/Zn-SOD) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), p38 mitogen-activated protein kinase (p38MAPK), and phosphorylated p38MAPK (p-p38MAPK) antibodies (Cell signaling technology, Danvers, MA, USA). HSP27 and HSP32 are induced by several stimuli, including heat stimulation, and reduce oxidative stress.[33–37] Band density was determined by densitometry using NIH image software. Results were expressed as the ratio of the density of specific bands to the corresponding β-actin.

Table 2. Changes in Clinical Variables at Baseline and After 4 Weeks

	Waon therapy (n=20)			Control (n=20)			Comparison at baseline P value
	Baseline	After 4 weeks	P value	Baseline	After 4 weeks	P value	
BW, kg	58.2±16.5	57.6±16.1	<0.05	56.9±9.1	56.3±8.7	0.44	0.76
HR, beats/min	78±12	71±11	0.058	70±12	70±10	0.85	0.065
SBP, mmHg	103±18	96±21	0.12	108±15	112±14	0.22	0.33
DBP, mmHg	61±11	56±14	0.082	65±11	69±10	0.18	0.25
CTR, %	56.3±6.2	53.0±5.6	<0.001	53.5±6.4	53.4±6.1	0.79	0.17
LVEF, %	31.8±11.3	35.8±13.1	<0.01	34.3±7.0	36.5±10.0	0.25	0.27
BNP, pg/ml	402±221	225±137	<0.001	374±235	362±291	0.85	0.70
UA, mg/dl	6.65±1.88	6.24±1.51	0.15	6.98±1.28	7.15±1.61	0.55	0.83

BW, body weight; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; CTR, cardiothoracic ratio; LVEF, left ventricular ejection fraction; BNP, brain natriuretic peptide; UA, uric acid.

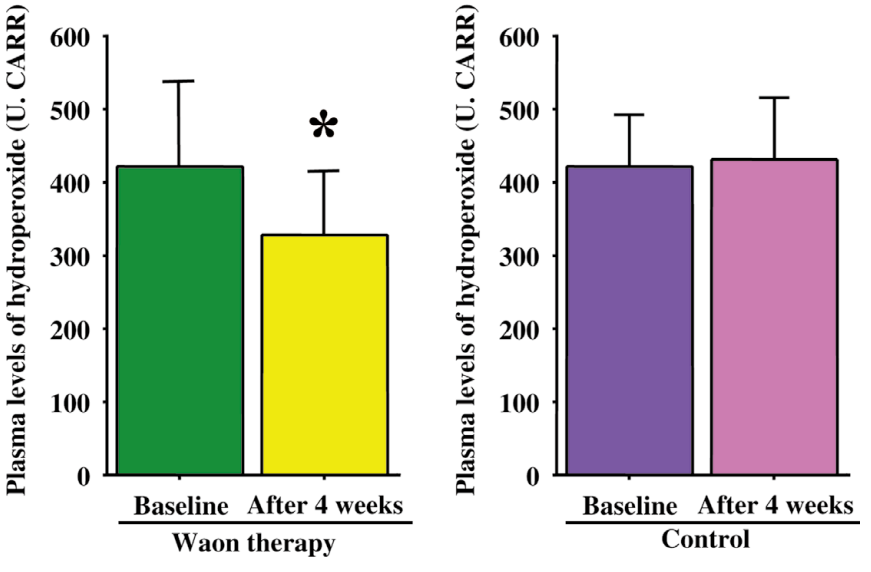

Figure 1. Changes in oxidative stress after 4 weeks of Waon treatment. The plasma concentrations of hydroperoxide decreased significantly after 4 weeks of Waon therapy. *P<0.001 vs. baseline.

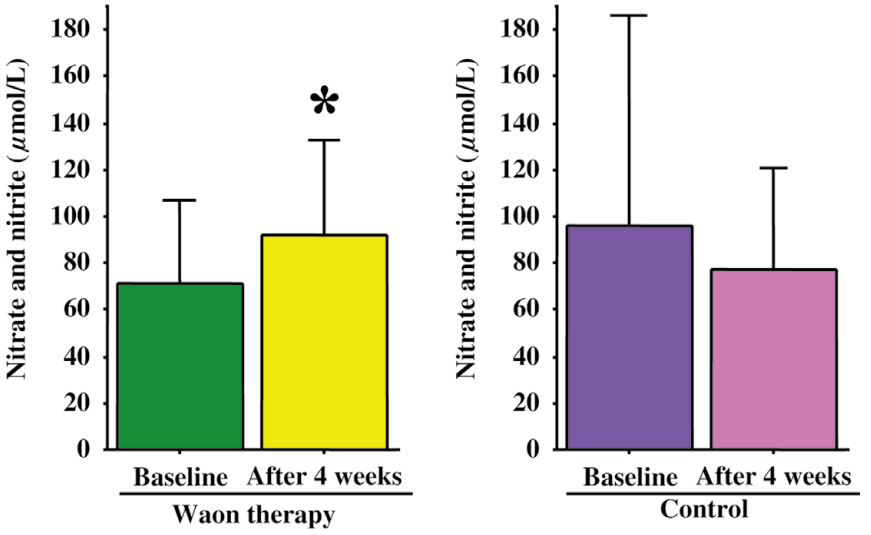

Figure 2. Changes in nitric oxide metabolites after 4 weeks of treatment. The plasma concentration of nitric oxide metabolites increased significantly in the Waon therapy group, but did not change in the control group. *P<0.05 vs. baseline.

