References

1 Gibbons RJ, Chatterjee K, Daley J, Douglas JS, Fihn SD, Gardin JM, *et al.* ACC/AHA/ACP-ASIM guidelines for the management of patients with chronic stable angina: a report of the American College of Cardiology/ American Heart Association Task Force on Practice Guidelines (Committee on Management of Patients With Chronic Stable Angina). *J Am Coll Cardiol* 1999; **33**:2092–2197.

2 Leschke M, Schoebel FC, Mecklenbeck W, Stein D, Jax TW, Muller-Gartner HW, *et al.* Long-term intermittent urokinase therapy in patients with end-stage coronary artery disease and refractory angina pectoris: a randomized dose–response trial. *J Am Coll Cardiol* 1996; **27**:575–584.

3 Isner JM, Pieczek A, Schainfeld R, Blair R, Haley L, Asahara T, *et al.* Clinical evidence of angiogenesis after arterial gene transfer of phVEGF165 in patient with ischaemic limb. *Lancet* 1996; **348**:370–374.

4 Losordo DW, Vale PR, Symes JF, Dunnington CH, Esakof DD, Maysky M, *et al.* Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. *Circulation* 1998; **98**:2800–2804.

5 Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, Masaki H, *et al.* Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *Lancet* 2002; **360**:427–435.

6 Haupt G, Haupt A, Ekkernkamp A, Gerety B, Chvapil M. Influence of shock waves on fracture healing. *Urology* 1992; **39**:529–532.

7 Rompe JD, Rumler F, Hopf C, Nafe B, Heine J. Extracorporal shock wave therapy for calcifying tendinitis of the shoulder. *Clin Orthop Relat Res* 1995; **321**:196–201.

8 Gutersohn A, Gaspari G. Shock waves upregulate vascular endothelial growth factor m-RNA in human umbilical vascular endothelial cells. *Circulation* 2000; **102 (Suppl I)**:I-18.

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; **110**:3055–3061.

10 Aoki M, Sakai K, Koyanagi S, Takeshita A, Nakamura M. Effect of nitroglycerin on coronary collateral function during exercise evaluated by quantitative analysis of thallium-201 single photon emission computed tomography. *Am Heart J* 1991; **121**:1361–1366.

11 Mohri M, Koyanagi M, Egashira K, Tagawa H, Ichiki T, Shimokawa H, *et al.* Angina pectoris caused by coronary microvascular spasm. *Lancet* 1998; **351**:1165–1169.

12 Apfel RE. Acoustic cavitation: a possible consequence of biomedical uses of ultrasound. *Br J Cancer Suppl* 1982; **45**:140–146.

13 Maisonhaute E, Prado C, White PC, Compton RG. Surface acoustic cavitation understood via nanosecond electrochemistry. Part III: shear stress in ultrasonic cleaning. *Ultrason Sonochem* 2002; **9**:297–303.

14 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.

15 Wang CJ, Wang FS, Yang KD, Weng LH, Hsu CC, Huang CS, *et al.* Shock wave therapy induces neovascularization at the tendon-bone junction. A study in rabbits. *J Orthop Res* 2003; **21**:984–989.

16 Szatkowski A, Ndubuka-Irobunda C, Oesterle SN, Burkhoff D. Transmyocardial laser revascularization: a review of basic and clinical aspects. *Am J Cardiovasc Drugs* 2002; **2**:255–266.

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

Toyokazu Uwatoku[a], Kenta Ito[b], Kohtaro Abe[a], Keiji Oi[a], Takatoshi Hizume[a], Kenji Sunagawa[a] and Hiroaki Shimokawa[a,b]

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 ± 1 vs. $32\pm1\%$, $P<0.001$) and left ventricular end-diastolic volume was smaller (95 ± 1 vs. 99 ± 2 ml, $P<0.05$) in the shock wave group compared with the control group. Furthermore, wall thickening fraction (32 ± 1 vs. $28\pm1\%$, $P<0.01$), regional myocardial blood flow (1.7 ± 0.2 vs. 1.0 ± 0.1 ml/min/g, $P<0.01$), and number of capillaries in the border zone (1348 ± 15 vs. 938 ± 34 mm^2, $P<0.0001$) 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.**

Coronary Artery Disease 2007, 18:397–404

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

[a]Department of Cardiovascular Medicine, Kyushu University Graduate School of Medical Sciences, Fukuoka and [b]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Correspondence to Dr Hiroaki Shimokawa, MD, PhD, Professor and Chairman, Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryomachi, Aoba-ku, Sendai 980-8574 Japan
Tel: +81 22 717 7151; fax: +81 22 717 7156;
e-mail: shimo@cardio.med.tohoku.ac.jp

Received 29 August 2006 Revised 23 December 2006
Accepted 3 January 2007

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.

