therapy enhanced angiogenesis and improved myocardial ischemia in a pig model of chronic myocardial ischemia,[3,4] and that SW therapy improved the symptoms and myocardial perfusion in patients with severe angina pectoris in an open trial.[3,5] The present double-blind and placebo-controlled study further demonstrates that our extracorporeal cardiac SW therapy is an effective therapeutic option for severe angina pectoris, providing convincing evidence for its effectiveness and safety.

During the past 2 decades, regenerative therapies using genes, cytokines, and progenitor cells have been under investigation for ischemic cardiovascular diseases.[8] However, these therapies have not been consistently effective in humans, despite promising results in early preclinical studies.[9–12] A potential explanation for these inconsistent results is the complex crosstalk among multiple pathways, in which enhancement of only 1 factor among numerous angiogenic factors may not be enough to achieve clinical benefit. Furthermore, animal studies of cell therapy have revealed that the number of newly generated vascular cells is too low to induce any functional improvement, suggesting that the paracrine action of transplanted cells stimulates intrinsic angiogenic capacity.[13] In contrast, low-energy SW upregulates multiple angiogenic pathways (eg, VEGF, flt-1, SDF-1, and nitric oxide synthase).[4,7,14]

There are several limitations to the present study. First, the number of patients is small. Although more than 150 patients with severe angina pectoris were reviewed as potential candidates for this study, most of them were excluded due to insufficient midication, potential indications of CABG or PCI, and co-existence of malignant tumor. However, we were able to reconfirm the beneficial effects of SW therapy in the present double-blind and placebo-control study, as we had observed in a previous open study.[5] A future large-scale trial would validate the present results. Second, maximum exercise capacity and pVO_2 were not significantly improved while the symptoms and 6-min walking distance were significantly improved by the SW therapy. In 6 of the 8 patients, exercise was stopped because of leg pain or fatigue before reaching the anaerobic threshold. Thus, exercise tolerance might have been underestimated because of arteriosclerosis obliterans and/or physical deconditioning. Another parameter, such as arteriovenous difference in lactate concentration under overdrive pacing, might have been a better index of ischemia. Third, the number of circulating progenitor cells in peripheral blood was not increased in the present study. Thus, it remains to be examined whether our SW therapy promotes recruitment of bone marrow-derived cells by the ischemic myocardium of humans.[14,15] Fourth, the detailed molecular mechanisms of the beneficial effects of SW in humans remain to be clarified in future studies.[3–7]

In conclusion, the present double-blind, placebo-controlled study further confirmed the effectiveness and safety of our extracorporeal cardiac SW therapy for the treatment of severe angina pectoris, although large-scale multi-center study is needed.

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Cardiac shock wave therapy ameliorates left ventricular remodeling after myocardial ischemia–reperfusion injury in pigs *in vivo*

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Objectives **Left ventricular (LV) remodeling after acute myocardial infarction (AMI) is associated with a poor prognosis and an impaired quality of life. We have shown earlier that low-energy extracorporeal cardiac shock wave (SW) therapy improves chronic myocardial ischemia in pigs and humans and also ameliorates LV remodeling in a pig model of AMI induced by permanent coronary ligation. However, in the current clinical setting, most of the patients with AMI receive reperfusion therapy. Thus, in this study we examined whether our SW therapy also ameliorates LV remodeling after myocardial ischemia–reperfusion (I/R) injury in pigs *in vivo*.**

Methods **Pigs were subjected to a 90-min ischemia and reperfusion using a balloon catheter and were randomly assigned to two groups with or without SW therapy to the ischemic border zone (0.09 mJ/mm^2, 200 pulses/spot, 9 spots/animal, three times in the first week) ($n=15$ each).**

Results **Four weeks after I/R, compared with the control group, the SW group showed significantly ameliorated LV remodeling in terms of LV enlargement (131 ± 9 vs. 100 ± 7 ml), reduced LV ejection fraction (28 ± 2 vs. 36 ± 3%), and elevated left ventricular end-diastolic pressure (11 ± 2 vs. 4 ± 1 mmHg) (all $P<0.05$, $n=8$ each). The SW group also showed significantly increased regional myocardial blood flow (−0.06 ± 0.11 vs. 0.36 ± 0.13 ml/min/g, $P<0.05$), capillary density (1.233 ± 31 vs. 1.560 ± 60/mm^2, $P<0.001$), and endothelial nitric oxide synthase activity (0.24 ± 0.03 vs. 0.41 ± 0.05, $P<0.05$) in the ischemic border zone compared with the control group ($n=7$ each).**

Conclusion **These results indicate that our SW therapy is also effective in ameliorating LV remodeling after myocardial I/R injury in pigs *in vivo*.** *Coron Artery Dis* **21:304–311 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: angiogenesis, reperfusion, shock wave, ventricular remodeling

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Introduction

Ischemic heart disease is the leading cause of death in western countries. The development of left ventricular (LV) remodeling after acute myocardial infarction (AMI) leads to sudden cardiac death, heart failure, and poor prognosis. Thus, it is important to improve LV remodeling after AMI to improve prognosis and the quality of life. Several regenerative therapies, such as gene [1–3] and cell therapies [4–8], are currently under development; however, most of these are invasive in nature and their effectiveness and safety have not yet been fully established. Thus, more effective and less invasive therapies need to be developed.

We have shown earlier that low-energy extracorporeal cardiac shock wave (SW) therapy effectively induces angiogenesis and improves cardiac functions in a porcine model of chronic myocardial ischemia [9], and that SW therapy improves symptoms, reduces the use of nitroglycerin, and improves myocardial perfusion in patients with end-stage coronary artery disease [10,11]. Furthermore, we have recently shown that SW therapy improves LV remodeling in a porcine model of AMI with permanent coronary ligation [12]. However, in the current clinical setting, most patients with AMI receive emergency reperfusion therapy with either percutaneous coronary intervention or thrombolytic agents. It remains to be determined whether our extracorporeal cardiac SW therapy also ameliorates myocardial ischemia–reperfusion (I/R) injury *in vivo*. Thus, in this study we examined whether our SW therapy also ameliorates LV remodeling after myocardial I/R injury in pigs *in vivo*, and if so, what mechanism(s) might be involved.

Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals established by the US National Institutes of Health (Publication No. 85-23, revised 1996). All procedures were performed according to the protocols approved by the Institutional Committee for Use and Care of Laboratory Animals at Tohoku University (20-Idou-151 and 21-Idou-156).

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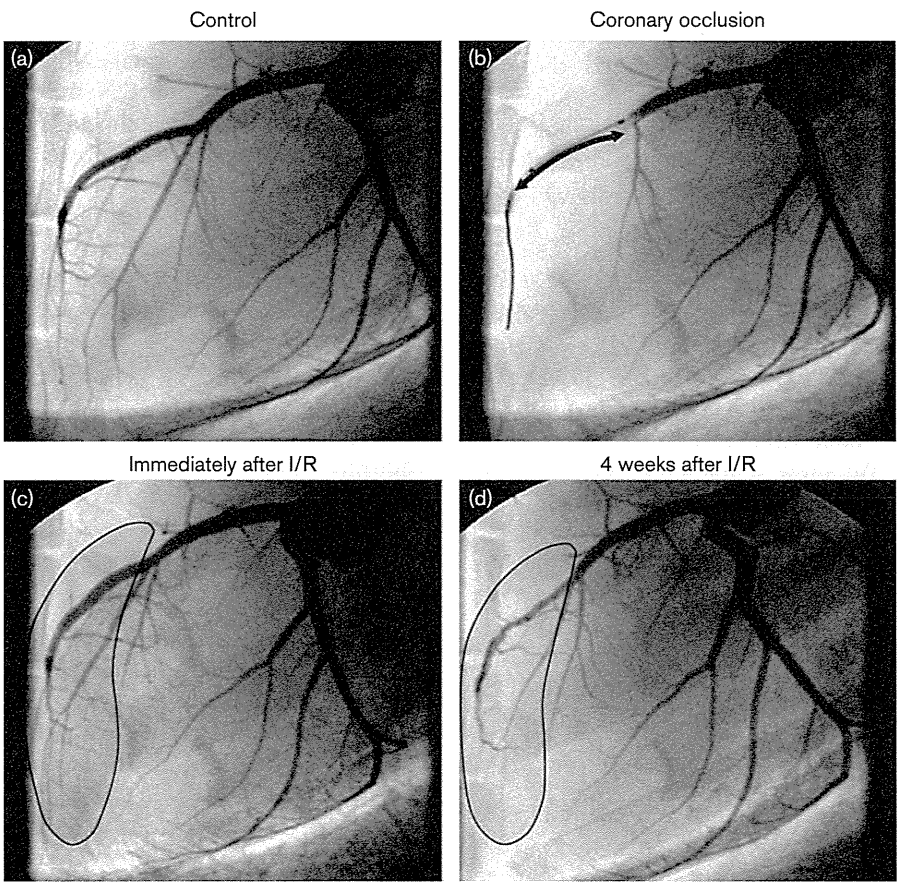
Porcine model of myocardial I/R

A total of 30 domestic male pigs (25–30 kg in body weight) were used in this study. They underwent myocardial I/R injury with and without SW therapy. They were subjected to cardiac catheterization and histology study at 4 weeks after I/R ($n = 8$ each) and to western blotting study at 1 week after I/R ($n = 7$ each). The animals were anesthetized with ketamine hydrochloride (15 mg/kg, intramuscular), and after intubation, they were kept anesthetized with an inhalation of 2.0% sevoflurane for cardiac catheterization and euthanization. We inserted a 7F sheath into the left carotid artery for cardiac catheterization. A 5000-IU bolus of heparin was administered intravenously and 2000-IU was injected every hour. We performed a left ventriculography (LVG) and coronary angiography (CAG) in a left oblique view with the use of a cineangiography system (Toshiba Medical, Tochigi, Japan) [9,12]. LV volume and LV ejection fraction (LVEF) were calculated using Simpson's method. A coronary angioplasty balloon (2.5–3.5 mm in diameter depending on the vessel size) was then introduced into the left anterior descending coronary artery (LAD) and inflated just distal to the first diagonal branch for 90 min, which has been shown earlier to effectively induce myocardial infarction [13,14], at the lowest pressure that completely occluded distal flow (Fig. 1). After 90 min of ischemia, the balloon was deflated and both CAG and LVG were reperformed to confirm the patency of distal LAD and the reduced LV wall motion, respectively. Cardiac catheterization was performed before ischemia, immediately after reperfusion, and 4 weeks after I/R. After the study, 4 weeks after I/R, the animals were euthanized by an overdose of pentobarbital.