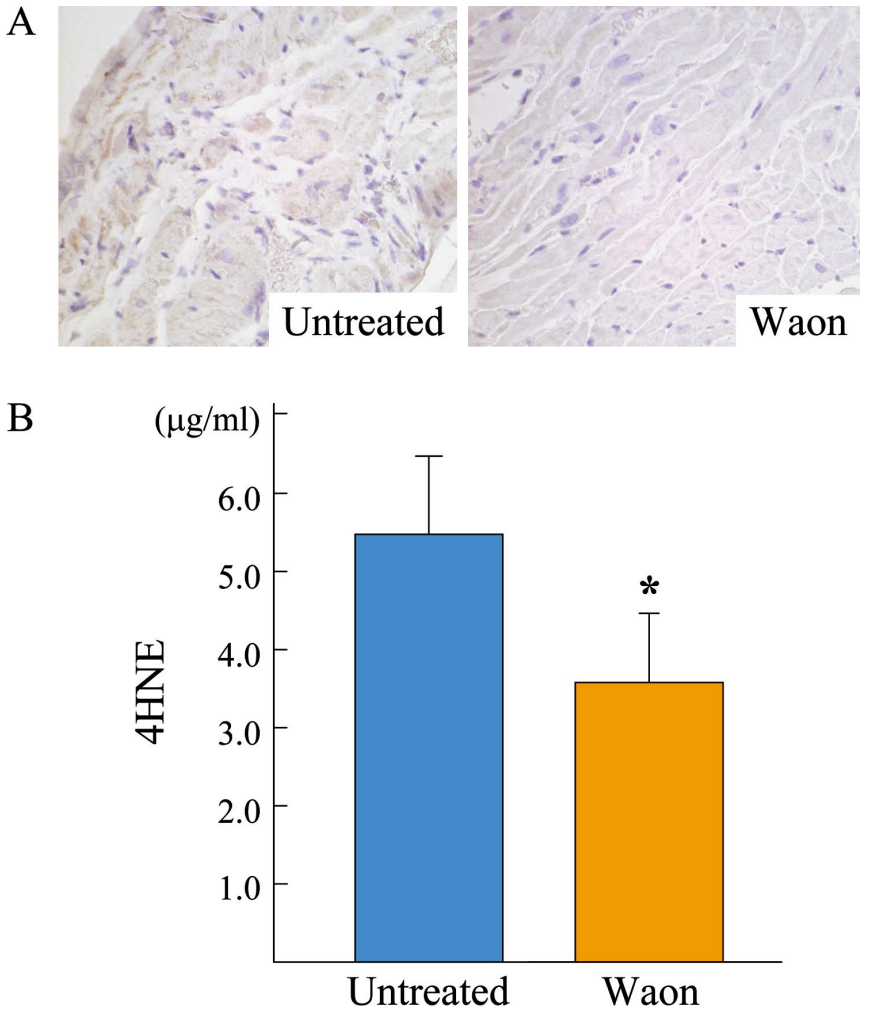

Figure 3. Effects of Waon therapy on the expression of 4-HNE in hearts. (**A**) Immunoreactivity of 4-hydroxy-2-nonenal (4-HNE) decreased in hearts of TO-2 hamsters treated by 4-week Waon therapy compared to the untreated hamsters. (**B**) ELISA revealed that 4-week Waon therapy decreased the concentration of cardiac 4-HNE of TO-2 hamsters significantly compared to untreated hamsters (Waon therapy group, 3.57±0.96 vs. untreated group, 5.53±1.17 μg/ml, P< 0.05, n=8 per group).

