

# Extracorporeal cardiac shock wave therapy improves left ventricular remodeling after acute myocardial infarction in pigs

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**Objective** We have recently demonstrated that low-energy extracorporeal shock wave therapy improves chronic myocardial ischemia in pigs and humans. In this study, we examined whether our shock wave therapy is also effective at improving left ventricular remodeling after acute myocardial infarction in pigs.

**Methods** Acute myocardial infarction was created by surgically excising the proximal segment of the left circumflex coronary artery ( $n=20$ ). In the early treatment protocol, the shock wave therapy was started 3 days after acute myocardial infarction, whereas in the late treatment protocol, the therapy was started 4 weeks after acute myocardial infarction ( $n=5$  each). The remaining animals were treated in the same manner, but without the shock wave treatment in each protocol ( $n=5$  each).

**Results** In the early treatment protocol, left ventricular ejection fraction was higher ( $42 \pm 1$  vs.  $32 \pm 1\%$ ,  $P < 0.001$ ) and left ventricular end-diastolic volume was smaller ( $95 \pm 1$  vs.  $99 \pm 2$  ml,  $P < 0.05$ ) in the shock wave group compared with the control group. Furthermore, wall thickening fraction ( $32 \pm 1$  vs.  $28 \pm 1\%$ ,  $P < 0.01$ ), regional myocardial blood flow ( $1.7 \pm 0.2$  vs.  $1.0 \pm 0.1$  ml/min/g,  $P < 0.01$ ), and number of capillaries in the border zone

( $1348 \pm 15$  vs.  $938 \pm 34$  mm<sup>2</sup>,  $P < 0.0001$ ) were all significantly improved in the shock wave group compared with the control group. By contrast, in the late treatment group, no such beneficial effects of the shock wave therapy were noted.

**Conclusion** These results suggest that our extracorporeal cardiac shock wave therapy is also an effective and noninvasive therapy for improving left ventricular remodeling after acute myocardial infarction when started in the early phase of the disorder. *Coron Artery Dis* 18:397–404 © 2007 Lippincott Williams & Wilkins.

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**Keywords:** acute myocardial infarction, angiogenesis, remodeling, shock wave therapy

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## Introduction

Ischemic heart disease is the leading cause of death in Western countries and the number of patients with end-stage coronary artery disease without an indication of percutaneous coronary intervention or coronary artery bypass grafting is increasing. The prognosis of such patients remains poor because medication is the only therapy to treat the disorder. Acute myocardial infarction (AMI) is associated with a loss of cardiomyocytes and subsequent development of left ventricular (LV) remodeling, both of which lead to heart failure, sudden cardiac death, and poor prognosis [1]. If sufficient angiogenesis can be induced in the border zone of infarcted myocardium, the progression of LV remodeling could be suppressed, with resultant improved prognosis. Although gene therapy and cell-based therapy for end-stage coronary artery disease are under development [2–4], these therapies are invasive in nature and their safety has not been established yet.

We have recently demonstrated that low-energy extracorporeal cardiac shock wave (SW) therapy effectively induces angiogenesis and improves myocardial perfusion and cardiac function in a porcine model of chronic myocardial ischemia [5] and in patients with end-stage coronary artery disease [6] without any major adverse effects. However, it remains to be examined whether our SW therapy also is effective at improving LV remodeling after AMI. In this study, we thus examined, in a porcine model of AMI, whether our SW therapy improves LV remodeling by inducing angiogenesis in the border zone of infarcted myocardium and, if so, whether there is an appropriate timing for the therapy after AMI.

## Methods

All procedures were approved by the Institutional Animal Care and Use Committee and were conducted in conformity with the institutional guidelines.

### Porcine model of myocardial infarction

A total of 20 domestic male pigs (20–25 kg in body weight) were used in this study. They were anesthetized with ketamine (15 mg/kg, intramuscular) and were maintained under general anesthesia with an inhalation of 1.5% isoflurane for coronary ligation, SW treatment, and euthanization [5]. After the chest was opened, the pericardium and the left atrial appendage were suspended. Then, the proximal segment (approximately 15 mm in length) of the left circumflex coronary artery (LCx) was ligated and was stripped to create AMI. In our preliminary study, we confirmed that this stripping method was more effective at preventing the development of intracoronary collaterals at the chronic stage (4 weeks after AMI) than a simple ligation of the coronary artery.