Extracorporeal cardiac SW therapy

On the basis of our earlier studies [9–12,15,16], we applied a low-energy SW (0.09 mJ/mm^2, approximately 10% of the energy used for the lithotripsy treatment, 200 shots/spot for 27 spots) to the border zone around the infarcted myocardium with the guidance of an echocardiogram equipped within the specially designed SW generator (Storz Medical AG, Kreuzlingen, Switzerland) in an R-wave-triggered manner to avoid ventricular

Fig. 1



Porcine model of myocardial ischemia–reperfusion (I/R). Coronary angiograms at baseline (a), during balloon inflation in the left anterior descending coronary artery (b), immediately after reperfusion (c), and 4 weeks after the I/R in the same pig. The inflated balloon is shown by an arrowed line (b) and the ischemic myocardial area by the shaded area (c and d).

arrhythmias. In a preliminary study, we confirmed that no adverse effects, such as cardiac rupture or tamponade, were noted even if we applied a SW to the infracted myocardium (data not shown). We examined LV wall motion by echocardiography during I/R and defined the border zone as the edge of the area where the LV wall motion was severely depressed after I/R. We were able to accurately focus a SW to any part of the heart under the guidance of echocardiography with a focus of approximately 2 mm [9–12]. We performed the SW treatment three times in the first week (day 1, 3, and 5), whereas the animals in the control group received the same procedures three times but without the SW treatment.

Cardiac enzymes

We measured serum concentrations of cardiac troponin T and creatinine kinase myocardial blood isoform (CK-MB) using an electrochemiluminescence immunoassay and a chemiluminescence immunoassay, respectively. Blood samples were serially collected before and 5, 12, 24, 48, and 72 h after the I/R injury, and the extent of myocardial infarction was expressed as the area under the curves of troponin T and CK-MB [12].

Echocardiography

We performed a transthoracic echocardiographic study (Aplio 80, Toshiba Medical). We calculated the wall thickening fraction (WTF, %) by using the following formula: WTF = 100 × (end-systolic wall thickness − end-diastolic wall thickness)/end-diastolic wall thickness [9]. We measured the WTF in the infarcted area and the border zone when the animals were sedated.

Regional myocardial blood flow

We evaluated regional myocardial blood flow (RMBF) with colored microspheres (Dye-Trak VII +, Triton Technology, San Diego, USA) ($n = 4$ each) [9,12]. We injected 6 million microspheres (diameter 15 μm) into the left atrium before the induction of myocardial ischemia and 4 weeks after I/R. We drew a reference arterial blood sample from the descending aorta at a constant rate of 12 ml/min for 90 s using a withdrawal pump. We extracted microspheres from the LV wall and blood samples by potassium hydroxide digestion, extracted the dyes from the microspheres with ethylene glycol monoethyl ether acetate (70 μl), and determined their concentrations by spectrophotometry. We calculated the change of myocardial blood flow (ml/min/g) in the infarcted region and border zone.

Myocardial capillary density

The heart was removed and 10% formaldehyde was injected into the left coronary artery with a pressure of 100–120 mmHg. After fixation, tissue specimens were obtained from the border zone of each animal. We treated the paraffin-embedded sections with a rabbit anti-factor VIII antibody (N1505, Dako, Copenhagen, Denmark), and counted the number of factor VIII-positive cells in 10 random fields of the border zone and the remote area in each heart at × 400 magnification, and calculated capillary density [9,12]. Ten random fields of each sample were examined in a blinded manner. Each field covered 0.036 mm^2.

Myocardial fibrosis

Masson-trichrome staining was performed using the paraffin-embedded sections. We evaluated the fibrosis area in 10 random fields of the border zone in each heart at × 200 magnification. A digital image processing software AxioVision 4.5.0.0 (Carl Zeiss, Göttingen, Germany) was used to detect the myocardial fibrosis area, and the ratio of the fibrosis area to the myocardial area was calculated.

Western blot analysis

To examine the mechanisms of the inhibitory effects of SW therapy on LV remodeling, another set of animals with I/R injury, with and without SW therapy, were made and they were euthanized at 1 week after the procedure. We performed western blot analysis for phosphorylated endothelial nitric oxide synthase (phospho-eNOS) and vascular endothelial growth factor (VEGF). Samples from the border zone were used and the extracted samples (50 μg of protein) were subjected to SDS-PAGE/immunoblot analysis by using the specific antibody for phospho-eNOS at Ser1177 (No. 9571, Cell Signaling Technology, Danvers, Massachusetts, USA), total-eNOS (No. 610296, Becton Dickinson, Franklin Lakes, New Jersey, USA), and VEGF (sc-152, Santa Cruz Biotechnology, Santa Cruz, California, USA). The regions containing proteins were visualized by an electrochemiluminescence western blotting luminal reagent (RPN2132, GE Healthcare Bioscience, Waukesha, Wisconsin, USA). The extents of eNOS phosphorylation and VEGF expression were normalized by that of total-eNOS and β-actin, respectively [9,17].

Statistical analysis

Results were expressed as mean ± SEM. We determined the statistical significance by an analysis of variance for multiple comparisons and the unpaired Student's t-test. Values of P less than 0.05 were considered to be statistically significant.

Results

Extent of myocardial infarction

The extent of myocardial infarction, when evaluated by the area under the curve of troponin T or CK-MB, was comparable between the control and the SW groups (troponin T, 296 ± 32 vs. 319 ± 30 ng/ml*h, $P = 0.61$; CK-MB, 1.641 ± 301 vs. 1.993 ± 353 ng/ml*h, $P = 0.46$), indicating that the extent of myocardial infarction was comparable between the two groups.

Safety of SW therapy

No procedural complications or adverse effects related to SW therapy were noted throughout the experiments.

Cardiac catheterization: CAG and LVG

At 4 weeks after the I/R injury, CAG confirmed the patency of reperfused LAD in all pigs (Fig. 1). Before and immediately after I/R (before the SW treatment), LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), and LVEF were all comparable between the two groups (Fig. 2). Four weeks after I/R, LVG showed marked LV enlargement and reduced LVEF in the control group (Fig. 2). In contrast, LV enlargement and reduced LVEF were significantly ameliorated in the SW group. (LVEDV, 100 ± 7 vs. 131 ± 9 ml, $P < 0.05$; LVESV, 65 ± 8 vs. 95 ± 7 ml, $P < 0.05$; LVEF, 36 ± 3 vs. 28 ± 2%, $P < 0.05$) (Fig. 2). Although LV end-diastolic pressure was comparable between the two groups before and immediately after I/R (before the SW treatment), it remained elevated in the control group but was normalized in the SW group 4 weeks after I/R (11 ± 2 vs. 4 ± 1 mmHg, $P < 0.05$) (Fig. 2).

Echocardiography

We measured the WTF of the infarcted region and the border zone by transthoracic echocardiography. The WTF in the infarcted region was significantly decreased to the same extent after I/R and was comparable between the control and the SW groups throughout the experimental period (before I/R, 22 ± 2 vs. 20 ± 1%; immediately after I/R, 2 ± 1 vs. 1 ± 0.3%; 4 weeks, 4 ± 2 vs. 5 ± 2%) (Fig. 3a). In contrast, 4 weeks after I/R, the WTF was significantly improved at the border zone in the SW group as compared with the control group (before I/R, 24 ± 2 vs. 22 ± 2%, $P = 0.54$; immediately after I/R, 16 ± 1 vs. 15 ± 2%, $P = 0.59$; and 4 weeks, 15 ± 2 vs. 24 ± 4%, $P < 0.05$) (Fig. 3b).

Regional myocardial blood flow

In the infarcted region, RMBF was equally decreased in the control and the SW groups at 4 weeks after I/R as compared with before I/R (−0.52 ± 0.22 vs. −0.49 ± 0.08 ml/min/g, $P = 0.89$), whereas RMBF at the border zone was significantly increased only in the SW group (control: −0.06 ± 0.11 vs. SW: 0.36 ± 0.13 ml/min/g, $P < 0.05$) (Fig. 4).