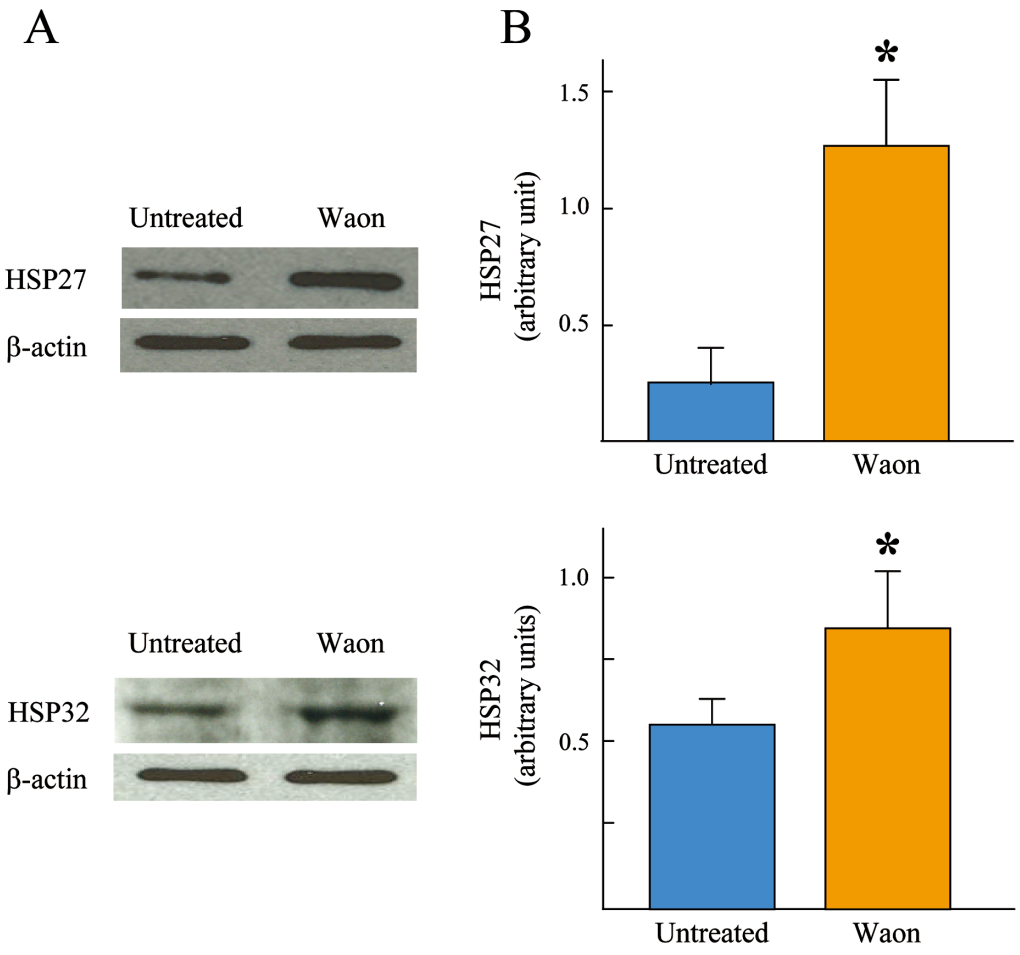

Figure 4. Effects of Waon therapy on the cardiac expressions of HSP27 and HSP32 assessed by Western blotting. (**A**) The representative Western blotting bands of HSP27 and HSP32 expressions in the whole hearts of TO-2 hamsters are shown. (**B**) Cardiac expressions of HSP27 and HSP32 in TO-2 hamsters increased significantly after 4 weeks of Waon therapy compared to those in the untreated group. *P<0.01 vs. untreated hamsters, n=8 per group.

Statistical Analysis

Values are expressed as means±SD. Statistical analysis was performed using Stat View Version 5.0 software. Comparisons of baseline clinical characteristics between the 2 groups were performed using Pearson's chi-square test or Student's unpaired t-test. Within-group changes between before and 4 weeks after treatment were evaluated by paired t-tests. In the animal experiments, the results of the 2 groups were compared by Student's unpaired t-test. Statistical significance was accepted when the P-value was <0.05.

Results

Clinical Examination

Patient Characteristics The patients' baseline characteristics are shown in Table 1. There were no significant differences in age, gender, NYHA functional class, causative heart diseases, atrial fibrillation and medication between the 2 groups at baseline. In addition, as shown in Table 2, there were no significant differences in body weight, HR, SBP, DBP, CTR, LVEF, BNP, and uric acid between the 2 groups at baseline.

Changes in Clinical Variables After 4 Weeks During the study, none of the patients treated with Waon therapy had worsened clinical symptoms. The changes in clinical variables after 4 weeks are summarized in Table 2. Body weight, CTR, and BNP decreased significantly after 4 weeks of Waon therapy compared to baseline, but they did not change in the control group. In addition, echocardiography demonstrated that LVEF increased significantly after 4 weeks of Waon therapy, but did not change in the control group. There were no significant differences in HR, SBP, DBP, and uric acid after 4 weeks of Waon therapy. In the control group, there were no significant differences in these clinical variables after 4 weeks of treatment.

Plasma Concentrations of Hydroperoxide The changes in plasma concentrations of hydroperoxide, which is an index of oxidative stress, are shown in Figure 1. The plasma concentration of hydroperoxide decreased significantly after 4 weeks of Waon therapy, whereas it did not change in the control group after 4 weeks of treatment (Waon therapy group, 422±116 to 327±88 U.CARR, P<0.001; control group, 422±71 to 431±85 U.CARR, P=0.59). There was no significant difference between the 2 groups at baseline (P=0.99).

