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

Yoshitaka Ito, Kenta Ito, Takashi Shiroto, Ryuji Tsuburaya, Gao Jun Yi, Morihiko Takeda, Yoshihiro Fukumoto, Satoshi Yasuda and Hiroaki Shimokawa

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.**

Coronary Artery Disease 2010, **21**:304–311

Keywords: angiogenesis, reperfusion, shock wave, ventricular remodeling

Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Correspondence to Dr Kenta Ito, MD, PhD, Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai, 980-8574, Japan
Tel: +81 22 717 7153; fax: +81 22 717 7156;
e-mail: ito-kenta@cardio.med.tohoku.ac.jp

Received 5 January 2010 **Revised** 20 March 2010
Accepted 2 April 2010

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).

0954-6928 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins DOI: 10.1097/MCA.0b013e32833aec62

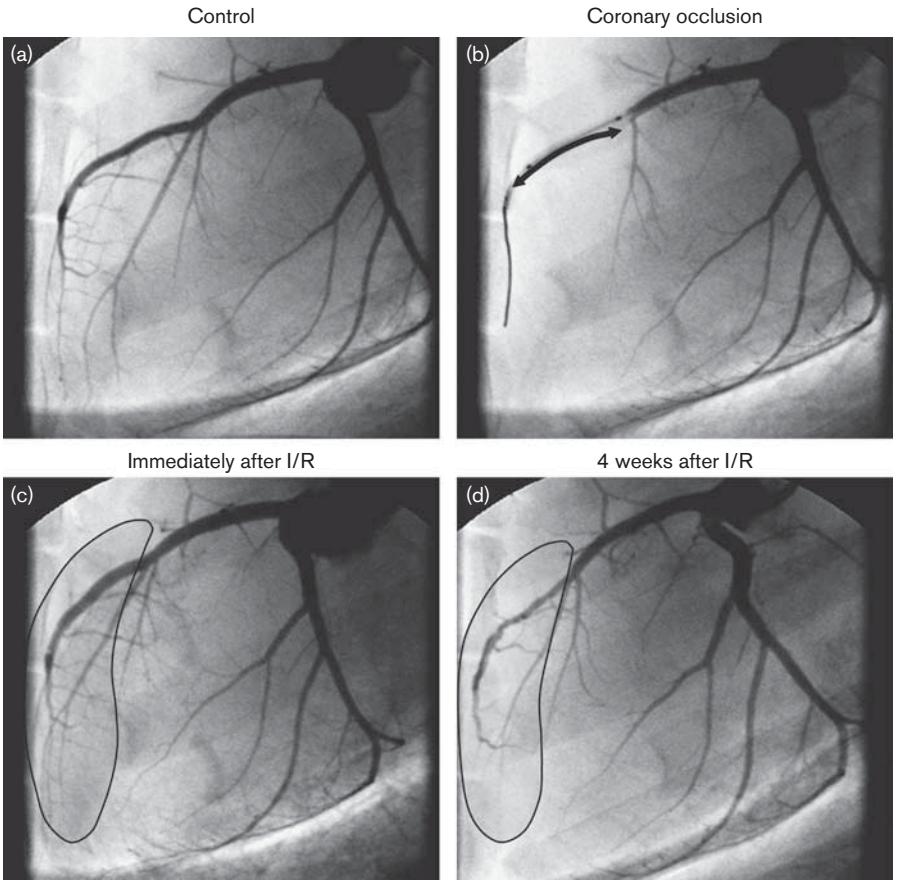
Porcine model of myocardial I/R

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. $28 \pm 2\%$, $P < 0.05$) (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. $20 \pm 1\%$; immediately after I/R, 2 ± 1 vs. $1 \pm 0.3\%$; 4 weeks, 4 ± 2 vs. $5 \pm 2\%$) (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. $22 \pm 2\%$, $P = 0.54$; immediately after I/R, 16 ± 1 vs. $15 \pm 2\%$, $P = 0.59$; and 4 weeks, 15 ± 2 vs. $24 \pm 4\%$, $P < 0.05$) (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).