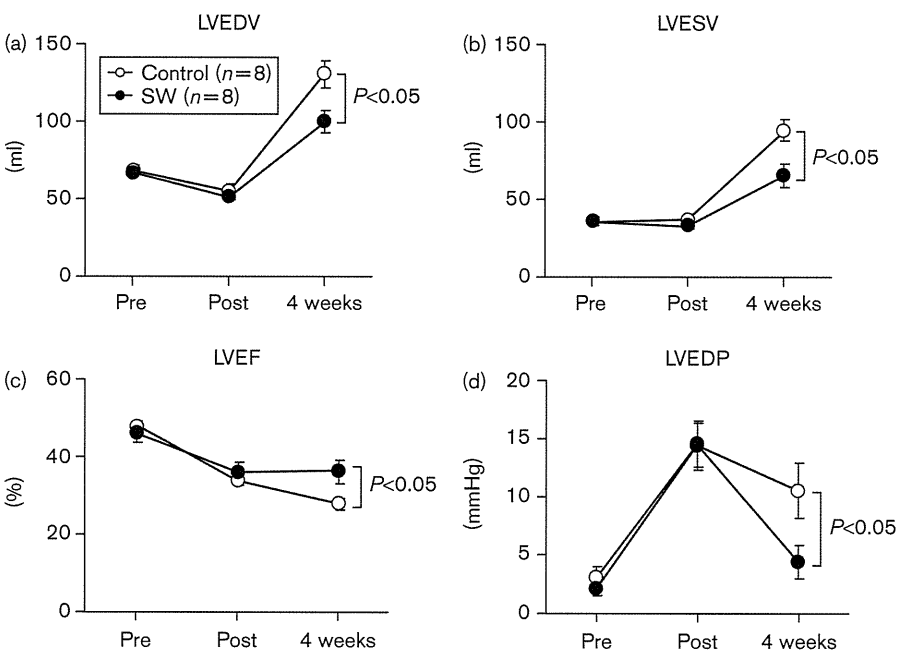
Histopathology

Factor VIII staining showed that 4 weeks after I/R the number of factor VIII-positive blood vessels at the border zone was significantly higher in the SW group than in the control group (1.560 ± 60 vs. 1.233 ± 31/mm^2, $P < 0.001$) (Fig. 5a–c). In the remote area, the number of vessels was comparable between the two groups (Fig. 5d). Masson-trichrome staining showed that there was no difference in the extent of myocardial fibrosis at the border zone between the two groups (control, 0.15 ± 0.02 vs. SW, 0.13 ± 0.03, $P = 0.72$).

Western blot analysis

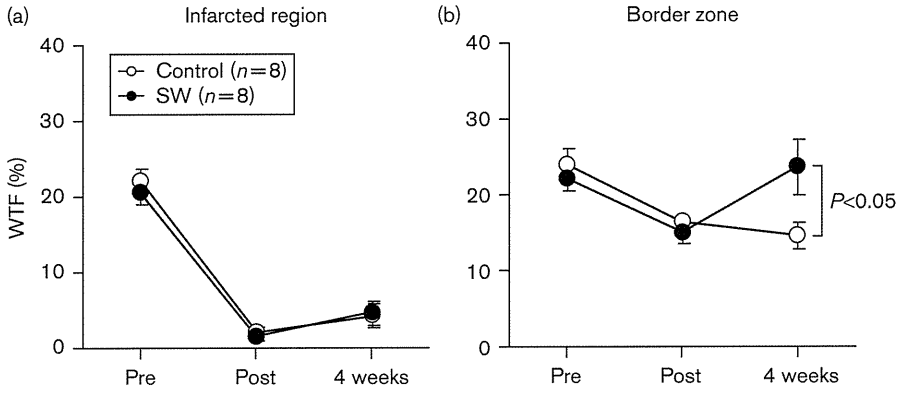
Western blot analysis showed that the ratio of phospho-eNOS to total-eNOS, a marker of eNOS activation, was

Fig. 2



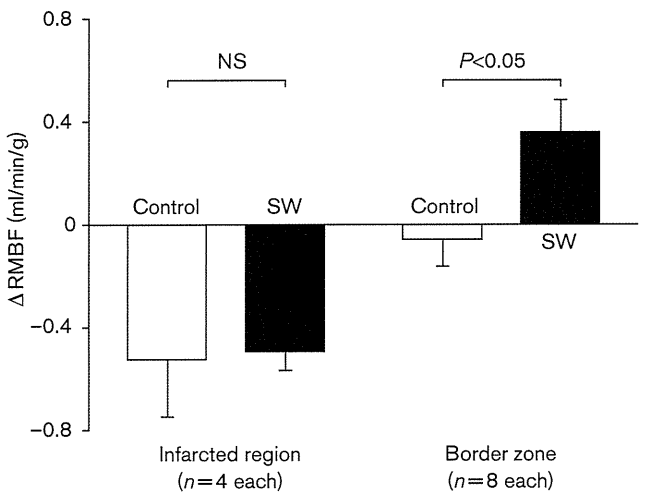
The shock wave (SW) therapy ameliorates left ventricular (LV) remodeling after myocardial ischemia–reperfusion (I/R). The SW therapy significantly ameliorated LV remodeling as evaluated by LV end-diastolic volume (LVEDV) (a), LV end-systolic volume (LVESV) (b) and LV ejection fraction (LVEF) (c) and also normalized LV end-diastolic pressure (LVEDP) (d). Pre, before I/R; Post, immediately after I/R; 4 weeks, 4 weeks after I/R.

Fig. 3



The shock wave (SW) therapy ameliorates left ventricular (LV) systolic function. An echocardiographic study showed that the wall thickening fraction (WTF) in the infarcted region was comparable between the two groups throughout the study period (a), whereas the WTF in the border zone was normalized by the SW therapy at 4 weeks after the ischemia–reperfusion (I/R) injury (b). Pre, before I/R; Post, immediately after I/R; 4 weeks, 4 weeks after I/R.

Fig. 4



The shock wave (SW) therapy ameliorates myocardial blood flow. At 4 weeks after ischemia–reperfusion, although regional myocardial blood flow (RMBF) in the infarcted area was equally reduced in the control and SW groups, the flow in the border zone was significantly increased only in the SW group.

significantly increased in the SW group than in the control group 1 week after I/R (0.41 ± 0.05 vs. 0.24 ± 0.03, $P < 0.05$) (Fig. 6a). The protein expression of VEGF also tended to be increased in the SW group compared with the control group 1 week after I/R (0.78 ± 0.26 vs. 0.40 ± 0.12, $P = 0.22$) (Fig. 6b).

Discussion

The novel finding of this study is that our extracorporeal cardiac SW therapy ameliorates LV remodeling after myocardial I/R injury in pigs *in vivo*. Importantly, no procedural complications or adverse effects with SW therapy were noted in this study, a consistent finding with our earlier studies for chronic myocardial ischemia in pigs and humans, AMI in pigs, and hind limb ischemia in rabbits [9–12,15,16,18].

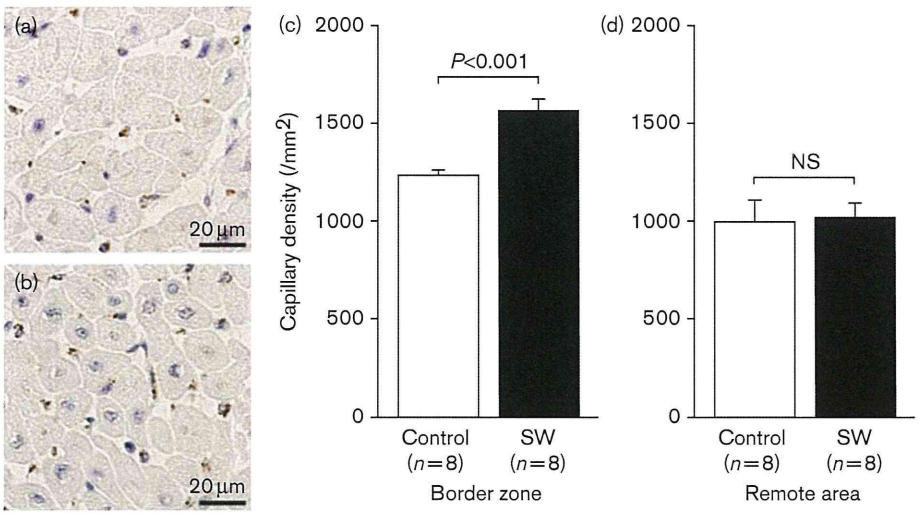
Inhibitory effects of the SW therapy on LV remodeling after I/R

Although short-term and long-term outcomes of patients with AMI have improved during the last decades as reperfusion therapy became widely available in emergency care [19–22], LV remodeling after AMI still remains one of its major complications [23]. We have recently shown that our SW therapy ameliorates LV remodeling after AMI with permanent coronary ligation in pigs *in vivo* [12]. However, in the current clinical setting, most of the AMI patients are treated with emergency reperfusion therapy. In this study, to simulate the current situation with reperfusion therapy, we examined the possible beneficial effects of our SW therapy in a porcine model of myocardial I/R *in vivo*. In this model, severe LV remodeling characterized by marked LV enlargement and reduced LVEF was noted 4 weeks after I/R in the control group, which, on the other hand, was effectively ameliorated by SW therapy. Echocardiographic study also showed that regional LV wall motion was normalized at the border zone accompanied with increased RMBF and capillary density. These results suggest that SW-induced angiogenesis at the border zone substantially contributes to the suppression of LV remodeling *in vivo*.