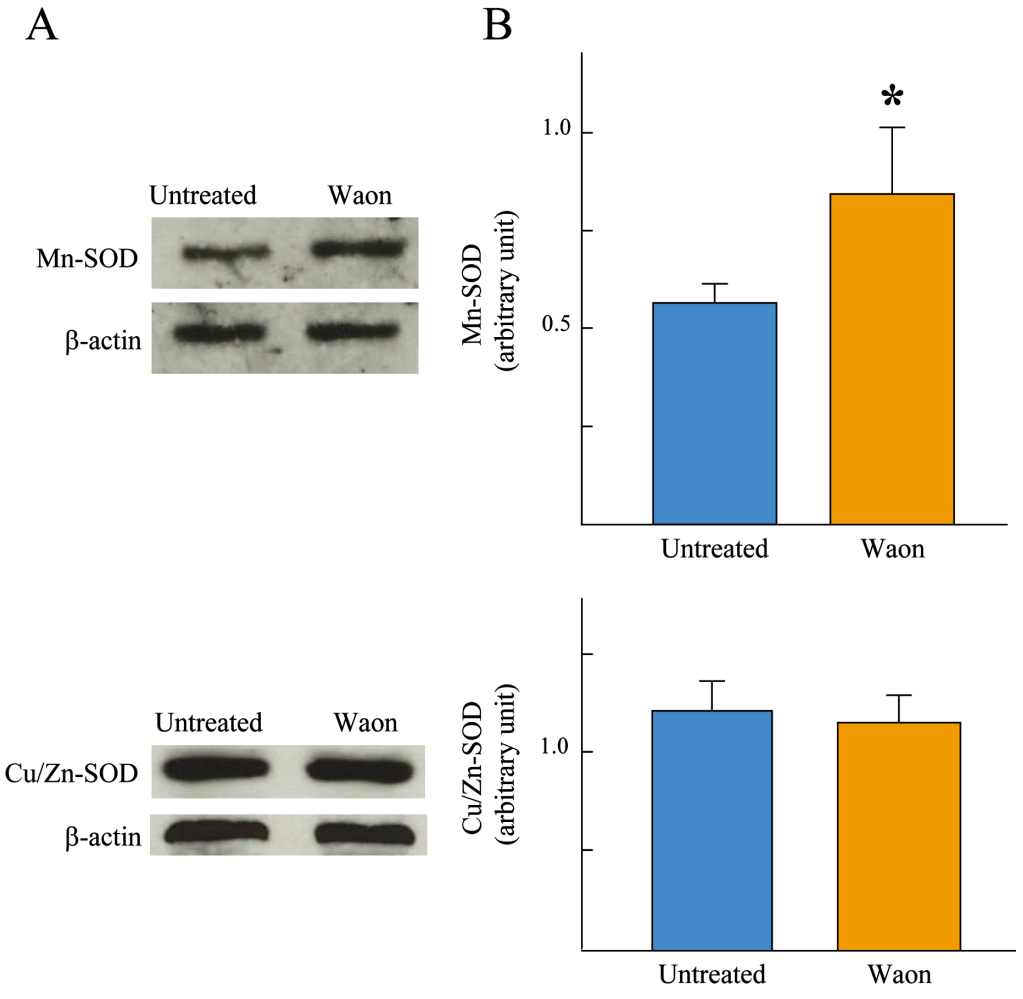

Figure 5. Effects of Waon therapy on the cardiac expressions of Mn-SOD and Cu/Zn-SOD assessed by Western blotting. (**A**) The representative Western blotting bands of Mn-SOD and Cu/Zn-SOD expressions in the whole hearts of TO-2 hamsters are shown. (**B**) Cardiac expression of Mn-SOD in TO-2 hamsters increased significantly after 4 weeks of Waon therapy compared to that in the untreated group, while cardiac expression of Cu/Zn-SOD did not change. *P<0.01 vs. untreated hamsters, n=8 per group.

Plasma Concentrations of Nitrate and Nitrite The nitric oxide metabolites, nitrate and nitrite, were measured at baseline and 4 weeks after treatment (Figure 2). The plasma concentration of nitric oxide metabolites increased significantly in the Waon therapy group, but did not change in the control group (Waon therapy group, 71.2±35.4 to 92.0±40.5 μmol/L, P<0.05; control group, 96.2±90.2 to 77.4±43.5 μmol/L, P=0.23). There was no significant difference between the 2 groups at baseline (P=0.26).

Animal Experiments

Effect of Waon Therapy on Cardiac Function In order to examine the effect of Waon therapy on oxidative stress in CHF, animal experiments using TO-2 cardiomyopathic hamsters were performed. First of all, the effect of Waon therapy on cardiac function in TO-2 hamsters was confirmed. Waon therapy significantly increased the LV +dP/dt of TO-2 hamsters compared to untreated hamsters (LV +dP/dt: Waon therapy group, 5,880±1,640 vs. untreated group, 4,180±660 mmHg/s, P<0.01, n=11 per group, %FS: Waon therapy group, 23.3±4.3 vs. untreated group, 16.5±4.2%, P<0.01, n=11 per group).

Effect of Waon Therapy on Oxidative Stress Immunohistochemistry using 4-HNE antibody, which is a marker of oxidative stress, was performed to analyze the effect of Waon therapy on oxidative stress in the failing heart. Cardiac 4-HNE immunoreactivities were lower in TO-2 hamsters following 4-week Waon therapy than in untreated hamsters (Figure 3A), which indicates that Waon therapy decreases oxidative stress in the failing heart.

ELISA of 4-HNE also revealed that 4-week Waon therapy decreased the concentration of cardiac 4-HNE of TO-2 hamsters significantly compared to untreated hamsters (Waon therapy group, 3.57±0.96 vs. untreated group, 5.53±1.17 μg/ml, P<0.05, n=8 per group, Figure 3B).