0954-6928

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 (end-systolic wall thickness − end-diastolic wall thickness)/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 μm; 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 (~5 × 10 × 10 mm) and blood samples by potassium hydroxide digestion, extracted the dyes from the spheres with dimethylformamide (200 μl), 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^2, 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 (~5 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 ~2 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 × magnification.

Statistical analysis

Results are expressed as mean ± 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 ± 199 vs. 2461 ± 199 ng/ml and CK-MB, 219 ± 24 vs. 197 ± 17 ng/ml) and 24 h after AMI (CK, 1219 ± 83 vs. 1161 ± 77 ng/ml, and CK-MB 116 ± 8 vs. 102 ± 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

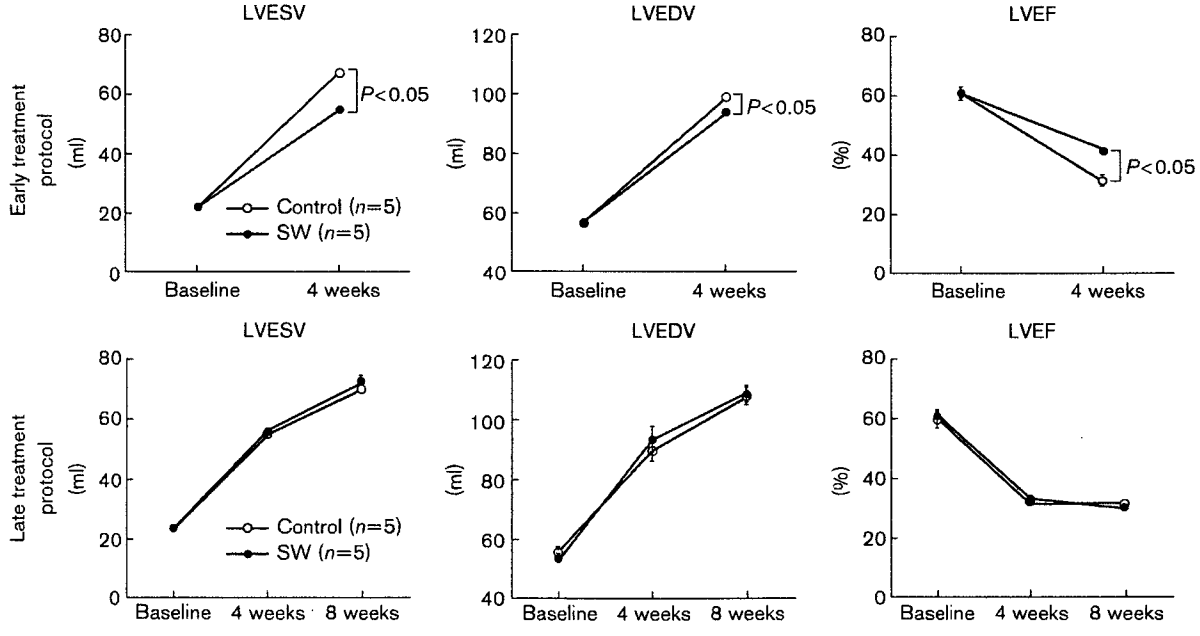
In the early treatment protocol, 4 weeks after AMI, CAG showed that the proximal segment of LCx was lacking whereas the distal segment of LCx was perfused through collateral flow. Figures 1 and 2 represent the results of LVG and echocardiography, respectively. In the control group, LVG and echocardiography 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 ± 1 vs. 67 ± 1 ml, $P < 0.0001$; end-diastolic LV volume, 95 ± 1 vs. 99 ± 1 ml, $P < 0.05$; LV ejection fraction 42 ± 1 vs. 32 ± 1%, $P < 0.001$) (Fig. 1) or by echocardiography (end-systolic LV volume, 46 ± 1 vs. 56 ± 1 ml, $P < 0.001$; end-diastolic LV volume, 87 ± 1 vs. 97 ± 2 ml, $P < 0.01$; LV ejection fraction 52 ± 1 vs. 45 ± 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 ± 2 vs. 70 ± 1 ml, $P = 0.21$; end-diastolic LV volume, 109 ± 3 vs. 108 ± 3 ml, $P = 0.84$; LV ejection fraction 31 ± 1 vs. 32 ± 1%, $P = 0.42$) (Fig. 1) or by echocardiography (end-systolic LV volume, 73 ± 3 vs. 67 ± 2 ml, $P = 0.18$; end-diastolic LV volume, 105 ± 4 vs. 108 ± 4 ml, $P = 0.62$; LV ejection fraction 45 ± 1 vs. 47 ± 2%, $P = 0.42$) (Fig. 2).