### Coronary angiography and left ventriculography

After systemic heparinization (10 000 U/body), we performed coronary angiography (CAG) and left ventriculography (LVG) in a left oblique view with the use of a cineangiography system (Toshiba Medical, Tokyo, Japan) [5]. The LV volume and ejection fraction were calculated by Simpson's method.

### Echocardiography

We performed transthoracic echocardiographic studies (Sonos 5500, Agilent Technology, Tokyo, Japan) [5]. The LV volume and ejection fraction were calculated by Teichholz's method. We calculated wall thickening fraction (WTF) by using the following formula:  $WTF = 100 \text{ (end-systolic wall thickness} - \text{end-diastolic wall thickness)}/\text{end-diastolic wall thickness}$ . We measured WTF in the infarcted area, the border zone, and the remote normal area when pigs were sedated.

### Regional myocardial blood flow

We evaluated the regional myocardial blood flow (RMBF) with colored microspheres (Dye-Trak, Triton Technology, Nottinghamshire, UK) [5,7]. We injected microspheres (diameter 15  $\mu\text{m}$ ; 6 000 000 spheres per animal) through the left atrium and aspirated a reference arterial blood sample from the descending aorta at a constant rate of 20 ml/min for 60 s using a withdrawal pump. We extracted microspheres from the LV samples ( $\sim 5 \times 10 \times 10 \text{ mm}$ ) and blood samples by potassium hydroxide digestion, extracted the dyes from the spheres with dimethylformamide (200  $\mu\text{l}$ ), and determined their concentrations by spectrophotometry. We calculated the myocardial blood flow (ml/min/g) of the LV wall in the infarcted area, the border zone, and the remote normal area.

### Cardiac enzymes

To estimate the size of infarcted regions, we measured serum concentrations of creatinine kinase (CK) and its cardiac isoform, CK-MB, by using chemiluminescence immunoassay before and 12 and 24 h after creating AMI.

### Extracorporeal cardiac shock wave therapy

On the basis of our previous study [5], we applied a low energy of SW (0.09 mJ/mm<sup>2</sup>, approximately 10% of the energy for the lithotripsy treatment, 200 shots/spot for 27 spots) to the border zone around the infarcted myocardium under the guidance of an echocardiogram located within the specially designed SW generator (Storz Medical AG, Kreuzlingen, Switzerland). We observed LV wall motion by echocardiography both before and after creating AMI; the edge of the risk area where the LV wall motion was severely depressed after creating AMI was defined as the border zone ( $\sim 5 \text{ mm}$  in width in the outer zone of the risk area). We were able to accurately focus SW in any part of the heart under the guidance of echocardiography with a focus of  $\sim 2 \text{ mm}$  [5,6]. We applied SW in R-wave-triggered manner to avoid ventricular arrhythmias [5,6]. The extracorporeal cardiac SW therapy in action in a pig or a patient was shown in our previous reports [5,6].

### Protocols

We performed the following two study protocols.

#### Protocol 1: early treatment protocol

The SW therapy was started 3 days after AMI and performed three times a week at days 3, 6, and 9, whereas the control group received the same procedures three times a week but without the SW treatment ( $n = 5$  each). In all animals, CAG, LVG, echocardiographic studies, and measurement of RMBF were performed before (baseline) and at 4 weeks after AMI.

#### Protocol 2: late treatment protocol

The SW therapy was started 4 weeks after AMI and performed three times a week at days 28, 31, and 34, whereas the control group received the same procedures 3 times a week but without the SW treatment ( $n = 5$  each). In all animals, CAG, LVG, and echocardiographic studies were performed before (baseline) and at 4 and 8 weeks after AMI.

### Factor VIII staining

Tissue specimens were obtained from the border zone and the remote area of each animal at the end of the study. We treated paraffin-embedded sections with a rabbit anti-factor VIII antibody (N1505, Dako, Copenhagen, Denmark) [5]. We counted the number of factor VIII-positive cells in 10 fields of the border zone in each heart at 400  $\times$  magnification.