Effect of Waon Therapy on HSP27 and HSP32 Expressions The representative Western blotting bands of HSP27 and HSP32 expression in the whole hearts of TO-2 hamsters are shown in Figure 4. Cardiac expressions of HSP27 and 32 were significantly upregulated in hamsters treated with 4-week

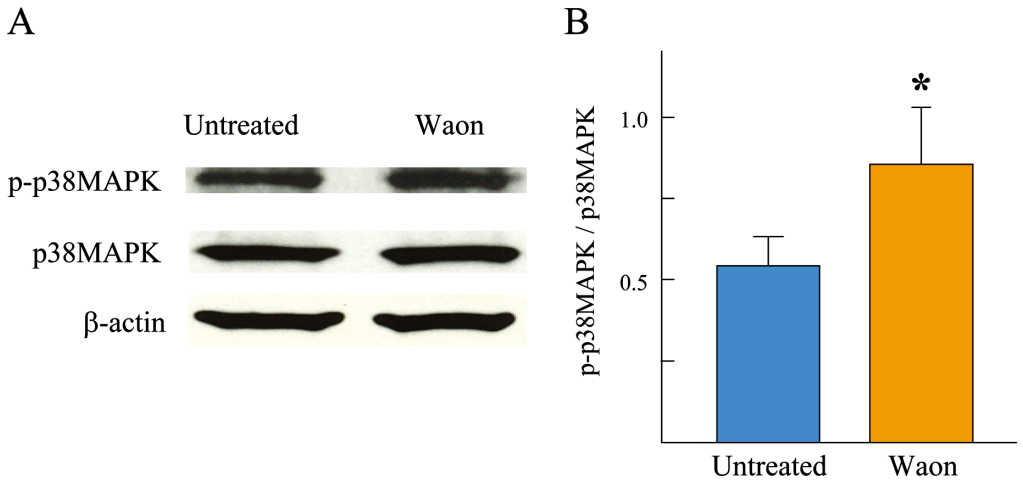

Figure 6. Effects of Waon therapy on the activation of cardiac p38MAPK signaling assessed by Western blotting. (**A**) The representative Western blotting bands of p38MAPK and p-p38MAPK expressions in the whole hearts of TO-2 hamsters are shown. (**B**) Cardiac expression of p-p38MAPK in TO-2 hamsters increased after 4 weeks of Waon therapy compared to that in the untreated group, while cardiac expression of p38MAPK did not change. The ratio of p-p38MAPK for p38MAPK in TO-2 hamsters was significantly higher in Waon therapy group than that in the untreated group. *P<0.01 vs. untreated hamsters, n=8 per group.

Waon therapy compared to untreated hamsters (HSP27, Waon therapy group, 1.25±0.30 vs. untreated group, 0.27±0.16 arbitrary units, n=8 per group, P<0.01; HSP32, Waon therapy group, 0.83±0.18 vs. untreated group, 0.54±0.12 arbitrary units, n=8 per group, P<0.01).

Effect of Waon Therapy on SOD Expression Cardiac expression of Mn-SOD was significantly upregulated in hamsters treated with 4-week Waon therapy compared to untreated hamsters (Waon therapy group, 0.81±0.20 vs. untreated group, 0.52±0.16 arbitrary units, n=8 per group, P<0.01; Figure 5). However, cardiac expression of Cu/Zn-SOD was not changed by Waon therapy (Cu/Zn-SOD, Waon therapy group, 1.14±0.08 vs. untreated group, 1.21±0.16 arbitrary units, n=8 per group, P=0.29, Figure 5).

Effect of Waon Therapy on the Activation of p38MAPK Waon therapy did not change cardiac expression of p38MAPK. However, cardiac expression of p-p38MAPK was upregulated in hamsters treated with 4-week Waon therapy compared to untreated hamsters. The ratio of p-p38MAPK for p38MAPK in TO-2 hamsters was significantly higher in the Waon therapy group than that in the untreated group. (Waon therapy group, 0.84±0.18 vs. untreated group, 0.53±0.14 arbitrary units, n=8 per group, P<0.01; Figure 6.)

Discussion

The present clinical study demonstrated that 4 weeks of Waon therapy improved cardiac function, decreased plasma hydroperoxide concentrations and increased plasma nitrite and nitrate concentrations. In addition, 4 weeks of Waon therapy improved cardiac function and decreased oxidative stress in failing hearts of TO-2 hamsters. Furthermore, it appears that Waon therapy reduces the oxidative stress through HSP27, HSP32 and Mn-SOD.

Oxidative stress is implicated in the pathogenesis of heart failure. Reactive oxygen species (ROS) are produced in the failing myocardium, and ROS causes progression of heart failure.[37] Increased ROS in CHF impairs vascular endothelial function, which is represented by the endothelium-dependent vasodilatory response, through decreased NO bioavailability induced by decreased eNOS activity, including decreased eNOS expression and increased eNOS uncoupling.[38–43] As vascular endothelial function is one of the most important factors affecting clinical symptoms in CHF, therapy that improves vascular endothelial dysfunction is considered to be an ideal treatment for CHF. Furthermore, increased oxidative stress induces apoptosis of cardiomyocytes, resulting in further impairment of the failing myocardium.[37] Therefore, therapies that decrease oxidative stress are important to improve vascular endothelial function and cardiac function in CHF.