Mechanisms for the inhibitory effects of the SW therapy on LV remodeling after I/R

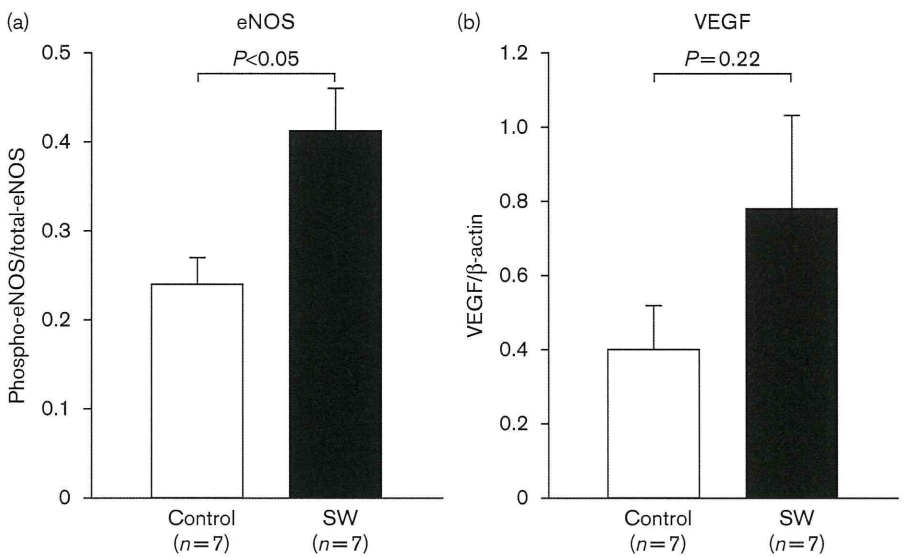
The precise mechanisms of SW-mediated suppression of LV remodeling after myocardial I/R remain to be fully elucidated. However, it is conceivable that multiple

Fig. 5



The shock wave (SW) therapy increases capillary density in the border zone. Representative factor VIII staining in the control group (a) and the SW group (b), and quantitative analysis of the vessel number in the border zone (c) and in the remote area (d). The SW therapy significantly increased the density of factor VIII-positive capillaries in the border zone, whereas the capillary density was comparable between two groups in the remote area.

Fig. 6



The shock wave (SW) enhances endothelial nitric oxide synthase (eNOS) activity. The SW therapy significantly enhanced eNOS phosphorylation, a marker of eNOS activation, in the border zone (a) and tended to do so for vascular endothelial growth factor (VEGF) protein expression (b) at 1 week after myocardial ischemia–reperfusion.

mechanisms are involved in the inhibitory effects of our SW therapy on LV remodeling after I/R. When a SW hits a tissue, the SW induces cavitation (a micrometer-sized violent collapse of bubbles) by the first compression by the positive pressure component and expansion with the tensile component of SW [24]. As the physical forces generated by cavitation are highly localized, the SW could induce localized stress on cell membranes, leading to a variety of biochemical effects including shear stress, hyperpolarization, and Ras activation [25], and the induction of stress fibers and intercellular gaps [26]. In addition, the SW induces nonenzymatic NO synthesis from L-arginine and hydrogen peroxide [27], upregulates eNOS, and suppresses nuclear factor-κB activation in the cultured human umbilical venous endothelial cells [28]. NO exerts a wide variety of biological effects including the regulation of vascular tone and angiogenesis [29–31]. In this study, we confirmed that SW therapy increases

eNOS activity and capillary density at the border zone, associated with an improvement of LV remodeling and dysfunction. These results suggest that our SW therapy improves LV remodeling after I/R, at least in part, by enhancing NO production.

Enhanced expression of multiple angiogenic factors, such as VEGF and stromal-derived factor 1, is crucial for the recruitment and incorporation of endothelial progenitor cells [32–34]. We have shown earlier that our SW therapy upregulates myocardial VEGF/Flt-1 expression in pigs *in vivo* [9]. A SW has also been reported to promote mobilization and differentiation of bone marrow-derived cells in a rat model of chronic hindlimb ischemia [35] and in rat bone marrow-derived mononuclear cells *in vitro* [36]. In this study, we confirmed that SW therapy increases RMBF, capillary density, and eNOS activity, and tended to increase the VEGF expression at the border zone. Although the renin–angiotensin system plays an important role in the pathogenesis of LV remodeling after AMI, partly because of enhanced myocardial fibrosis [37–40], the extent of the fibrosis at the border zone was comparable between the two groups in this study.

Advantage of the non-invasive SW therapy

The gene [1–3] and cell therapies [4–8], although worthy of development, require invasive procedures such as general anesthesia, cardiac catheterizations, and open chest surgery [4–8,41,42] to deliver the genes or cells to the ischemic myocardium, which may limit the usefulness of these therapies in the clinical setting. Our extracorporeal cardiac SW therapy is quite noninvasive and safe without any adverse effects, which is a major advantage of our SW therapy. This is an important point in determining the clinical usefulness of angiogenic therapies, especially in elderly patients with severe ischemic heart disease.

Limitations of the study

Several limitations of this study should be mentioned. First, we observed a trend for but not a significant increase in the VEGF level in the SW group compared with the control group, although we have earlier shown a significant increase in the VEGF expression by SW therapy in a porcine model of chronic myocardial ischemia [9]. This discrepancy may be partly because of the different stage of myocardial ischemia examined. In our earlier study, the VEGF protein level was evaluated 8 weeks after creating a chronic myocardial ischemia, when the VEGF level might have returned to the normal level, and therefore the SW-induced enhancement of the VEGF expression was clearly detected [9]. On the other hand, in this study, the VEGF level was studied 1 week after creating a myocardial I/R, when the expression of VEGF was still strongly enhanced even in the control group. Importantly, however, we were able to show the significant upregulation of eNOS by the SW therapy in this study, which we did not examine in the earlier study [9]. Second, although we were able to show that our SW therapy enhances angiogenesis at the border zone of the LV, the effects of the SW therapy on each component of the myocardial tissue, including vascular endothelial cells, vascular smooth muscle cells, cardiomyocytes, extracellular matrix, and inflammatory cells, remain to be clarified in future studies. Third, although we studied the expression of eNOS and VEGF in this study, there are many other growth factors and chemokines that could enhance angiogenesis such as stromal-derived factor 1/CXCR4 system and angiopoietin/Tie-2 system. This point also remains to be examined in future studies.

Conclusion

We were able to show that our low-energy extracorporeal cardiac SW therapy effectively induces angiogenesis and ameliorates LV remodeling after I/R in pigs *in vivo* without any adverse effects. Thus, our SW therapy could be a novel and safe strategy for the prevention of LV remodeling after AMI in humans.

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There is no conflict of interest.

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体外衝撃波を用いた非侵襲性血管新生治療

伊藤　健太　　下川　宏明

要　旨

我が国では，人口の高齢化や生活習慣の欧米化に伴い，虚血性心疾患や閉塞性動脈硬化症といった動脈硬化性疾患患者が増加してきている．我々は，基礎研究の結果を基に，低出力の衝撃波を用いた血管新生療法(「低出力体外衝撃波治療」)を開発し，①重症狭心症，②急性心筋梗塞，③下肢閉塞性動脈硬化症を対象に臨床試験を行っている．重症狭心症に対しては，第1次臨床試験（オープン試験）と第2次臨床試験（二重盲検プラセボ対照試験）を行い，本治療法の有効性と安全性を確認し，論文報告している．本治療法は，麻酔や侵襲的な処置を伴わずに，体外から治療を行うことができる非侵襲的な治療法であり，繰り返し行うことも可能である．今後幅広い疾患への応用が期待される．

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Key words：虚血性心疾患，閉塞性動脈硬化症，血管新生，衝撃波治療

はじめに

我が国では，人口の高齢化や生活習慣の欧米化・糖尿病患者の増加に伴い，虚血性心疾患や閉塞性動脈硬化症といった動脈硬化性疾患の患者数が増加の一途をたどっている．動脈硬化性疾患に対する治療は，生活習慣の改善をベースに，①薬物療法，②カテーテル治療，③バイパス手術の3本柱から成るが，近年これら従来の治療法では十分な治療効果を得られない重症例（例えば，びまん性狭窄病変を持つ症例）が増加してきている．このような症例は，胸痛や下肢痛・潰瘍形成のため生活の質（QOL）が低下するのみならず，予後も不良である．

近年，閉塞性動脈硬化症や虚血性心疾患に対して，遺伝子治療や細胞移植治療が試みられ，日本を含めた世界各国で臨床試験が行われている．これらの治療では，骨髄細胞の採取や遺伝子・細胞の送達のために全身麻酔下での骨髄穿刺や開胸操作といった大きな侵襲を伴う．そのため患者への身体的負担は大きく，また繰り返し行うことは困難である．また，有効性・安全性が認められたとしても，費用が高額になることは避けられない．一方，遺伝子治療や細胞移植治療について，動物実験の結果から期待されたほどの有効性が，臨床試験において認められていないことも指摘されている．そのため，低侵襲で，かつ有効性の期待できる新しい治療法の開発が望まれている．我々は，培養細胞や大型動物（ブタ）を用いた検討の結果を基に，低出力の衝撃波を用いた血管新生療法(「低出力体外衝撃波治療」)を開発し，現在，①重症狭心症，②急性心筋梗塞，③下肢閉塞性動脈硬化症を対象に臨床試験を行っている[1]．