### Statistical analysis

Results are expressed as mean  $\pm$  SEM. We determined the statistical significance by analysis of variance for multiple comparisons. A value of  $P < 0.05$  was considered to be statistically significant.

## Results

### Size of myocardial infarction

The size of myocardial infarction as evaluated by serum concentrations of CK and CK-MB was comparable between the control and the SW groups at 12 h after (CK,  $2572 \pm 199$  vs.  $2461 \pm 199$  ng/ml and CK-MB,  $219 \pm 24$  vs.  $197 \pm 17$  ng/ml) and 24 h after AMI (CK,  $1219 \pm 83$  vs.  $1161 \pm 77$  ng/ml, and CK-MB  $116 \pm 8$  vs.  $102 \pm 13$  ng/ml). The SW therapy was started 3 days after AMI in the early treatment protocol and 28 days after AMI in the late treatment protocol.

### Effect of the shock wave therapy on left ventricular remodeling after acute myocardial infarction

In the early treatment protocol, 4 weeks after AMI, CAG showed that the proximal segment of LCx was lacking whereas the distal segment of LCx was perfused through collateral flow. Figures 1 and 2 represent the results of LVG and echocardiography, respectively. In the control group, LVG and echocardiography showed increased end-systolic and end-diastolic LV volume and decreased LV ejection fraction compared with baseline values before AMI (Figs 1 and 2). By contrast, in the SW group, LV volume and LV ejection fraction were significantly improved compared with the control group, when

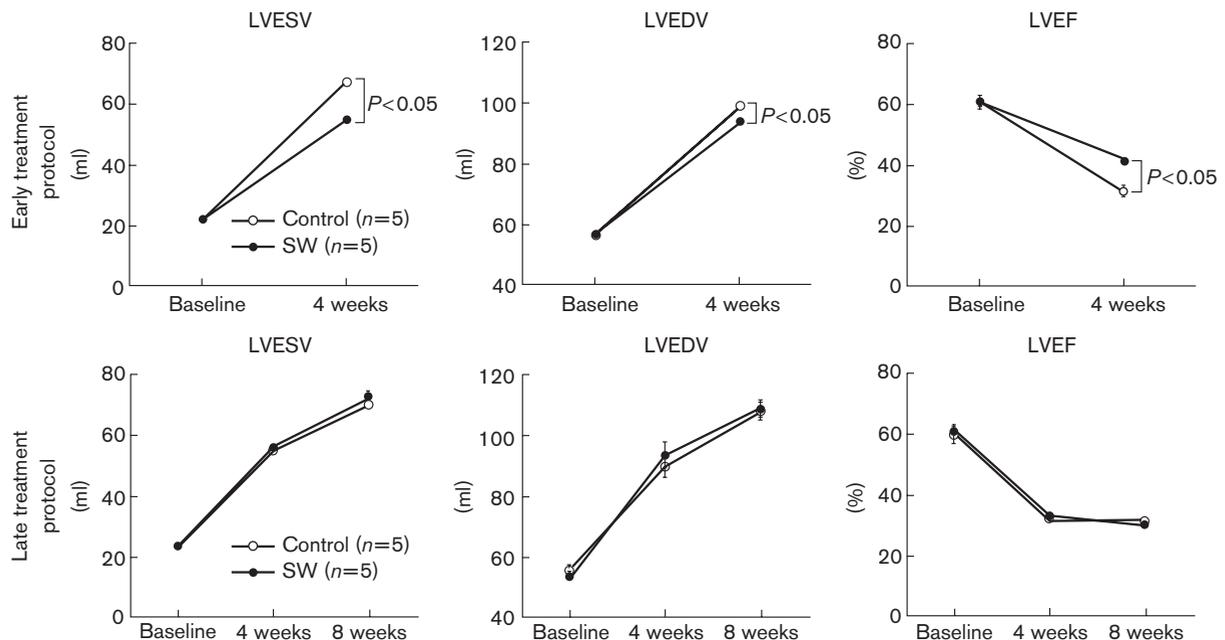
evaluated either by LVG (end-systolic LV volume,  $55 \pm 1$  vs.  $67 \pm 1$  ml,  $P < 0.0001$ ; end-diastolic LV volume,  $95 \pm 1$  vs.  $99 \pm 1$  ml,  $P < 0.05$ ; LV ejection fraction  $42 \pm 1$  vs.  $32 \pm 1\%$ ,  $P < 0.001$ ) (Fig. 1) or by echocardiography (end-systolic LV volume,  $46 \pm 1$  vs.  $56 \pm 1$  ml,  $P < 0.001$ ; end-diastolic LV volume,  $87 \pm 1$  vs.  $97 \pm 2$  ml,  $P < 0.01$ ; LV ejection fraction  $52 \pm 1$  vs.  $45 \pm 1\%$ ,  $P < 0.005$ ) (Fig. 2).