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

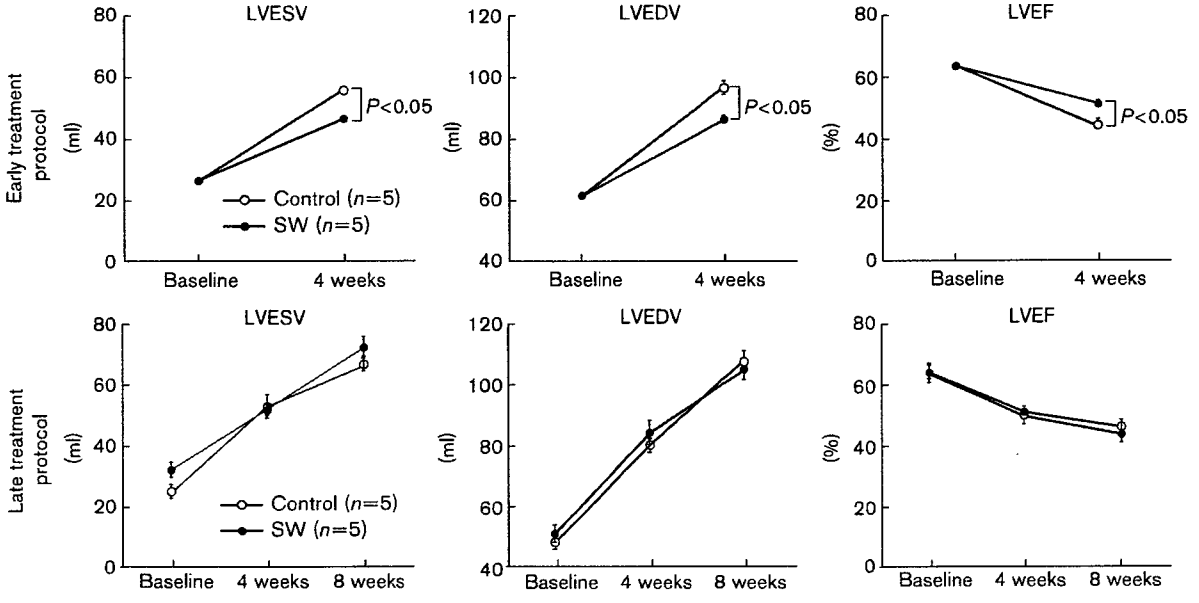
As a significant improvement was noted in the early treatment protocol for LV volumes and functions, we

Fig. 1



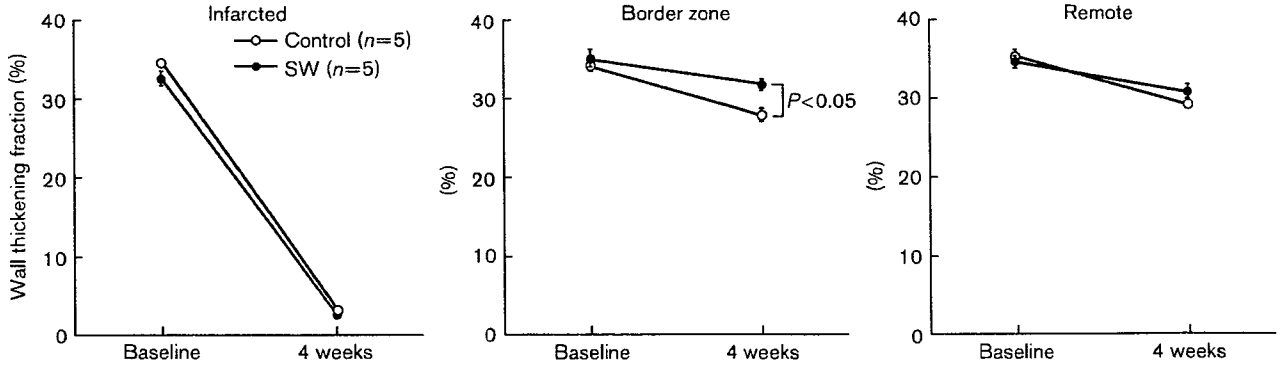
Results of left ventriculography for the inhibitory effects of the cardiac shock wave (SW) therapy on the development of left ventricular (LV) remodeling after AMI. The inhibitory effects of the SW therapy were noted in the early treatment protocol (upper panel) but not in the late treatment protocol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