SOD is well known as a key anti-oxidant enzyme, and some kinds of HSP, such as HSP27 and HSP32, reduce oxidative stress.[33–37] It has been reported that overexpression of HSP27 attenuated doxorubicin-induced cardiac dysfunction through the decreases of oxidative stress and apoptosis in hearts of HSP27 transgenic mice.[44] We demonstrated that Waon therapy upregulated the cardiac expressions of HSP27 and Mn-SOD in TO-2 cardiomyopathic hamsters. In addition, Waon therapy was shown to increase cardiac expression of HSP32 in TO-2 hamsters. HSP32 is also known as Heme Oxygenase-1, and it plays a role in cellular protection against injury caused by ROS. HSP32 degrades the pro-oxidant heme and catalyzes it into biliverdin and bilirubin, which function as anti-oxidants.[35,36] Hearts of heterozygous HSP32 knockout mice, subjected to ischemia/reperfusion injury, had increased oxidative stress and infarct size compared to wild type mice.[45] In contrast, hearts of HSP32 transgenic mice had reduced oxidative stress and infarct size compared to wild type mice when they were subjected to ischemia/reperfusion injury.[46] These results clarified the cardio-protective effect of HSP32. Given the results of the clinical study and the animal experiments presented in this paper, the increases of HSP27, Mn-SOD and HSP32 by Waon therapy appear to reduce oxidative stress and improve cardiac function in CHF.

It is reported that whole-body hyperthermia with 15 min

42°C hot water bathing increases cardiac Mn-SOD through the production of TNF-α and IL-1β of normal rats.[47] TNF-α activates the translocation of NF-κB and increases Mn-SOD.[48] However, Waon therapy did not modulate the cardiac expression of TNF-α and NF-κB signaling in TO-2 hamsters with heart failure (data not shown). We think that TNF-α and NF-κB have already increased in heart failure, therefore, the mechanisms by which Waon therapy increases Mn-SOD in failing hearts might not involve TNF-α/NF-κB pathway. It is reported that p38MAPK is activated by several stress factors, and is involved in the induction of HSP and Mn-SOD.[49] In this study, we demonstrated that Waon therapy increased cardiac p-p38MAPK in TO-2 hamsters with heart failure. We believe that Waon therapy activates p38MAPK signaling, which leads to the induction of HSP and Mn-SOD.

Oxidative stress is also involved in the pathogenesis of arteriosclerosis and major cardiovascular diseases.[50] We reported that Waon therapy for 2 weeks improved impaired vascular endothelial function in the setting of atherosclerotic risk factors, such as hypertension, hypercholesterolemia, diabetes mellitus, obesity, and smoking.[12] In addition, we demonstrated that 2 weeks of Waon therapy significantly decreased urinary 8-epi-PGF$_{2\alpha}$ levels in patients with at least 1 atherosclerotic risk factor, when compared to those of patients who did not undergo Waon therapy.[13] Thus, Waon therapy reduces oxidative stress and improves vascular function in patients with atherosclerotic risk factors.

Although the plasma concentrations of nitrite and nitrate were significantly lower in patients with atrial fibrillation than in the control subjects,[51] there is no significant difference in the incidence of atrial fibrillation between 2 groups in the present study.

There are limitations in this study. It is difficult to get rid of the bias in data, because this study is not blind or a cross over test.

Conclusion

Waon therapy decreases oxidative stress and is an innovative non-pharmacological therapy for patients with CHF.