By contrast, in the late treatment protocol, when the SW was started 4 weeks after AMI, no such improvement was noted at 4 weeks after the SW therapy for LV volume or the depressed cardiac performance, when evaluated either by LVG (end-systolic LV volume,  $73 \pm 2$  vs.  $70 \pm 1$  ml,  $P = 0.21$ ; end-diastolic LV volume,  $109 \pm 3$  vs.  $108 \pm 3$  ml,  $P = 0.84$ ; LV ejection fraction  $31 \pm 1$  vs.  $32 \pm 1\%$ ,  $P = 0.42$ ) (Fig. 1) or by echocardiography (end-systolic LV volume,  $73 \pm 3$  vs.  $67 \pm 2$  ml,  $P = 0.18$ ; end-diastolic LV volume,  $105 \pm 4$  vs.  $108 \pm 4$  ml,  $P = 0.62$ ; LV ejection fraction  $45 \pm 1$  vs.  $47 \pm 2\%$ ,  $P = 0.42$ ) (Fig. 2).

### Effects of the shock wave therapy on myocardial function and blood flow (early treatment protocol)

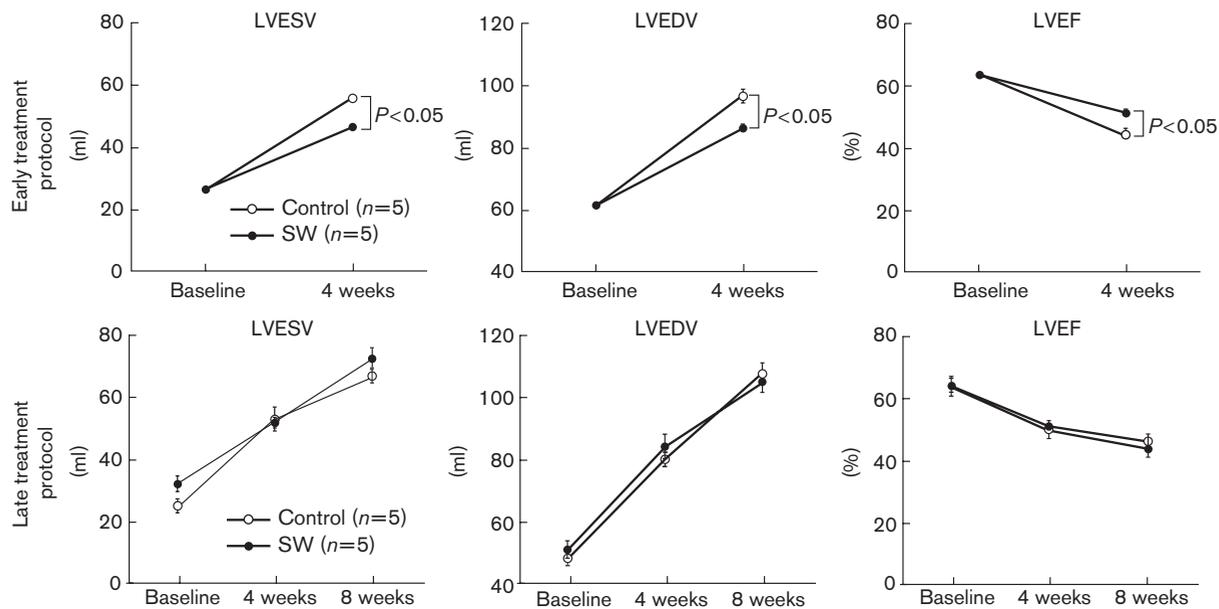
As a significant improvement was noted in the early treatment protocol for LV volumes and functions, we