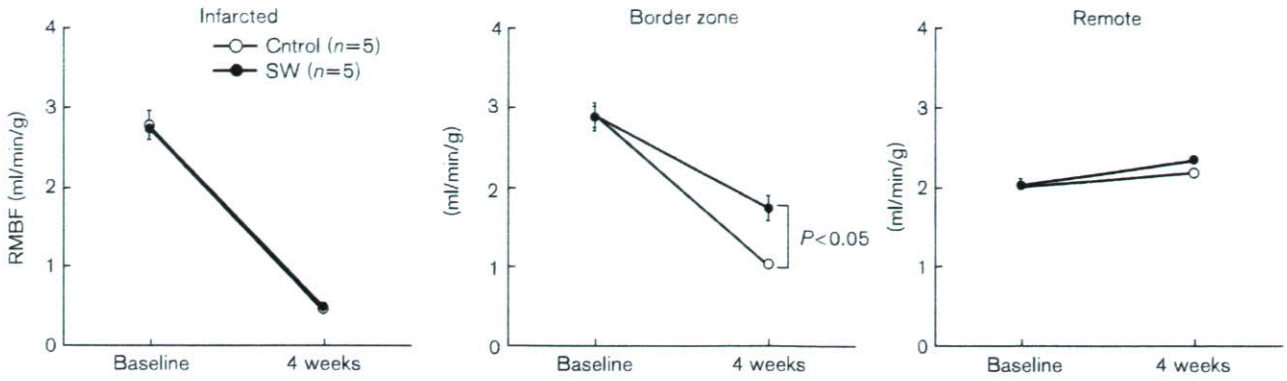
Fig. 3



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 ± SEM ($n=5$ each). Border zone, myocardium in the border zone; infarcted, infarcted myocardium; remote, myocardium in the remote, normal area.

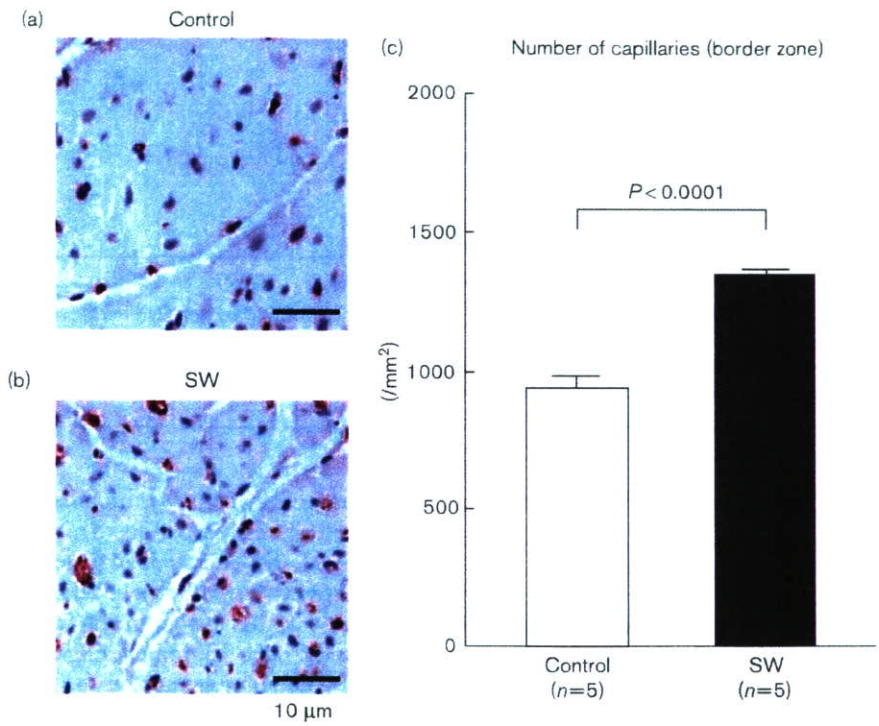
further measured WTF of the infarcted region, the border zone, and remote area by epicardiac echocardiography in this protocol. Four weeks after AMI, we observed a significant reduction in WTF in the border zone in the control group, which was maintained in the SW group (28 ± 1 vs. $32 \pm 1\%$; $P < 0.01$) (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 ± 0.1 vs. 1.7 ± 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 ± 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