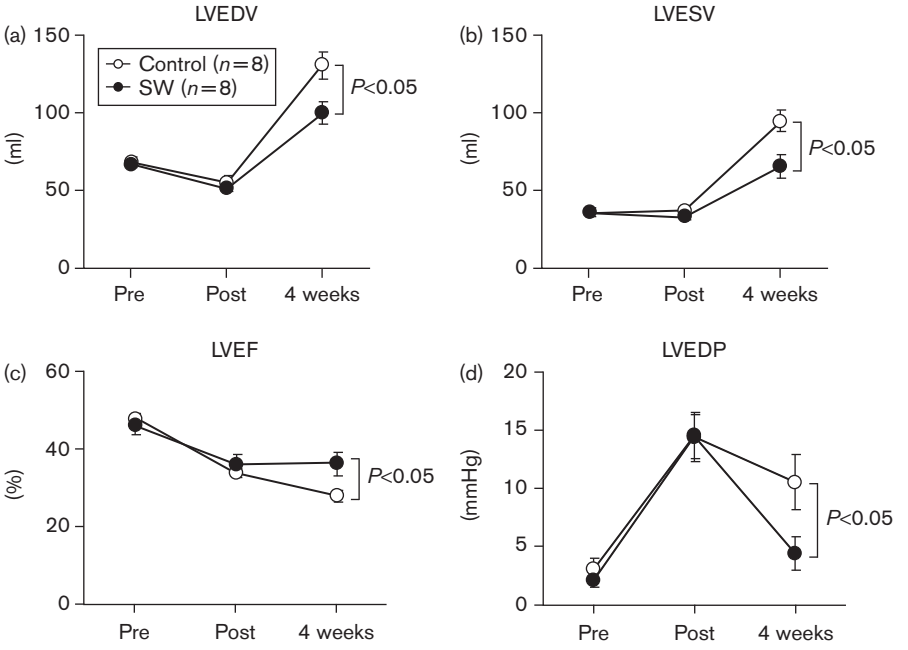
Histopathology

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. $1.233 \pm 31/mm^2$, $P < 0.001$) (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

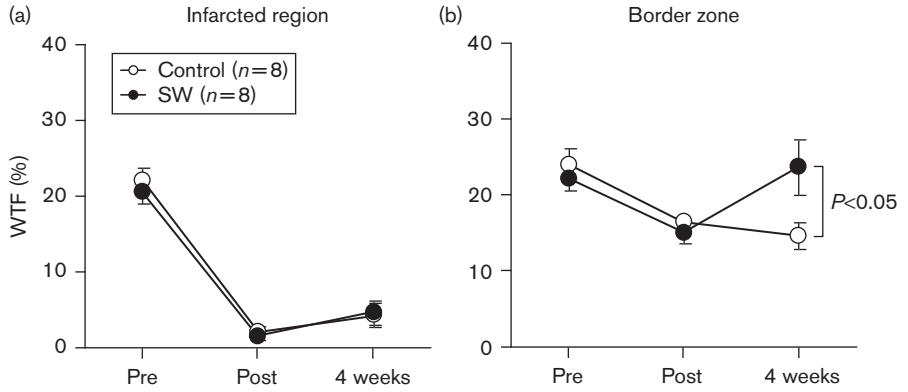
Western blot analysis showed that the ratio of phospho-eNOS to total-eNOS, a marker of eNOS activation, was

Fig. 2



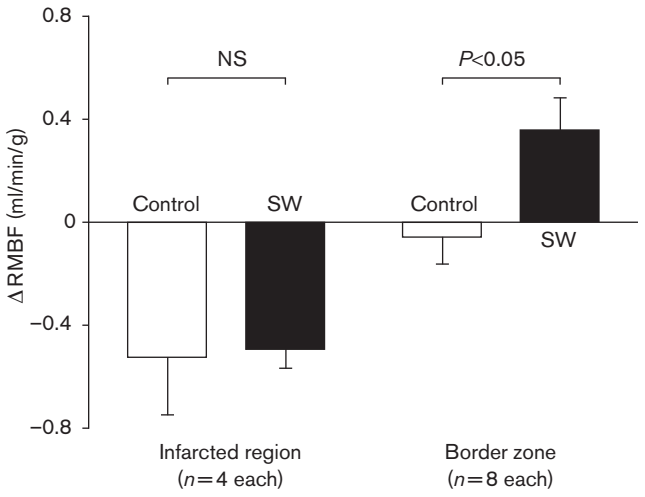
The shock wave (SW) therapy ameliorates left ventricular (LV) remodeling after myocardial ischemia–reperfusion (I/R). The SW therapy significantly ameliorated LV remodeling as evaluated by LV end-diastolic volume (LVEDV) (a), LV end-systolic volume (LVESV) (b) and LV ejection fraction (LVEF) (c) and 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].