Fig. 1



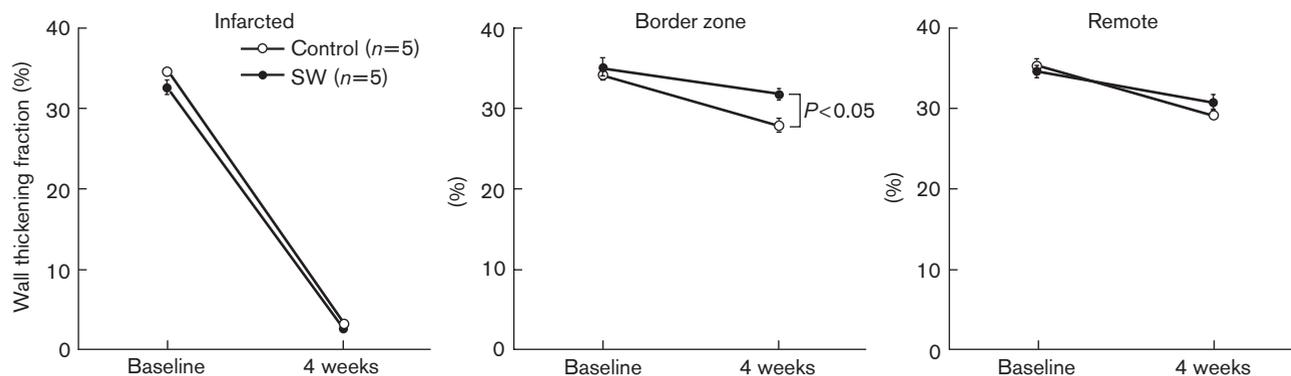
Results of left ventriculography for the inhibitory effects of the cardiac shock wave (SW) therapy on the development of left ventricular (LV) remodeling after AMI. The inhibitory effects of the SW therapy were noted in the early treatment protocol (upper panel) but not in the late treatment protocol (lower panel). Results are expressed as mean  $\pm$  SEM ( $n = 5$  each). AMI, acute myocardial infarction; Control, control group without the SW therapy; LVEDV, LV end-diastolic volume; LVEF, LV ejection fraction; LVESV, LV end-systolic volume; SW, SW group.

Fig. 2



Results of echocardiography for the inhibitory effects of the cardiac shock wave (SW) therapy on the development of left ventricular (LV) remodeling after AMI. The inhibitory effects of the SW therapy were noted in the early treatment protocol (upper panel) but not in the late treatment protocol (lower panel). Results are expressed as mean  $\pm$  SEM ( $n=5$  each). AMI, acute myocardial infarction; LVEDV, LV end-diastolic volume; LVEF, LV ejection fraction; LVESV, LV end-systolic volume.