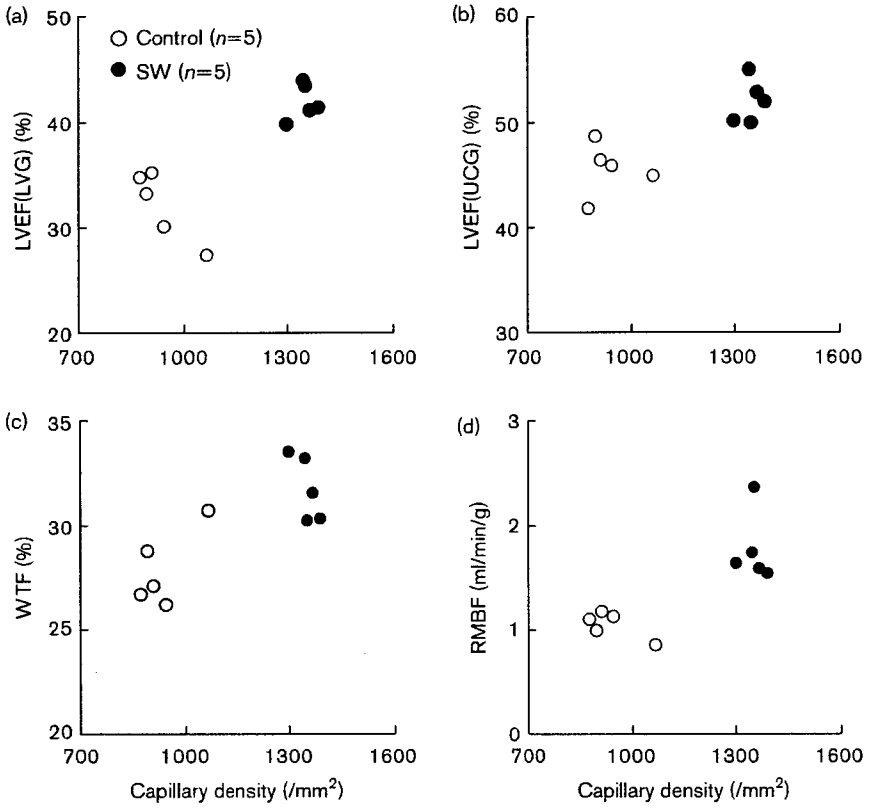


Capillary density in the early treatment protocol. Representative factor VIII staining of the border zone myocardium in the control group (a) and in the shock wave (SW) group (b), and quantitative analysis of the number of capillaries in the border zone (c). Extracorporeal cardiac SW therapy significantly increased the density of factor VIII-positive capillaries in the border zone. Scale bar, 10 μm. Results are expressed as mean ± 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 ± 15 vs. $938 \pm 34/mm^2$, $P < 0.0001$), 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, β-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.

Acknowledgements

We thank Dr Ernest H. Marlinghaus, Storz Medical AG, Switzerland, for valuable comments on our study. This study was supported in part by 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.

References

1 Jessup M, Brozena S. Heart failure. *N Engl J Med* 2003; **348**:2007–2018.
2 Dimmeler S, Zeiher AM, Schneider MD. Unchain my heart: the scientific foundations of cardiac repair. *J Clin Invest* 2005; **115**:572–583.
3 Forrester JS, Price MJ, Makkar RR. Stem cell repair of infarcted myocardium: an overview for clinicians. *Circulation* 2003; **108**:1139–1145.
4 Mathur A, Martin JF. Stem cells and repair of the heart. *Lancet* 2004; **364**:183–192.
5 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; **110**:3055–3061.
6 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.
7 Prinzen FW, Bassingthwaighte JB. Blood flow distributions by microsphere deposition methods. *Cardiovasc Res* 2000; **45**:13–21.
8 Vandervelde S, Van Luyn MJ, Tio RA, Harmsen MC. Signaling factors in stem cell-mediated repair of infarcted myocardium. *J Mol Cell Cardiol* 2005; **39**:363–376.
9 Massa M, Rosti V, Ferrario M, Campanelli R, Ramajoli I, Rosso R, *et al.* Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction. *Blood* 2005; **105**:199–206.
10 Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, *et al.* Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. *Circulation* 2001; **103**:2776–2779.
11 Minatoguchi S, Takemura G, Chen XH, Wang N, Uno Y, Koda M, *et al.* Acceleration of the healing process and myocardial regeneration may be important as a mechanism of improvement of cardiac function and remodeling by postinfarction granulocyte colony-stimulating factor treatment. *Circulation* 2004; **109**:2572–2580.
12 Asahara T, Murohara T, Sullivan A, Silver M, Van der Zee R, Li T, *et al.* Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 1997; **275**:964–967.
13 Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med* 2003; **9**:702–712.
14 Millauer B, Wizigmann-Voos S, Schnurch H, Martinez R, Moller 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.
15 Grunewald M, Avraham I, Dor Y, Bachar-Lustig E, Itin A, Yung S, *et al.* VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell* 2006; **124**:175–189.
16 Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, *et al.* Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 2004; **10**:858–864.
17 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.

18 Gutersohn A, Gaspari G. Shock waves upregulate vascular endothelial growth factor m-RNA in human umbilical vascular endothelial cells. *Circulation* 2000; **102 (Suppl I)**:1–18.
19 Smith RS Jr, Agata J, Xia CF, Chao L, Chao J. Human endothelial nitric oxide synthase gene delivery protects against cardiac remodeling and reduces oxidative stress after myocardial infarction. *Life Sci* 2005; **76**: 2457–2471.
20 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.
21 Antman EM, Anbe DT, Armstrong PW, Bates ER, Green LA, Hand M, *et al.* American College of Cardiology; American Heart Association Task Force on Practice Guidelines; Canadian Cardiovascular Society. ACC/AHA guidelines for the management of patients with ST-elevation myocardial infarction: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Revise the 1999 Guidelines for the Management of Patients with Acute Myocardial Infarction). *Circulation* 2004; **110**: e82–e292.
22 Bahlmann FH, De Groot K, Mueller O, Hertel B, Haller H, Fliser D. Stimulation of endothelial progenitor cells: a new putative therapeutic effect of angiotensin II receptor antagonists. *Hypertension* 2005; **45**: 526–529.
23 Dimmeler S, Aicher A, Vasa M, Mildner-Rihm C, Adler K, Tiemann M, *et al.* HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. *J Clin Invest* 2001; **108**: 391–397.