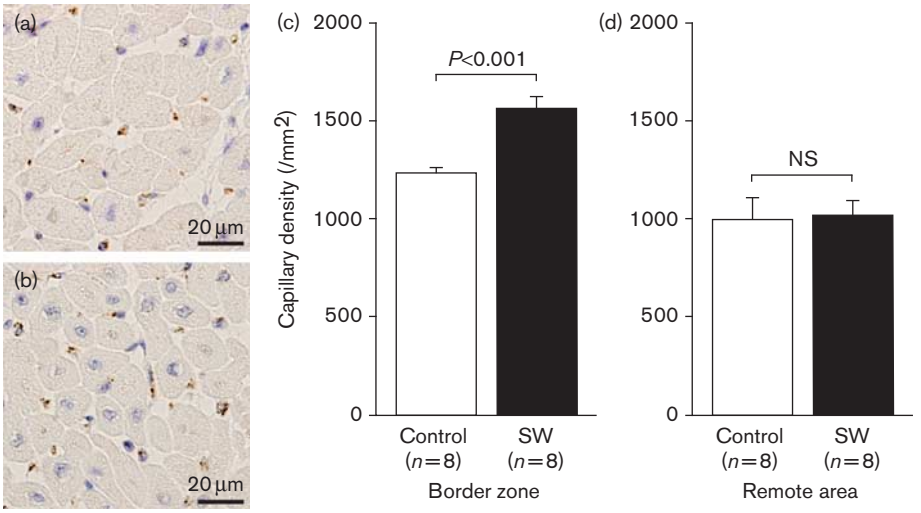
Inhibitory effects of the SW therapy on LV remodeling after I/R

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

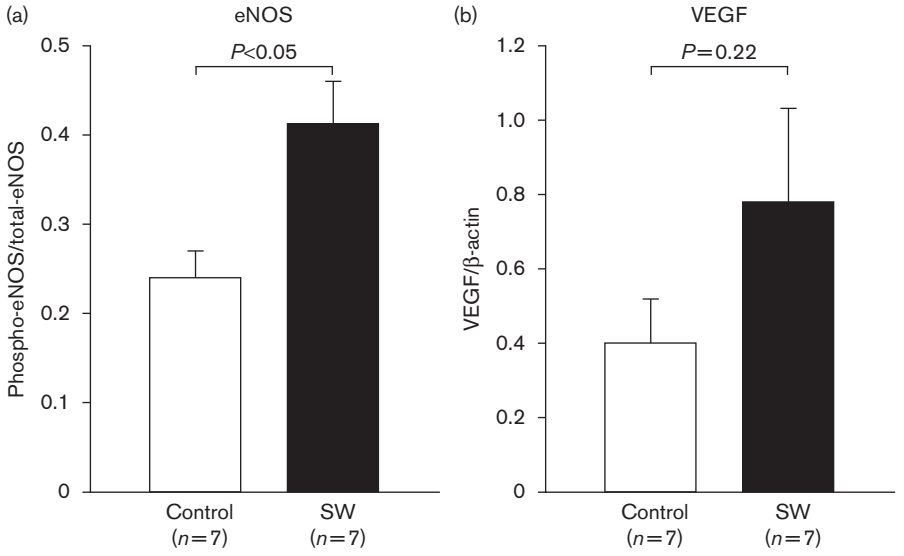
The precise mechanisms of SW-mediated suppression of LV remodeling after myocardial I/R remain to be fully elucidated. However, it is conceivable that multiple

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.

Acknowledgements

The authors thank Dr Ernest H. Marlinghaus, Storz Medical AG, Switzerland, for valuable comments on our study. The authors also thank Fumie Tatebayashi and Naomi Yamaki for their excellent technical assistance. This study was supported in part by the Global COE Program and Grants-in-Aid from the Japanese Ministry of Education, Culture, Sports, Science, and Technology, Tokyo, Japan, and the Japanese Ministry of Health, Labor, and Welfare, Tokyo, Japan.

There is no conflict of interest.

References

1 Rutanen J, Rissanen TT, Markkanen JE, Gruchala M, Silvennoinen P, Kivelä A, *et al.* Adenoviral catheter-mediated intramyocardial gene transfer using the mature form of vascular endothelial growth factor-D induces transmural angiogenesis in porcine heart. *Circulation* 2004; **109**:1029–1035.

2 Kastrup J, Jørgensen E, Rück A, Tägil K, Glogar D, Ruzyllo W, *et al.* Direct intramyocardial plasmid vascular endothelial growth factor-A165 gene therapy in patients with stable severe angina pectoris. A randomized double-blind placebo-controlled study: the Euroinject One trial. *J Am Coll Cardiol* 2005; **45**:982–988.