Fig. 3

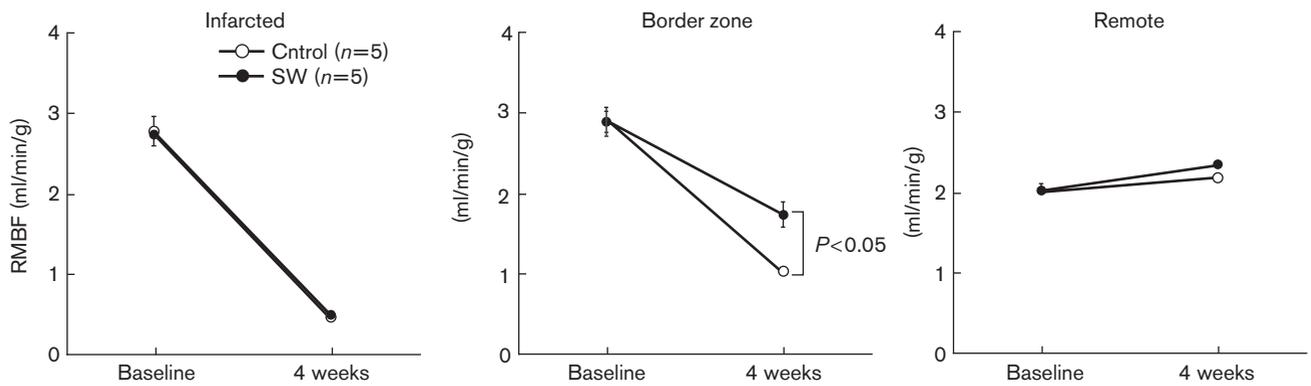


Wall thickening fraction (WTF) in the early treatment protocol. Extracorporeal cardiac shock wave (SW) therapy improved regional myocardial function in the border zone. Results are expressed as mean  $\pm$  SEM ( $n=5$  each). Border zone, myocardium in the border zone; infarcted, infarcted myocardium; remote, myocardium in the remote, normal area.

further measured WTF of the infarcted region, the border zone, and remote area by epicardial echocardiography in this protocol. Four weeks after AMI, we observed a significant reduction in WTF in the border zone in the control group, which was maintained in the SW group ( $28 \pm 1$  vs.  $32 \pm 1\%$ ;  $P < 0.01$ ) (Fig. 3). The WTF in infarcted area and remote

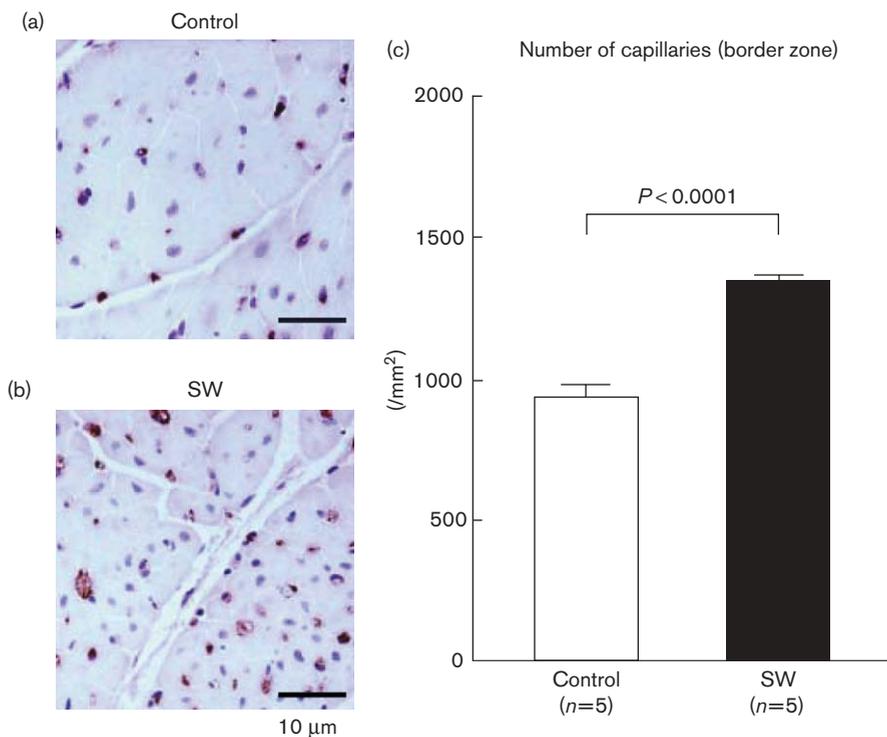
area was comparable between the two groups. Four weeks after AMI, RMBF in the border zone was decreased in the control group, which was significantly improved in the SW group (control group vs. SW group;  $1.0 \pm 0.1$  vs.  $1.7 \pm 0.2$  ml/min/g;  $P < 0.01$ ) (Fig. 4). The RMBF in infarcted and remote area was comparable between the two groups.

Fig. 4



Regional myocardial blood flow (RMBF) in the early treatment protocol. Results are expressed as mean  $\pm$  SEM ( $n=5$  each). Border zone, myocardium in the border zone; infarcted, infarcted myocardium; remote, myocardium in the remote, normal area. Extracorporeal cardiac shock wave (SW) therapy improved RMBF in the border zone.