Shock Waves
DOI 10.1007/s00193-008-0122-5

ORIGINAL ARTICLE

Extracorporeal cardiac shock wave therapy for ischemic heart disease

Hiroaki Shimokawa · Kenta Ito · Yoshihiro Fukumoto · Satoshi Yasuda

Received: 12 September 2007 / Accepted: 26 December 2007

Abstract Prognosis of severe ischemic heart disease with no indication of percutaneous coronary intervention or coronary artery bypass grafting still remains poor. Extracorporeal shock wave therapy was introduced for medical therapy more than 20 years ago to break up kidney stones. We have demonstrated that extracorporeal cardiac shock wave therapy at a low level of ~10% of energy density that used for urinary lithotripsy treatment, effectively induces coronary angiogenesis and improves myocardial ischemia in a porcine model of chronic myocardial ischemia in vivo. On the basis of the promising results in animal studies, we have recently developed a new, non-invasive angiogenic therapy with low-energy shock waves for ischemic heart disease. Our extracorporeal cardiac shock wave therapy improved symptoms and myocardial ischemia in patients with severe coronary artery disease. These beneficial effects of the shock wave therapy persisted for at least 12 months. Importantly, no procedural complications or adverse effects were noted. These results indicate that our extracorporeal cardiac shock wave therapy is an effective and non-invasive treatment for ischemic heart disease. To further confirm the usefulness and safety of our SW therapy, we are currently conducting the second clinical trial in a randomized and placebo-controlled manner.

Communicated by K. Takayama.

H. Shimokawa (✉) · K. Ito · Y. Fukumoto · S. Yasuda
Department of Cardiovascular Medicine,
Tohoku University Graduate School of Medicine,
1-1 Seiryomachi, Aoba-ku,
Sendai 980-8574, Japan
e-mail: shimo@cardio.med.tohoku.ac.jp

Keywords Cardiac shock wave therapy · Angiogenesis · Ischemic heart disease · Myocardial ischemia · Growth factors

PACS 42.62.Be

1 Introduction

Ischemic heart disease is the leading cause of death in developed countries and the number of patients is increasing worldwide [1]. Since the coronary arteries supply blood to the heart muscle, narrowing or closure of the arteries by atheromatous plaques limits blood flow to a part of heart muscle, causing an imbalance between oxygen supply and demand with a resultant development of myocardial ischemia. Myocardial ischemia can cause temporary chest pain (angina pectoris), permanent heart muscle damage (acute myocardial infarction), lethal arrhythmia, and sudden cardiac death. The current management of ischemic heart disease has three major therapeutic options, including medical treatment (drug therapy), percutaneous coronary intervention (PCI: balloon dilation of narrowed arteries), and coronary artery bypass grafting (CABG; heart surgery). However, prognosis of patients with severe coronary artery disease without indication of PCI or CABG still remains poor because medication is the only therapy to treat the disorder. Thus, it is crucial to develop an alternative therapy for severe ischemic heart disease. Currently, gene therapy and cell-based therapy for those patients are under development, however, most of these therapies are invasive in nature and their effectiveness and safety have not been established yet [2–4]. We have recently developed a new, non-invasive angiogenic therapy using low-energy shock waves (SW) [5–7]. In this article, we outline our recent works in animals and humans, and then discuss the

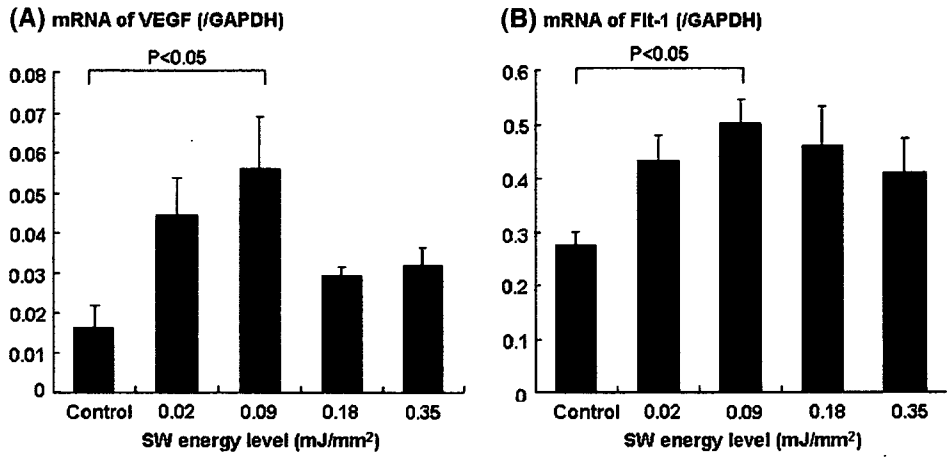


Fig. 1 SW treatment up-regulates mRNA expression of VEGF a and Flt-1 b in HUVECs in vitro with a maximum effect noted at 0.09 mJ/mm^2. Results are expressed as mean ± SEM (n = 10 each) (quoted from Ref. [5] with permission)