いとう　けんた，しもかわ　ひろあき：東北大学大学院循環器病態学分野

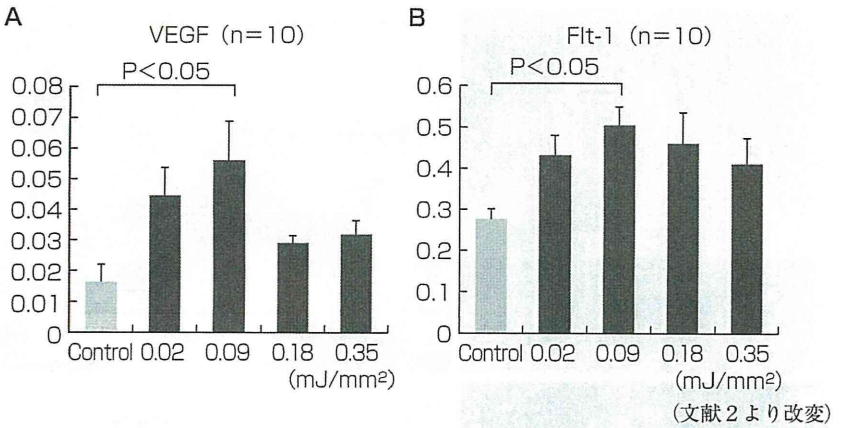

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図1. 衝撃波による血管増殖因子の発現亢進：(A) VEGF, (B) Flt-1
HUVECに衝撃波を照射すると，VEGFとFlt-1の発現が亢進した．発現の亢進は，結石破砕に用いる出力の約10%という弱い出力（0.09 mJ/mm²）の時に最大となった．

1. 衝撃波による血管新生作用

衝撃波とは音速を超えて伝わる圧力波で，同じような音響的特性を持つ媒体内を直線的に伝播していくことから，体外で発生させた衝撃波を皮膚表面から脂肪・筋肉などの体組織を通って伝播させ，体内深部の一点に収束させることができる．衝撃波を用いた治療法としては，尿路結石などの結石破砕治療が確立しており，既に20年以上前から標準的治療の1つとして保険適応にもなっている．我々は培養細胞を用いた基礎実験により，ヒト臍帯静脈内皮細胞(Human Umbilical Vein Endothelial Cells：HUVEC)に衝撃波を照射すると，結石破砕に用いる出力の約10%という弱い出力（0.09 mJ/mm²）をピークに，主要な血管新生因子の1つである血管内皮増殖因子（Vascular Endothelial Growth Factor：VEGF）およびその受容体であるFlt-1の発現が増加することを確認した(図1)[2]．そこで，ブタ慢性心筋虚血モデルにおいて体外衝撃波治療の効果を検討した．慢性虚血心筋に1日おきに3回衝撃波を照射し，4週間後に評価を行った．その結果，体外衝撃波治療により，虚血心筋組織におけるVEGFの発現が遺伝子レベル・蛋白レベルのいずれにおいても亢進していた[2]．さらに，毛細血管数の増加と冠血流の有意な増加，それに伴う左室壁運動の改善を認めた[2]．衝撃波治療中および治療後3日間のホルター心電図では重篤な不整脈を認めず，突然死も認めなかった．組織学的検討においても，出血などの組織損傷は認めなかった．以上の結果から，低出力の衝撃波を用いた体外衝撃波治療は，安全で有効な血管新生療法であることが示唆された．

2. 狭心症に対する低出力体外衝撃波治療

上記の基礎的検討に基づき，我々は，重症狭心症に対して低出力体外衝撃波治療の臨床試験を行っている．対象は以下の4条件を満たす狭心症症例とした．①20歳以上，②最大量の薬物治療に抵抗性で，かつ経皮的冠動脈形成術(percutaneous coronary intervention：PCI）や冠動脈バイパス手術（coronary artery bypass grafting：CABG）による完全な血行再建が不可能，③カナダ心臓協会（Canadian cardiovascular society：CCS)分類でClass II～IV，④負荷心筋シンチグラムや負荷心エコーで明らかな虚血領域

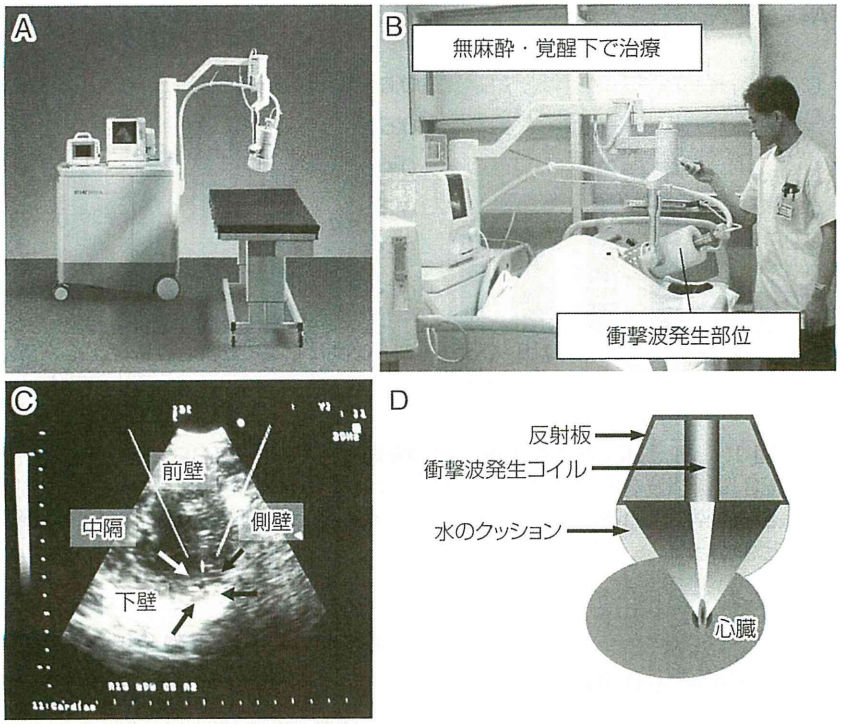

図 2. 体外衝撃波治療装置と治療風景：(A) 体外衝撃波治療装置，(B) 治療風景，(C) 心臓超音波検査画面，(D) 概念図

を認める．治療には，スイスのメーカーと共同開発した心臓病治療専用の衝撃波治療装置を用いている（図 2）．衝撃波発生ヘッドを患者の前胸壁に当て，装置に内蔵された超音波プローブで心臓を観察しながら，虚血部位に照準を合わせ，衝撃波を照射する．1 カ所につき 200 発の衝撃波を，虚血領域の広さに応じて 20～120 カ所照射する．1 回の治療時間は約 3 時間で，1～2 日おきに計 3 回治療を行う．痛みや苦痛を伴わないため，麻酔や鎮静薬の投与は必要ない．2003 年から重症狭心症患者 9 名を対象に実施した第 1 次臨床試験では，個人差はあるものの全例で狭心症症状が軽減し，ニトログリセリンの使用量が激減するなどの効果を認め，その効果は 1 年以上にわたって持続した（図 3）[3]．また，負荷心筋シンチグラムで評価した心筋血流も，衝撃波を照射した部位においてのみ改善を認めた（図 3）[3]．この結果から，衝撃波を照射した部位のみで血管新生が生じ，心筋灌流が改善したと考えられた．一方，治療に伴う合併症や副作用は全く認めなかった．さらに，2005 年から，低出力体外衝撃波治療とプラセボ治療を比較する第 2 次臨床試験を実施し，その結果，低出力体外衝撃波治療後には，CCS分類による狭心症の重症度，ニトログリセリンの使用頻度，6 分間歩行距離が有意に改善し，MRIで測定した左室一回拍出量，左室駆出率も有意に増加した（図 4）[4]．これらの効果はプラセボ治療後では認められなかった．本年 7 月，狭心症に対する低出力体外衝撃波治療は，厚生労働省の高度医療に承認された．