Fig. 5



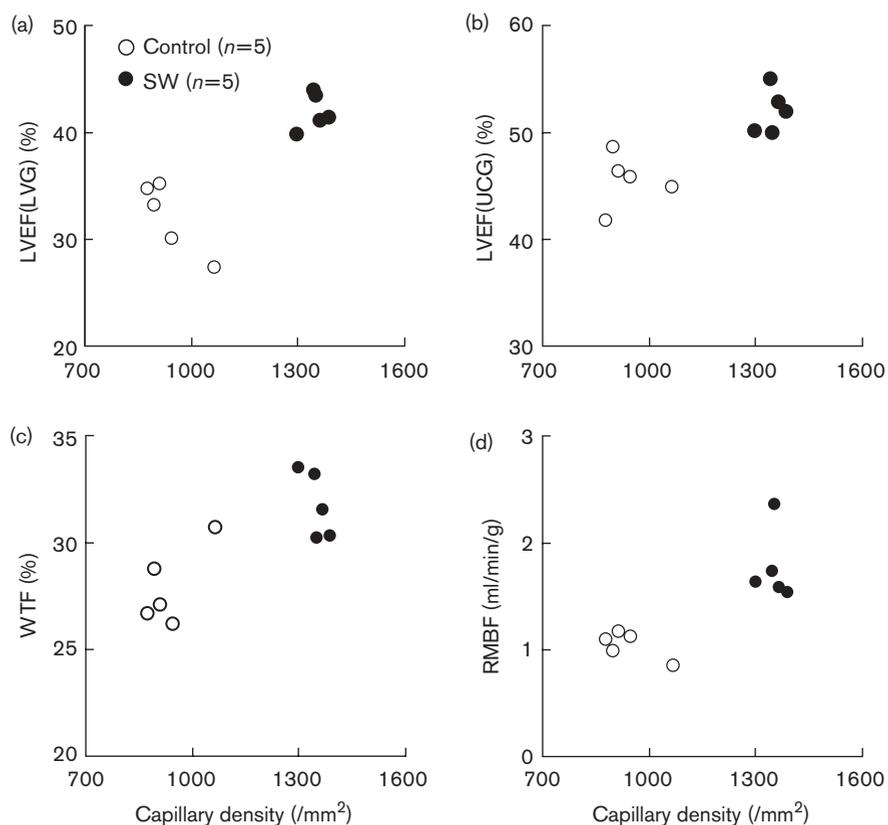
Capillary density in the early treatment protocol. Representative factor VIII staining of the border zone myocardium in the control group (a) and in the shock wave (SW) group (b), and quantitative analysis of the number of capillaries in the border zone (c). Extracorporeal cardiac SW therapy significantly increased the density of factor VIII-positive capillaries in the border zone. Scale bar, 10  $\mu$ m. Results are expressed as mean  $\pm$  SEM ( $n=5$  each).

#### Effects of the shock wave therapy on capillary density (early treatment protocol)

In the early treatment protocol, factor VIII staining showed that the number of factor VIII-positive capillaries in the border zone was higher in the SW group

than in the control group at 4 weeks after AMI ( $1348 \pm 15$  vs.  $938 \pm 34/\text{mm}^2$ ,  $P < 0.0001$ ), whereas the capillary density in the infarcted and remote areas was comparable between the two groups (Fig. 5). The relationships between the capillary density in the

Fig. 6



Relationship between capillary density in the border zone and left ventricular ejection fraction (LVEF) evaluated by left ventriculography (LVG) (a) and echocardiography (UCG) (b), wall thickening fraction (WTF), and regional myocardial blood flow (RMBF) (d).

border zone and LVEF, WTF, or RMBF are shown in Fig. 6.

## Discussion

The novel finding of this study is that our extracorporeal cardiac SW therapy improves LV remodeling after AMI in pigs when the therapy is started in the early phase, but not in the chronic phase, of the disorder. No procedural complications or adverse effects with the SW therapy were noted in this study, which was consistent with our previous studies for chronic myocardial ischemia in pigs and humans [5,6].