3 Gupta R, Tongers J, Losordo DW. Human studies of angiogenic gene therapy. *Circ Res* 2009; **105**:724–736.

4 van Ramshorst J, Bax JJ, Beeres SL, Dibbets-Schneider P, Roes SD, Stokkel MP, *et al.* Intramyocardial bone marrow cell injection for chronic myocardial ischemia: a randomized controlled trial. *JAMA* 2009; **301**:1997–2004.

5 Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, *et al.* Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet* 2004; **364**:141–148.

6 Schächinger V, Assmus B, Britten MB, Honold J, Lehmann R, Teupe C, *et al.* Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final 1-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol* 2004; **44**:1690–1699.

7 Schächinger V, Erbs S, Elsässer A, Haberbosch W, Hambrecht R, Hölschermann H, *et al.* Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial

infarction: final 1-year results of the REPAIR-AMI trial. *Eur Heart J* 2006; **27**:2775–2783.
8 Davani S, Deschaseaux F, Chalmers D, Tiberghien P, Kantelip JP. Can stem cells mend a broken heart? *Cardiovasc Res* 2005; **65**:305–316.
9 Nishida T, Shimokawa H, Oi K, Tatewaki H, Uwatoku T, Abe K, *et al.* Extracorporeal cardiac shock wave therapy markedly ameliorates ischemia-induced myocardial dysfunction in pigs in vivo. *Circulation* 2004; **364**:183–192.
10 Fukumoto Y, Ito A, Uwatoku T, Matoba T, Kishi T, Tanaka H, *et al.* Extracorporeal cardiac shock wave therapy ameliorates myocardial ischemia in patients with severe coronary artery disease. *Coron Artery Dis* 2006; **17**:63–70.
11 Kikuchi Y, Ito K, Ito Y, Shiroto T, Tsuburaya R, Aizawa K, *et al.* Double-blind and placebo-controlled study of the effectiveness and safety of extracorporeal cardiac shock wave therapy for severe angina pectoris. *Circ J* 2010; **74**:589–591.
12 Uwatoku T, Ito K, Abe K, Oi K, Hizume T, Sunagawa K, *et al.* Extracorporeal cardiac shock wave therapy improves left ventricular remodeling after acute myocardial infarction in pigs. *Coron Artery Dis* 2007; **18**:397–404.
13 Klein HH, Schubothe M, Nebendahl K, Kreuzer H. Temporal and spatial development of infarcts in porcine hearts. *Basic Res Cardiol* 1984; **79**:440–447.
14 Pich S, Klein HH, Lindert S, Nebendahl K, Kreuzer H. Cell death in ischemic, reperfused porcine hearts: a histochemical and functional study. *Basic Res Cardiol* 1988; **83**:550–559.
15 Shimokawa H, Ito K, Fukumoto Y, Yasuda S. Extracorporeal cardiac shock wave therapy for ischemic heart disease. *Shock Waves* 2008; **17**:449–455.
16 Ito K, Fukumoto Y, Shimokawa H. Extracorporeal shock wave therapy as a new and non-invasive angiogenic therapy. *Tohoku J Exp Med* 2009; **219**:1–9.
17 Versteilen AM, Korstjens IJ, Musters RJ, Groeneveld AB, Sipkema P. Rho kinase regulates renal blood flow by modulating eNOS activity in ischemia-reperfusion of the rat kidney. *Am J Physiol Renal Physiol* 2006; **291**:F606–F611.
18 Oi K, Fukumoto Y, Ito K, Uwatoku T, Abe K, Hizume T, *et al.* Extracorporeal shock wave therapy ameliorates hindlimb ischemia in rabbits. *Tohoku J Exp Med* 2008; **214**:151–158.
19 Kushner FG, Hand M, Smith SC Jr, King SB III, Anderson JL, Antman EM, *et al.* 2009 Focused Updates: ACC/AHA Guidelines for the Management of Patients With ST-Elevation Myocardial Infarction (updating the 2004 Guideline and 2007 Focused Update) and ACC/AHA/SCAI Guidelines on Percutaneous Coronary Intervention (updating the 2005 Guideline and 2007 Focused Update): a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *Circulation* 2009; **120**:2271–2306.
20 Van de Werf F, Bax J, Betriu A, Blomstrom-Lundqvist C, Crea F, Falk V, *et al.* Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation: the Task Force on the Management of ST-Segment Elevation Acute Myocardial Infarction of the European Society of Cardiology. *Eur Heart J* 2008; **29**:2909–2945.