mechanisms for SW-induced angiogenesis and advantages of our cardiac SW therapy.

2 Extracorporeal cardiac SW therapy for angina pectoris

2.1 In vitro study

Extracorporeal SW therapy has been introduced for medical therapy more than 20 years ago to break up kidney stones, which has markedly improved the treatment of urolithiasis. Furthermore, the lithotripsy therapy with SW is indicated for gallstones, pancreatic and salivary stones, and is also used for the treatment of certain orthopedic conditions, such as bone fracture and calcifying tendonitis [8,9].

It was demonstrated that a low level of SW could up-regulate angiogenic factors in vitro [10] and induce localized stress on cell membranes that resembles shear stress [11]. We have recently reported that a low level of SW enhances the expression of vascular endothelial growth factor (VEGF) and its receptor, Flt-1, in cultured human endothelial cells (HUVEC) in vitro with a maximum effect noted at 0.09 mJ/mm^2, which level is approximately 10% of that used for urinary lithotripsy treatment (Fig. 1) [5].

2.2 Animal studies

In the clinical setting, the goal for the treatment of ischemic heart disease should include not only enhancement of angiogenesis but also recovery of ischemia-induced myocardial dysfunction. We performed in vivo animal experiments with pigs, in which myocardial ischemia was made by placing an ameroid constrictor at the proximal segment of the left circumflex coronary artery (LCX) that gradually induced a total occlusion of the artery with sustained myocardial dysfunction but without myocardial infarction in 4 weeks [5]. Thereafter, we performed extracorporeal SW therapy to the ischemic myocardial region (n = 8). On the basis of our in vitro experiment, we applied a low energy of SW (0.09 mJ/mm^2) to nine spots in the ischemic LCX region (200 shots/spot) with a guidance of an echocardiogram equipped within a specially designed SW generator (Storz Medical AG, Tägerwilen, Switzerland). We were able to focus SW on any part of the heart under the guidance of echocardiography. In order to treat the targeted ischemic myocardium without inducing ventricular arrhythmia, we applied SW at end-diastole during cardiac cycle with a R-wave-triggered system. We performed the SW treatment (n = 8) at 4 weeks after the implantation of an ameroid constrictor 3 times within 1 week, whereas animals in the control group (n = 8) received the same anesthesia procedures 3 times a week but without the SW treatment. We evaluated cardiac function at ameroid implantation (baseline) and at 4 and 8 weeks after the implantation.

Four weeks after the implantation of an ameroid constrictor, wall motion of the LCX (posterolateral) region in the left ventricle (LV) was equally reduced in both the control and the SW group before the SW therapy (Fig. 2a, c). However, 4 weeks after the SW therapy, left ventriculography showed marked improvement of LV wall motion only in the SW (Fig. 2b, d). The SW therapy normalized LV ejection fraction in the SW group but not in the control group (Fig. 2e). In this study, the SW treatment normalized global and regional myocardial functions as well as regional myocardial blood flow in the chronic ischemic region evaluated with colored microspheres. In addition, the SW treatment increased vascular density in the SW-treated region and up-regulated VEGF expression in the ischemic myocardium in vivo (Fig. 3). Importantly, no procedural complications or adverse effects were noted during or after the SW treatment. These data indicate that the SW treatment up-regulated the endogenous angiogenic system in pigs in vivo, suggesting its usefulness for the treatment of ischemic heart disease in humans.