3. 急性心筋梗塞に対する低出力体外衝撃波治療

我が国では，急性心筋梗塞の発症早期にPCIによる再灌流療法が行われ，心筋梗塞発症早期

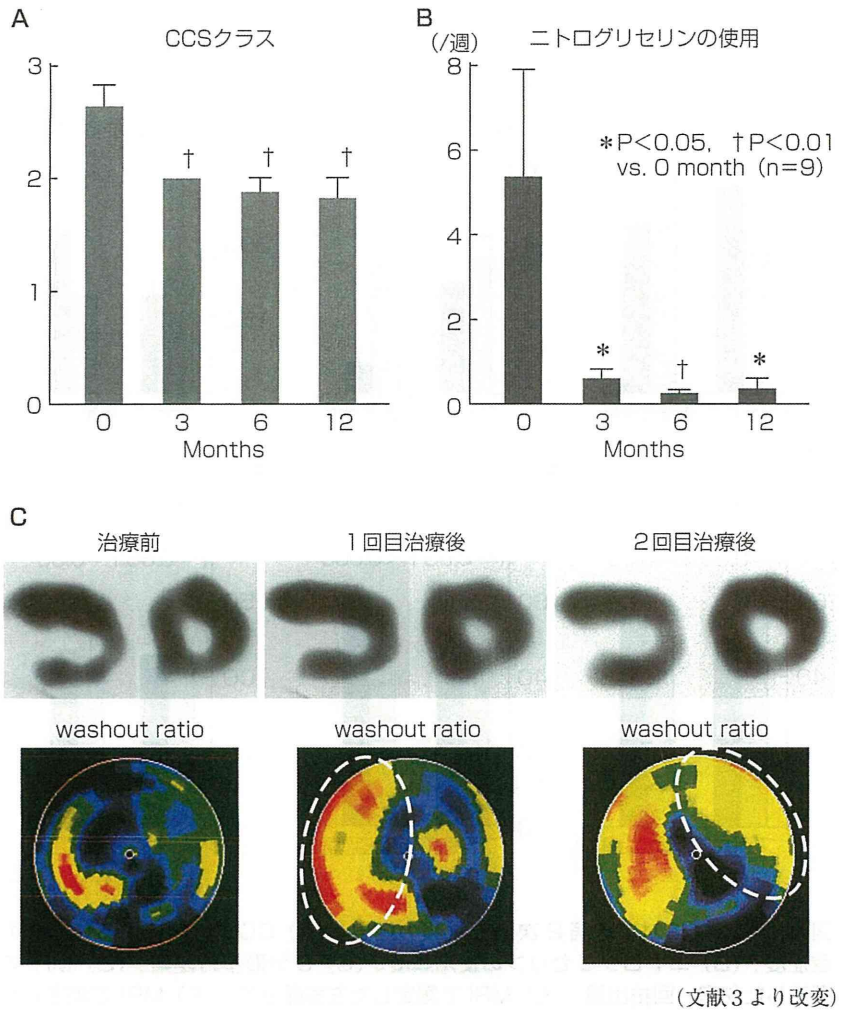

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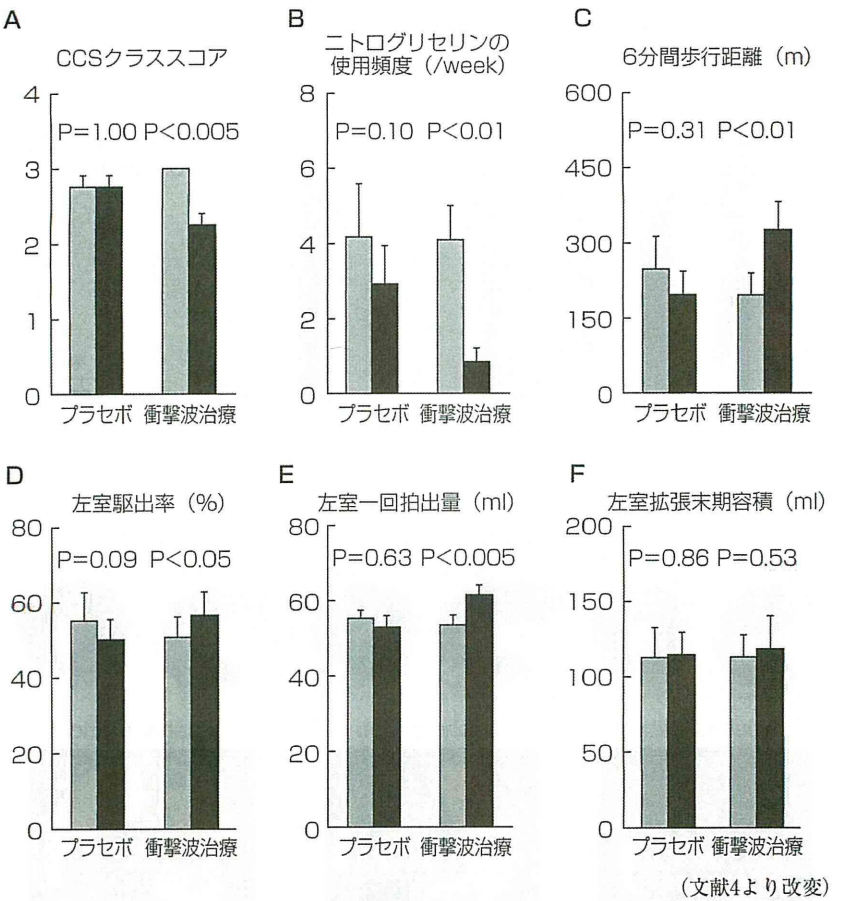
図3. 狭心症に対する第1次臨床試験の結果：(A) CCS分類による狭心症の重症度，(B) ニトログリセリンの使用頻度，(C) 負荷心筋シンチグラムによる心筋血流（青が虚血，黄～赤が血流良好を意味する）

自覚症状の改善を認めた（A，B）．また，衝撃波治療を受けた領域（破線で示された領域）でのみ血流の改善を認めた（C）．

の死亡率低下に貢献している．しかし十分な薬物治療を受けていても，慢性期には左室リモデリング（心拡大と収縮能低下）が進行し，重篤な心不全へ進行する例が少なくない．再灌流療法成功後も梗塞巣周囲では組織の浮腫や炎症により微小循環障害が遷延しており，これが左室リモデリング進行の一因と考えられている．そこで我々は，ブタ急性心筋梗塞モデルを用いて，低出力体外衝撃波治療が，慢性期の左室リモデリングを抑制するか検討した．急性心筋梗塞作成急性期に梗塞境界領域に対して低出力の衝撃波を照射し，4週間後に評価を行ったところ，左室リモデリングが軽減されることが確認された[5,6]．さらに，梗塞巣周囲の毛細血管数の増加していたことから，急性期の低出力体外衝撃波治療により境界領域での血管新生が促進されて梗塞サイズの拡大が抑制されたと考えられた．この結果を基に，2007年から急性心筋梗塞に対



（文献4より改変）

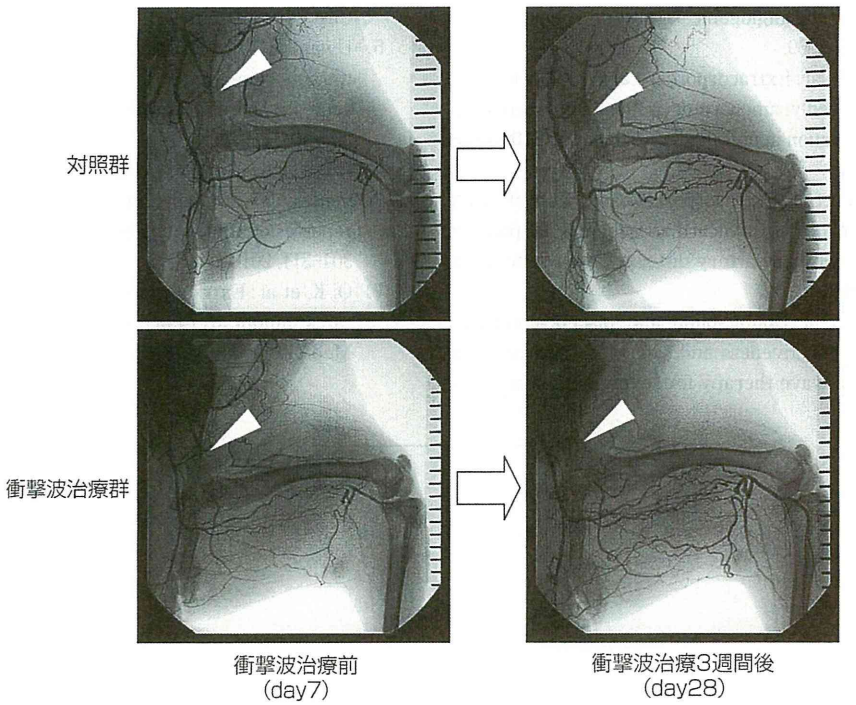
図4. 狭心症に対する第2次臨床試験の結果：(A) CCS分類による狭心症の重症度，(B) ニトログリセリンの使用頻度，(C) 6分間歩行距離，(D) MRIで測定した左室一回拍出量，(E) MRIで測定した左室駆出率，(F) MRIで測定した左室拡張末期容積

自覚症状の改善（A，B），運動耐用能の改善（C），心機能の改善（D，E）を認めた．

する体外衝撃波治療の臨床試験を行っている．この試験では，PCIによる再灌流療法が成功した症例に対して，心筋梗塞発症72時間以内に衝撃波治療を開始し，1〜2日おきに計3回行い，慢性期の左室リモデリングや心不全発症の抑制効果を評価する．

4. 下肢閉塞性動脈硬化症に対する低出力体外衝撃波治療

我々は，ウサギの大腿動脈〜膝窩動脈を摘出することにより下肢虚血を作成し，下肢虚血作成1週間後に低出力体外衝撃波治療を行った．治療3週間後に評価を行った結果，対照群に比して，低出力体外衝撃波治療群では虚血肢における側副血行路の発達が促進され，血管造影上の血流スコアや下肢血圧の改善効果を認めた（図5）[7]．これらの結果を基に，2007年から，間歇性跛行症状を有する慢性閉塞性動脈硬化症症例を対象に，低出力体外衝撃波治療の臨床試験を行っている．



(文献5より改変)

図5. 下肢虚血に対する体外衝撃波治療の効果
体外衝撃波治療群（下段）では，対照群（上段）に比して，虚血肢における側副血行路の発達が促進された．

おわりに

我々が開発している低出力体外衝撃波治療は極めて低侵襲であり，血管新生を促進することにより組織灌流を改善することが期待される新しい治療法である．本治療法の着想や至適治療条件の決定，動物実験および臨床試験などの先駆的な研究は，すべて我々がトップランナーとして，世界で最初に論文発表を行ってきた．狭心症に対する低出力体外衝撃波治療については，欧州などで医療機器として認可され，既に世界10カ国以上で500名以上の狭心症症例の治療に用いられている．我が国においては，厚生労働省の高度医療評価制度へ申請中である．近年，整形外科領域でも肘や肩，足底等の疼痛治療や難治性の骨折の治療にも低出力の衝撃波を用いた治療の有効性が報告されており，今後，幅広い疾患への応用が期待される．

本治療法の開発は，厚生労働科学研究費補助金を得て行っており，平成21年度には，研究課題「低侵襲性体外衝撃波治療法の実用化を目指したエビデンス確立のための拠点形成」が厚生労働省の先端医療開発特区（スーパー特区）に採択された．現在，幅広い疾患への適応拡大を目指して，様々な専門領域の研究者との共同研究による基礎研究および臨床試験を進めている．治療法の詳細並びに最新情報に関しては，以下のホームページを参照されたい（http://www.cardio.med.tohoku.ac.jp/shockwave/index.html）．

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REVIEW ARTICLE

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Extracorporeal Shock Wave Therapy for Ischemic Cardiovascular Disorders