21 Yasuda S, Shimokawa H. Acute myocardial infarction: the enduring challenge for cardiac protection and survival. *Circ J* 2009; **73**:2000–2008.
22 Takii T, Yasuda S, Takahashi J, Ito K, Shiba N, Shirato K, *et al.* Trends in acute myocardial infarction incidence and mortality over 30 years in Japan: report from the MIYAGI-AMI registry study. *Circ J* 2010; **74**:93–100.
23 Jessup M, Brozena S. Heart failure. *N Engl J Med* 2003; **348**:2007–2018.
24 Apfel RE. Acoustic cavitation: a possible consequence of biomedical uses of ultrasound. *Br J Cancer* 1982; **45** (**Suppl**):140–146.
25 Wang FS, Wang CJ, Huang HJ, Chung H, Chen RF, Yang KD. Physical shock wave mediates membrane hyperpolarization and Ras activation for osteogenesis in human bone marrow stromal cells. *Biochem Biophys Res Commun* 2001; **287**:648–655.
26 Seidl M, Steinbach P, Wörle K, Hofstädter F. Induction of stress fibers and intercellular gaps in human vascular endothelium by shock-waves. *Ultrasonics* 1994; **32**:397–400.
27 Gotte G, Amelio E, Russo S, Marlinghaus E, Musci G, Suzuki H. Short-time non-enzymatic nitric oxide synthesis from L-arginine and hydrogen peroxide induced by shock waves treatment. *FEBS Lett* 2002; **520**:153–155.
28 Mariotto S, Cavalieri E, Amelio E, Ciampa AR, de Prati AC, Marlinghaus E, *et al.* Extracorporeal shock waves: from lithotripsy to anti-inflammatory action by NO production. *Nitric Oxide* 2005; **12**:89–96.
29 Amano K, Matsubara H, Iba O, Okigaki M, Fujiyama S, Imada T, *et al.* Enhancement of ischemia-induced angiogenesis by eNOS overexpression. *Hypertension* 2003; **41**:156–162.
30 Murohara T, Asahara T, Silver M, Bauters C, Masuda H, Kalka C, *et al.* Nitric oxide synthase modulates angiogenesis in response to tissue ischemia. *J Clin Invest* 1998; **101**:2567–2578.
31 Shimokawa H. Primary endothelial dysfunction: atherosclerosis. *J Mol Cell Cardiol* 1999; **31**:23–37.
32 Millauer B, Wizigmann-Voos S, Schnürch H, Martinez R, Møller NP, Risau W, *et al.* High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell* 1993; **72**:835–846.
33 Grunewald M, Avraham I, Dor Y, Bachar-Lusting E, Itin A, Yung S, *et al.* VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell* 2006; **124**:175–189.
34 Askari AT, Unzek S, Popovic ZB, Goldman CK, Forudi F, Kiedrowski M, *et al.* Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet* 2003; **362**:697–703.
35 Aicher A, Heeschen C, Sasaki K, Urbich C, Zeiher AM, Dimmeler S. Low-energy shock wave for enhancing recruitment of endothelial progenitor cells: a new modality to increase efficacy of cell therapy in chronic hind limb ischemia. *Circulation* 2006; **114**:2823–2830.
36 Yip HK, Chang LT, Sun CK, Youssef AA, Sheu JJ, Wang CJ. Shock wave therapy applied to rat bone marrow-derived mononuclear cells enhances formation of cells stained positive for CD31 and vascular endothelial growth factor. *Circ J* 2008; **72**:150–156.
37 Pfeffer MA, McMurray JJ, Velazquez EJ, Rouleau JL, Køber L, Maggioni AP, *et al.* Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. *N Engl J Med* 2003; **349**:1893–1906.
38 Latini R, Maggioni AP, Flather M, Sleight P, Tognoni G. ACE inhibitor use in patients with myocardial infarction. Summary of evidence from clinical trials. *Circulation* 1995; **92**:3132–3137.
39 Weber KT. Extracellular matrix remodeling in heart failure: a role for de novo angiotensin II generation. *Circulation* 1997; **96**:4065–4082.
40 Ertl G, Frantz S. Healing after myocardial infarction. *Cardiovasc Res* 2005; **66**:22–32.
41 Dohmann HF, Perin EC, Takiya CM, Silva GV, Silva SA, Sousa AL, *et al.* Transendocardial autologous bone marrow mononuclear cell injection in ischemic heart failure: postmortem anatomicopathologic and immunohistochemical findings. *Circulation* 2005; **112**:521–526.
42 Stamm C, Westphal B, Kleine HD, Petzsch M, Kittner C, Klinge H, *et al.* Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet* 2003; **361**:45–46.