### Optimal timing of the shock wave therapy in acute myocardial infarction

In this study, the SW therapy improved LV remodeling after AMI when the therapy was started at 3 days, but not at 4 weeks after AMI. These results suggest that there is a therapeutic window for the SW-induced angiogenesis after AMI. In response to acute ischemia, the expression of multiple angiogenic factors and the mobilization of endothelial progenitor cells (EPCs) are enhanced [8,9]. For example, the number of circulating EPCs increases after the onset of AMI, peaks on day 7, and then gradually

decreases [10]. As the mobilized progenitor cells are reported to be involved in the healing process after AMI [2–4,8,11], SW-induced angiogenesis is expected to be more effective when the SW therapy is started in the earlier phase. As shown in the late treatment protocol in this study, once LV remodeling is established after AMI, our SW therapy may not be so effective at improving LV remodeling. It remains to be examined whether the beneficial effects of our SW therapy are more pronounced when the therapy is started earlier than 3 days after AMI. Further studies are needed to determine the best optimal timing of SW therapy in AMI.

### Mechanisms for the inhibitory effects of the shock wave therapy on left ventricular remodeling

It is highly possible that multiple mechanisms are involved in the inhibitory effects of our SW therapy on LV remodeling after AMI. Enhanced expression of multiple angiogenic factors, such as vascular endothelial growth factor (VEGF) and stromal-derived factor 1 (SDF-1), is crucial for the recruitment and incorporation of EPCs [12–17]. VEGF is known to induce angiogenesis by activating mobilization and homing of EPCs from the bone marrow to ischemic tissue [12–15]. We have

previously demonstrated that our SW therapy upregulates the expression of both VEGF and its receptor Flt-1, with a resultant increase in the capillary density and RMBF in ischemic myocardium [5]. In this study, we confirmed that the SW therapy also increased capillary density and RMBF in the border zone of the infarcted myocardium and that the improved myocardial perfusion was associated with an improvement in LV function. These results suggest that SW-induced angiogenesis effectively contributes to salvaging myocardium in the border zone and therefore improves LV remodeling characterized by LV enlargement and dysfunction.

The VEGF/Flt-1 system is essential in initiating vasculogenesis and angiogenesis [12–15]; however, other endogenous angiogenic systems, such as the endothelial nitric oxide synthase (eNOS)/cGMP system and the angiopoietin/tie-2 system, may also be involved in the beneficial effects of our SW therapy. Indeed, it has been reported that SW upregulates the expression of eNOS in vascular endothelial cells *in vitro* [18] and that eNOS gene delivery attenuates LV remodeling after AMI in rats *in vivo* [19]. Therefore, it is conceivable that SW-induced enhancement of endothelial nitric oxide production also plays an important role in suppressing the development of LV remodeling and functional deterioration after AMI. Recently, it was reported that SDF-1 is essential for the retention of proangiogenic stem cells in peripheral organs, although the upregulation of VEGF is sufficient to mobilize stem or progenitor cells from the bone marrow to the systemic circulation [16,17]. Therefore, it is possible that our SW therapy enhances the incorporation of circulating EPCs by repeatedly pronouncing the expression of SDF-1 in the border zone of the infarcted heart. This notion has been supported by the recent study by Aicher *et al.* [20] of SW therapy in conjunction with cell therapy. Further studies are needed to elucidate the molecular mechanisms involved in the beneficial effects of SW in suppressing LV remodeling after AMI.

### Limitations of the study

Several limitations should be mentioned for this study. First, although we were able to demonstrate that our SW therapy enhances angiogenesis in the border zone, the effects of the SW therapy on vascular smooth muscle cells, cardiomyocytes, or extracellular matrix remain to be examined in future studies. Second, the inhibitory effects of our SW therapy on LV remodeling after AMI need to be compared with those of drugs that are proven for the effects (e.g. ACE inhibitors). In the clinical setting with AMI, the use of ACE inhibitors,  $\beta$ -blockers, aspirin, and statins is recommended [21], and some of them are also reported to enhance the recruitment of EPCs [22,23]. Additional experiments are needed to examine whether our SW therapy is more effective at suppressing LV remodeling after AMI when combined with those drugs.

In summary, we were able to demonstrate that our low-energy extracorporeal cardiac SW therapy effectively induces angiogenesis, resulting in an increase in RMBF and suppression of the development of LV remodeling after AMI without any adverse effects. Thus, our extracorporeal cardiac SW therapy may be an effective, safe, and noninvasive therapeutic strategy for AMI in humans.

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