References

1. Tei C. Waon therapy: Soothing warmth therapy. *J Cardiol* 2007; **49:** 301–304.
2. Tei C, Horikiri Y, Park JC, Jeong JW, Chang KS, Toyama Y, et al. Acute hemodynamic improvement by thermal vasodilation in congestive heart failure. *Circulation* 1995; **91:** 2582–2590.
3. Tei C, Tanaka N. Thermal vasodilation as a treatment of congestive heart failure: A novel approach. *J Cardiol* 1996; **27:** 29–30.
4. Kihara T, Biro S, Imamura M, Yoshifuku S, Takasaki K, Ikeda Y, et al. Repeated thermal therapy treatment improves vascular endothelial and cardiac function in patients with chronic heart failure. *J Am Coll Cardiol* 2002; **39:** 754–759.
5. Kihara T, Biro S, Ikeda Y, Fukudome T, Shinsato T, Masuda A, et al. Effects of repeated sauna treatment on ventricular arrhythmias in patients with chronic heart failure. *Circ J* 2004; **68:** 1146–1151.
6. Miyata M, Kihara T, Kubozono T, Ikeda Y, Shinsato T, Izumi T, et al. Beneficial effects of Waon therapy on patients with chronic heart failure: Results of a prospective multicenter study. *J Cardiol* 2008; **52:** 79–85.
7. Basford JR, Oh JK, Allison TG, Sheffield CG, Manahan BG, Hodge DO, et al. Safety, acceptance, and physiologic effects of sauna bathing in people with chronic heart failure: A pilot report. *Arch Phys Med Rehabil* 2009; **90:** 173–177.
8. Kihara T, Miyata M, Fukudome T, Ikeda Y, Shinsato T, Kubozono T, et al. Waon therapy improves the prognosis of patients with chronic heart failure. *J Cardiol* 2009; **53:** 214–218.
9. Miyata M, Tei C. Waon therapy for cardiovascular disease: Innovative therapy for the 21st century. *Circ J* 2010; **74:** 617–621.
10. Tei C, Shinsato T, Miyata M, Kihara T, Hamasaki S. Waon therapy improves peripheral arterial disease. *J Am Coll Cardiol* 2007; **50:** 2169–2171.
11. Tei C, Shinsato T, Kihara T, Miyata M. Successful thermal therapy for end-stage peripheral artery disease. *J Cardiol* 2006; **47:** 163–164.
12. Imamura M, Biro S, Kihara T, Yoshifuku S, Takasaki K, Otsuji Y, et al. Repeated thermal therapy improves impaired vascular endothelial function in patients with coronary risk factors. *J Am Coll Cardiol* 2001; **38:** 1083–1088.
13. Masuda A, Miyata M, Kihara T, Minagoe S, Tei C. Repeated sauna therapy reduces urinary 8-epi-prostaglandin F (2alpha). *Jpn Heart J* 2004; **45:** 297–303.
14. Ikeda Y, Biro S, Kamogawa Y, Yosifuku S, Eto H, Orihara K, et al. Repeated sauna therapy increases arterial endothelial nitric oxide synthase expression and nitric oxide production in cardiomyopathic hamsters. *Circ J* 2005; **69:** 722–729.
15. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and mitochondrial DNA damage in heart failure. *Circ J* 2008; **72**(Suppl A)**:** A-31–A-37.
16. Belch JJ, Bridges AB, Scott N, Chopra M. Oxygen free radicals and congestive heart failure. *Br Heart J* 1991; **65:** 245–248.
17. Mallat Z, Philip I, Lebret M, Chatel D, Maclouf J, Tedgui A. Elevated levels of 8-iso-prostaglandin F$_{2\alpha}$ in pericardial fluid of patients with heart failure. *Circulation* 1998; **97:** 1536–1539.
18. Keith M, Geranmayegan A, Sole MJ, Kurian R, Robinson A, Omran AS, et al. Increased oxidative stress in patients with congestive heart failure. *J Am Coll Cardiol* 1998; **31:** 1352–1356.
19. Hornig B, Arakawa N, Kohler C, Drexler H. Vitamin C improves endothelial function of conduit arteries in patients with chronic heart failure. *Circulation* 1998; **97:** 363–368.
20. Nakamura K, Kusano K, Nakamura Y, Kakishita M, Ohta K, Nagase S, et al. Carvedilol decreases elevated oxidative stress in human failing myocardium. *Circulation* 2002; **105:** 2867–2871.
21. Nickenig G. Should angiotensin Ⅱ receptor blockers and statins be combined? *Circulation* 2004; **110:** 1013–1020.
22. George J, Carr E, Davies J, Belch JJ, Struthers A. High-dose allopurinol improves endothelial function by profoundly reducing vascular oxidative stress and not by lowering uric acid. *Circulation* 2006; **114:** 2508–2516.
23. Kato Y, Iwase M, Ichihara S, Kanazawa H, Hashimoto K, Noda A, et al. Beneficial effects of growth hormone-releasing peptide on myocardial oxidative stress and left ventricular dysfunction in dilated cardiomyopathic hamsters. *Circ J* 2010; **74:** 163–170.
24. Linke A, Adams V, Schulze PC, Erbs S, Gielen S, Fiehn E, et al. Antioxidative effects of exercise training in patients with chronic heart failure: Increase in radical scavenger enzyme activity in skeletal muscle. *Circulation* 2005; **111:** 1763–1770.
25. Mantovani G, Macciò A, Madeddu C, Mura L, Massa E, Gramignano G, et al. Reactive oxygen species, antioxidant mechanisms and serum cytokine levels in cancer patients: Impact of an antioxidant treatment. *J Cell Mol Med* 2002; **6:** 570–582.
26. Shimano M, Shibata R, Inden Y, Yoshida N, Uchikawa T, Tsuji Y, et al. Reactive oxidative metabolites are associated with atrial conduction disturbance in patients with atrial fibrillation. *Heart Rhythm* 2009; **6:** 935–940.
27. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite and [^{15}N]nitrate in biological fluids. *Anal Biochem* 1982; **126:** 131–138.
28. Factor SM, Minase T, Cho S, Dominitz R, Sonnenblick EH. Microvascular spasm in the cardiomyopathic Syrian hamster: A preventable cause of focal myocardial necrosis. *Circulation* 1982; **66:** 342–354.
29. Ichihara S, Yamada Y, Ichihara G, Kanazawa H, Hashimoto K, Kato Y, et al. Attenuation of oxidative stress and cardiac dysfunction by bisoprolol in an animal model of dilated cardiomyopathy. *Biochem Biophys Res Commun* 2006; **350:** 105–113.
30. Yu B, Otsuji Y, Yoshifuku S, Ikeda Y, Kamogawa Y, Yuasa T, et al. Prediction of prognosis in the UM-X7.1 hamster model of congestive heart failure using the Tei index. *Circ J* 2005; **69:** 991–993.
31. Toyokuni S, Miyake N, Hiai H, Hagiwara M, Kawakishi S, Osawa T, et al. The monoclonal antibody specific for the 4-hydroxy-2-nonenal histidine adduct. *FEBS Lett* 1995; **359:** 189–191.
32. Maki RA, Tyurin VA, Lyon RC, Hamilton RL, DeKosky ST, Kagan VE, et al. Aberrant expression of myeloperoxidase in astrocytes promotes phospholipid oxidation and memory deficits in a mouse model of Alzheimer disease. *J Biol Chem* 2009; **284:** 3158–3169.
33. Ilangovan G, Venkatakrishnan CD, Bratasz A, Osinbowale S, Cardounel AJ, Zweier JL, et al. Heat shock-induced attenuation of