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Contents

Abstract

Ischemic heart disease is the leading cause of death and a major cause of hospital admissions, with the number of affected patients increasing worldwide. The current management of ischemic heart disease has three major therapeutic options: medication, percutaneous coronary intervention (PCI), and coronary artery bypass grafting (CABG). However, the prognosis for patients with severe ischemic heart disease without indications for PCI or CABG still remains poor due to the lack of effective treatments. It is therefore crucial to develop alternative therapeutic strategies for severe ischemic heart disease. Extracorporeal shock wave (SW) therapy was introduced clinically more than 20 years ago to fragment kidney stones, which has markedly improved the treatment of urolithiasis. We found that a low-energy SW (about 10% of the energy density used for urolithiasis) effectively increases the expression of vascular endothelial growth factor (VEGF) in cultured endothelial cells. Based on this *in vitro* study, we initiated *in vivo* studies and have demonstrated that extracorporeal cardiac SW therapy with a low-energy SW up-regulates the expression of VEGF, induces neovascularization, and improves myocardial ischemia in a porcine model of chronic myocardial ischemia, without any adverse effects *in vivo*. On the basis of promising results in animal studies, we performed a series of clinical studies in patients with severe coronary artery disease without indication for PCI or CABG, including, firstly, an open trial followed by a placebo-controlled, double-blind study. In both studies, our extracorporeal cardiac SW therapy improved symptoms, exercise capacity, and myocardial perfusion in patients with severe coronary artery disease. Importantly, no procedural complications or adverse effects were noted. The SW therapy was also effective in ameliorating left ventricular remodeling after acute myocardial infarction (MI) in pigs and in enhancing angiogenesis in hind-limb ischemia in rabbits. Based on these animal studies, we are also conducting clinical studies in patients with acute MI and in those with peripheral artery disease. Thus, our extracorporeal cardiac SW therapy appears to be an effective, safe, and non-invasive angiogenic approach in cardiovascular medicine and its indication could be extended to a variety of ischemic diseases in the near future. In this article, we briefly summarize our work in animals and humans, and discuss the advantages and perspectives of our extracorporeal SW therapy.

1. Introduction

Ischemic heart disease is the leading cause of death and a major cause of hospital admissions, with the number of affected patients increasing worldwide.[1] The current management of ischemic heart disease has three major therapeutic options: medication, percutaneous coronary intervention (PCI), and coronary artery bypass grafting (CABG). However, the prognosis for patients with severe ischemic heart disease without indications for PCI or CABG still remains poor due to the lack of effective treatments. Therefore, it is crucial to develop alternative therapeutic strategies for severe ischemic heart disease. During this decade, a variety of regenerative therapies, such as gene and cell therapies, have been investigated.[2-12] However, most of these regenerative therapies are invasive in nature. In addition, although many of these therapies have been shown to be effective in animal models, their efficacy and safety have not yet been fully established in clinical trials.[13-20]

Extracorporeal shock wave (SW) therapy was introduced clinically more than 20 years ago to fragment kidney stones, and has markedly improved the treatment of urolithiasis. Extracorporeal SW lithotripsy with high-energy SW is also indicated for gallstones and pancreatic and salivary stones. We have previously reported that low-energy cardiac SW therapy effectively induces neovascularization and improves myocardial ischemia in a porcine model of chronic myocardial ischemia.[21,22] Based on the promising results from animal studies, we first reported that low-energy cardiac SW therapy significantly improved symptoms and myocardial perfusion and reduced the use of nitroglycerin.[23] In this article, we briefly summarize our work in animals and humans, and discuss the advantages and perspectives of our low-energy SW therapy for ischemic diseases.

2. *In vitro* Study

SW is a longitudinal acoustic wave that propagates through water or soft tissue as ultrasound does. In contrast to ultrasound, SW is a single pressure pulse with a short needle-like positive spike <1 μsec in duration and up to 100 MPa in amplitude, followed by a tensile wave of several μsec with lower amplitude. We and others demonstrated that low-energy SW enhances nitric oxide (NO) production[24] and the expression of vascular endothelial growth factor (VEGF) and its receptor, fms-related tyrosine kinase 1 (Flt-1), in cultured human umbilical vein endothelial cells (HUVECs) *in vitro* (figure 1).[21]

Importantly, we demonstrated that the expression of VEGF peaked at 0.09 mJ/mm^2 in cultured endothelial cells, at approximately 10% of the energy used for lithotripsy treatment in the clinical setting (figure 1).[21] Subsequently, Yip et al.[25] reported that low-energy SW applied to bone-marrow-derived mononuclear cells (BMDMNCs) enhanced VEGF production from BMDMNCs and their differentiation into endothelial phenotype cells.[25] In addition, Nurzynska et al.[26] reported that low-energy SW activated proliferation and differentiation in cardiac primitive cells. Tamma et al.[27] also reported that SW induced the proliferation and differentiation of osteoblasts and reduced their secretion of pro-osteoclastogenic factors.

SW exerts a 'cavitation effect' (a μm-sized violent collapse of bubbles inside and outside the cells)[28] and was shown to induce localized stress on cell membranes that resembles shear stress,[29] due to the localized nature of the physical forces generated by cavitation.[30] Several biochemical effects of SW have been reported including hyperpolarization, Ras activation, non-enzymatic NO synthesis, and induction of stress fibers and intercellular gaps.[31-33] However, detailed intracellular mechanisms of SW action remain to be elucidated.

3. Extracorporeal Cardiac Shock Wave (SW) Therapy for Angina Pectoris

3.1 Animal Studies

Based on our *in vitro* study, we examined whether low-energy SW could ameliorate myocardial ischemia in a porcine model *in vivo*. A porcine model of chronic myocardial ischemia was made by placing an ameroid constrictor at the proximal segment of the left circumflex (LCX) coronary artery. This gradually induced a total occlusion of the artery with sustained myocardial dysfunction but without myocardial infarction in 4 weeks.[21] At 4 weeks after the implantation of the ameroid

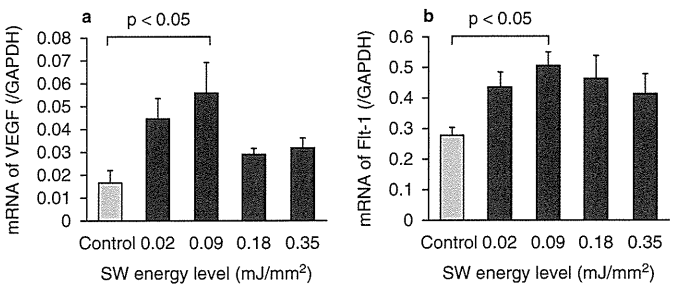

Fig. 1. Effects of shock wave (SW) therapy on mRNA expression in human umbilical vein endothelial cells (HUVECs) *in vitro*. SW treatment up-regulated mRNA expression as a proportion of glyceraldehyde dehydrogenase (GAPDH) mRNA expression of (**a**) vascular endothelial growth factor (VEGF) and (**b**) VEGF receptor, Flt-1, with a maximum effect noted at 0.09 mJ/mm^2, a level that is approximately 10% of that used for urinary lithotripsy. Results are expressed as mean ± SEM (n = 10 in each group). From Nishida et al.,[21] with permission.

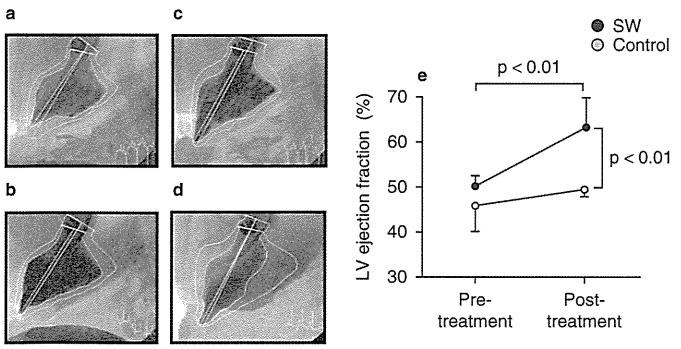

Fig. 2. Effects of shock wave (SW) therapy on left ventricular (LV) function in pigs *in vivo*. The extracorporeal cardiac SW therapy improved ischemia-induced myocardial dysfunction *in vivo* as evaluated by left ventriculography. Four weeks after the implantation of an ameroid constrictor, LV wall motion of the LCX (posterolateral) region was reduced in both (**a**) the control and (**c**) the SW group (before SW therapy). Eight weeks after the implantation of an ameroid constrictor, no significant change in LV wall motion was noted in the control group (**b**), whereas marked recovery was noted in the SW group (**d**). (**e**) SW therapy normalized LV ejection fraction in the SW group but not in the control group. Results are expressed as mean ± SEM (n = 8 in each group). From Nishida et al.,[21] with permission.

constrictor, we performed extracorporeal SW therapy to the ischemic myocardium three times during the first week (n = 8), whereas animals in the control group (n = 8) received the same anesthesia procedures three times a week but without the SW treatment. Based on our *in vitro* experiments, we applied low-energy SW (0.09 mJ/mm^2) to nine spots in the ischemic LCX region (200 shots/spot) with the guidance of an echocardiogram equipped with a specially designed SW generator (Storz Medical AG, Tägerwilen, Switzerland). In order to treat the targeted ischemic myocardium without inducing ventricular arrhythmia, we applied SW at end-diastole during the cardiac cycle with an R-wave-triggered system. We evaluated cardiac function before (baseline) and at 4 and 8 weeks after the ameroid implantation.