hydroxyl radical generation and mitochondrial aconitase activity in cardiac H9c2 cells. *Am J Physiol Cell Physiol* 2006; **290:** C313–C324.
34. Turakhia S, Venkatakrishnan CD, Dunsmore K, Wong H, Kuppusamy P, Zweier JL, et al. Doxorubicin-induced cardiotoxicity: Direct correlation of cardiac fibroblast and H9c2 cell survival and aconitase activity with heat shock protein 27. *Am J Physiol Heart Circ Physiol* 2007; **293:** H3111–H3121.
35. Morita T. Heme oxygenase and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2005; **25:** 1786–1795.
36. Siow RC, Sato H, Mann GE. Heme oxygenase-carbon monoxide signalling pathway in atherosclerosis: Anti-atherogenic actions of bilirubin and carbon monoxide? *Cardiovasc Res* 1999; **41:** 385–394.
37. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. *J Clin Invest* 2005; **115:** 500–508.
38. Smith CJ, Sun D, Hoegler C, Roth BS, Zhang X, Zhao G, et al. Reduced gene expression of vascular endothelial NO synthase and cyclooxygenase-1 in heart failure. *Circ Res* 1996; **78:** 58–64.
39. Katz SD. Mechanisms and implications of endothelial dysfunction in congestive heart failure. *Curr Opin Cardiol* 1997; **12:** 259–264.
40. Ferrari R, Bachetti T, Agnoletti L, Comini L, Curello S. Endothelial function and dysfunction in heart failure. *Eur Heart J* 1998; **19:** G41–G47.
41. Agnoletti L, Curello S, Bachetti T, Malacarne F, Gaia G, Comini L, et al. Serum from patients with severe heart failure downregulates eNOS and is proapoptotic: Role of tumor necrosis factor-alpha. *Circulation* 1999; **100:** 1983–1991.
42. Fukai T. Endothelial GTPCH in eNOS uncoupling and atherosclerosis. *Arterioscler Thromb Vasc Biol* 2007; **27:** 1493–1495.
43. Higashi Y, Noma K, Yoshizumi M, Kihara Y. Endothelial function and oxidative stress in cardiovascular diseases. *Circ J* 2009; **73:** 411–418.
44. Liu L, Zhang X, Qian B, Min X, Gao X, Li C, et al. Over-expression of heat shock protein 27 attenuates doxorubicin-induced cardiac dysfunction in mice. *Eur J Heart Fail* 2007; **9:** 762–769.
45. Yoshida T, Maulik N, Ho YS, Alam J, Das DK. H(mox-1) constitutes an adaptive response to effect antioxidant cardioprotection: A study with transgenic mice heterozygous for targeted disruption of the Heme oxygenase-1 gene. *Circulation* 2001; **103:** 1695–1701.
46. Yet SF, Tian R, Layne MD, Wang ZY, Maemura K, Solovyeva M, et al. Cardiac-specific expression of heme oxygenase-1 protects against ischemia and reperfusion injury in transgenic mice. *Circ Res* 2001; **89:** 168–173.
47. Yamashita N, Hoshida S, Otsu K, Taniguchi N, Kuzuya T, Hori M. Involvement of cytokines in the mechanism of whole-body hyperthermia-induced cardioprotection. *Circulation* 2000; **102:** 452–457.
48. Lee YJ, Cho HN, Jeoung DI, Soh JW, Cho CK, Bae S, et al. HSP25 overexpression attenuates oxidative stress-induced apoptosis: Roles of ERK1/2 signaling and manganese superoxide dismutase. *Free Radic Biol Med* 2004; **36:** 429–444.
49. Banerjee Mustafi S, Chakraborty PK, Dey RS, Raha S. Heat stress upregulates chaperone heat shock protein 70 and antioxidant manganese superoxide dismutase through reactive oxygen species (ROS), p38MAPK, and Akt. *Cell Stress Chaperones* 2009; **14:** 579–589.
50. Heistad DD, Wakisaka Y, Miller J, Chu Y, Pena-Silva R. Novel aspects of oxidative stress in cardiovascular diseases. *Circ J* 2009; **73:** 201–207.
51. Minamino T, Kitakaze M, Sato H, Asanuma H, Funaya H, Koretsune Y, et al. Plasma levels of nitrite/nitrate and platelet cGMP levels are decreased in patients with atrial fibrillation. *Arterioscler Thromb Vasc Biol* 1997; **17:** 3191–3195.