Four weeks after the implantation of an ameroid constrictor, wall motion of the posterolateral (LCX) region in the left ventricle (LV) was reduced in both the control and the SW groups to the same extent (figure 2a,c). However, 4 weeks after the SW therapy, left ventriculography showed marked improvement of LV wall motion only in the SW group (figure 2b,d). The SW therapy normalized the LV ejection fraction in the SW group but not in the control group (figure 2e). In this study, the SW treatment normalized global and regional myocardial function as well as regional myocardial blood flow in the chronic ischemic region, evaluated using colored microspheres (Dye-Trak, Triton Technology) and spectrophotometry. In addition, the SW therapy increased capillary density and up-regulated VEGF expression in the ischemic myocardium *in vivo* (figure 3). Importantly, no procedural complications or adverse effects, such as tissue injury, hemorrhage, or arrhythmia, were noted during or after the SW therapy. These results suggest that the low-energy cardiac SW therapy activates the endogenous angiogenic system in pigs *in vivo*.[21] This was the first report to demonstrate the potential usefulness of extracorporeal cardiac SW therapy as a non-invasive treatment for chronic myocardial ischemia.

3.2 Clinical Studies

Based on the promising results in animal studies, we performed the first clinical trial of extracorporeal cardiac SW therapy in an open-labeled manner.[23] We performed cardiac SW therapy (200 shots/spot at 0.09 mJ/mm^2 for 20–40 spots, three times a week/series) in nine patients with end-stage coronary artery disease (CAD) with no indication for PCI or CABG (55–82 years old, five men and four women). During the therapy, the patients lay on the bed in a supine position without any anesthesia (figure 4). Importantly, our SW therapy significantly improved symptoms and reduced nitroglycerin use

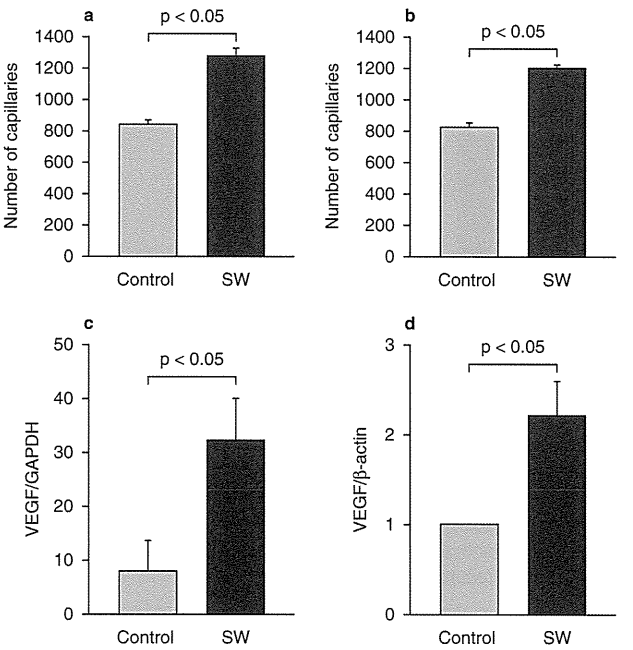

Fig. 3. Effects of shock wave (SW) therapy on capillary density and vascular endothelial growth factor (VEGF) expression in the ischemic myocardium in pigs *in vivo*. The extracorporeal cardiac SW therapy increased the density of factor VIII-positive capillaries and VEGF expression in the ischemic myocardium. Capillary density was significantly greater in the SW group than in the control group in both (**a**) the endocardium and (**b**) the epicardium. The (**c**) mRNA expression and (**d**) protein levels of VEGF as proportions of glyceraldehyde dehydrogenase (GAPDH) mRNA expression and β-actin, respectively, were significantly higher in the SW group than in the control group. Results are expressed as mean ± SEM (n = 6 in each group). From Nishida et al.,[21] with permission.

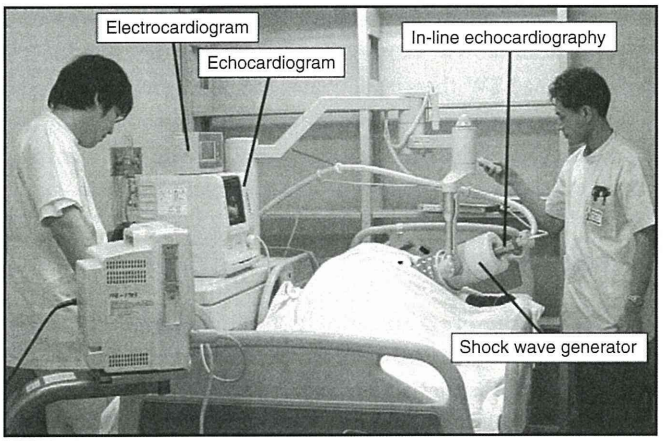

Fig. 4. Extracorporeal cardiac shock wave (SW) therapy in action in a patient with severe coronary artery disease. The machine is equipped with a SW generator and in-line echocardiography. The SW generator is attached to the chest wall of the patient when used. The SW pulse is easily focused on the ischemic myocardium under the guidance of echocardiography. There is no need for anesthesia or sedatives.

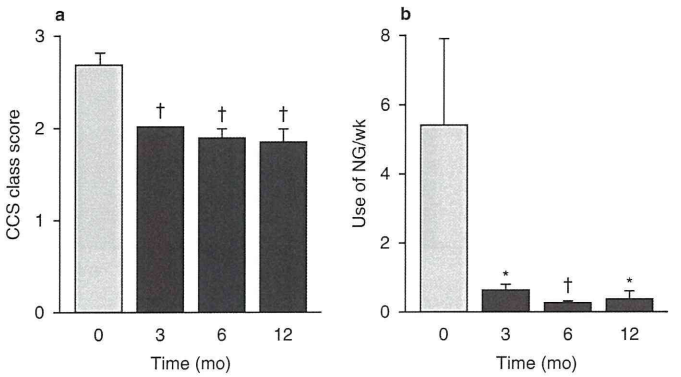

Fig. 5. Effects of extracorporeal cardiac shock wave (SW) therapy on symptoms and the use of nitroglycerin. Extracorporeal cardiac SW therapy significantly improved (**a**) the Canadian Cardiovascular Society (CCS) class scores and (**b**) number of nitroglycerin (NG) uses per week in patients with severe angina pectoris. Results are expressed as mean ± SEM. * $p < 0.05$, † $p < 0.01$ vs 0 month (statistically analyzed by a *post hoc* test after one-way ANOVA). From Fukumoto et al.,[23] with permission.

(figure 5) and improved myocardial perfusion as assessed by dipyridamole stress thallium scintigraphy only in the ischemic area treated with the SW therapy (figure 6). These beneficial effects of the SW therapy persisted for at least 12 months. No procedural complications or adverse effects were noted. These results indicated that our extracorporeal cardiac SW therapy was a safe, effective, and non-invasive therapeutic strategy for severe ischemic heart disease.[23] Following our initial report, several clinical studies with positive results were reported worldwide.[34-37] To confirm the usefulness and safety of our SW therapy, we performed a second clinical trial in a randomized and placebo-controlled manner. In this second trial, again we were able to demonstrate that the low-energy SW therapy not only improved symptoms and reduced nitroglycerin use, but also improved LV function (figure 7), establishing cardiac SW therapy as an effective and safe angiogenic strategy for severe ischemic heart disease.[38] As described above, extracorporeal cardiac SW therapy improved the quality of life in patients with angina pectoris. However, it is still not known whether our SW therapy improves the long-term prognosis of those patients. Further studies are needed.

4. Extracorporeal Cardiac SW Therapy for Acute Myocardial Infarction

The development of emergent reperfusion therapy has dramatically reduced the mortality of patients with acute myocardial infarction (AMI). However, LV remodeling following AMI, which leads to heart failure, sudden cardiac death, and poor prognosis,[39] still needs to be addressed. It was reported that capillary density in the border zone is negatively correlated with infarct size 1 month after AMI, suggesting the importance of adequate growth of the capillary microvasculature.[40] It is highly expected that enhancing neovascularization in the

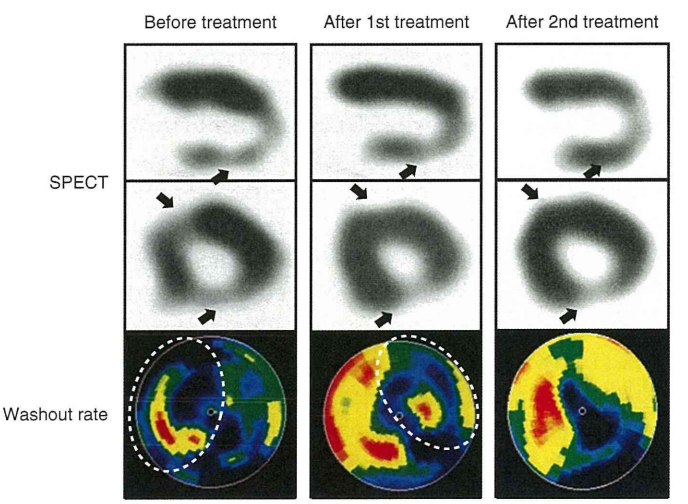

Fig. 6. Effects of extracorporeal cardiac shock wave (SW) therapy on myocardial perfusion in patients with severe angina pectoris. Dipyridamole stress thallium-201 single photon emission computed tomography (SPECT) imaging and polar map in a patient with severe three-vessel coronary artery disease before and after SW therapy. The results clearly demonstrated that SW therapy ameliorated myocardial perfusion only where SW was applied; in the anteroseptal wall after the first treatment and in the lateral wall after the second treatment (arrows) in a step-wise manner after the staged SW treatment. The areas treated with SW therapy are indicated with dotted lines. From Fukumoto et al.,[23] with permission.