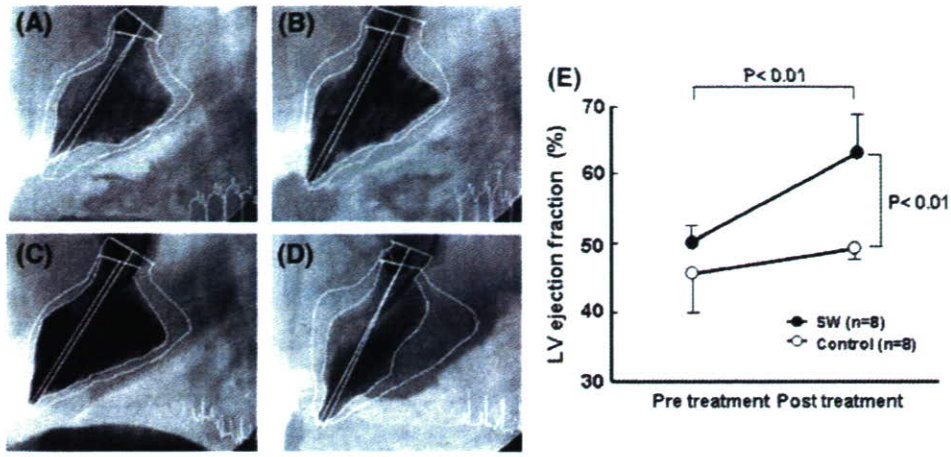


Fig. 2 Extracorporeal cardiac SW therapy improves ischemia-induced myocardial dysfunction in vivo as evaluated with left ventriculography. Four weeks after the implantation of an ameroid constrictor, LV wall motion of the LCX (posterolateral) region was reduced in both the control **a** and the SW group (before the SW therapy) **c**. 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** The SW therapy normalized LV ejection fraction in the SW group but not in the control group. Results are expressed as mean ± SEM ($n = 8$ each) (quoted from Ref. [5] with permission)

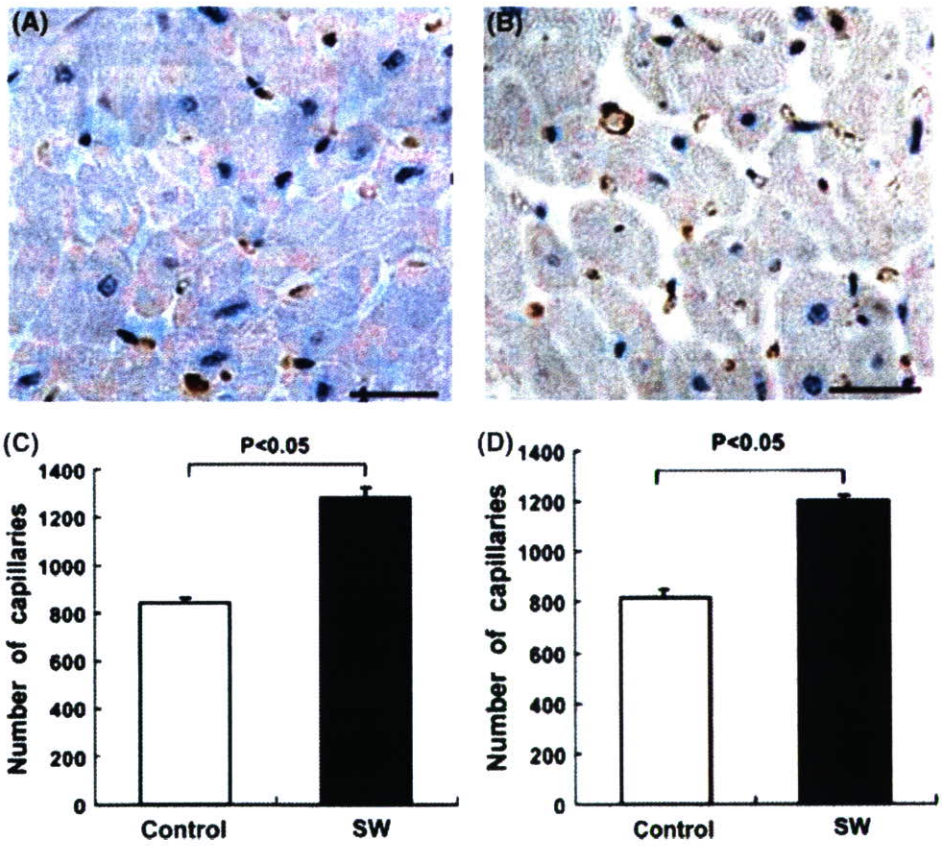


Fig. 3 Extracorporeal cardiac SW therapy increases the density of factor VIII-positive capillaries in the ischemic myocardium. Factor VIII staining of the LCX region from the control **a** and the SW group **b**. *Scale bar* represents 20 μm. Capillary density was significantly greater in the SW group (SW) than in the control group (Control) in both the endocardium **c** and the epicardium **d**. Results are expressed as mean ± SEM ($n = 6$ each) (quoted from Ref. [5] with permission)

2.3 Clinical studies

On the basis of the promising results in animal studies, we started the first clinical trial of cardiac SW therapy in humans in 2003. We treated nine patients with end-stage ischemic heart disease with no indication of PCI or CABG (55–82 years old, five men and four women) with our cardiac SW therapy (200 shots/spot at 0.09 mJ/mm^2 for 20–40 spots, 3 times a week/series) (Fig. 4) [6]. As shown in Fig. 4a, patients just lied down on the bed without anesthesia during

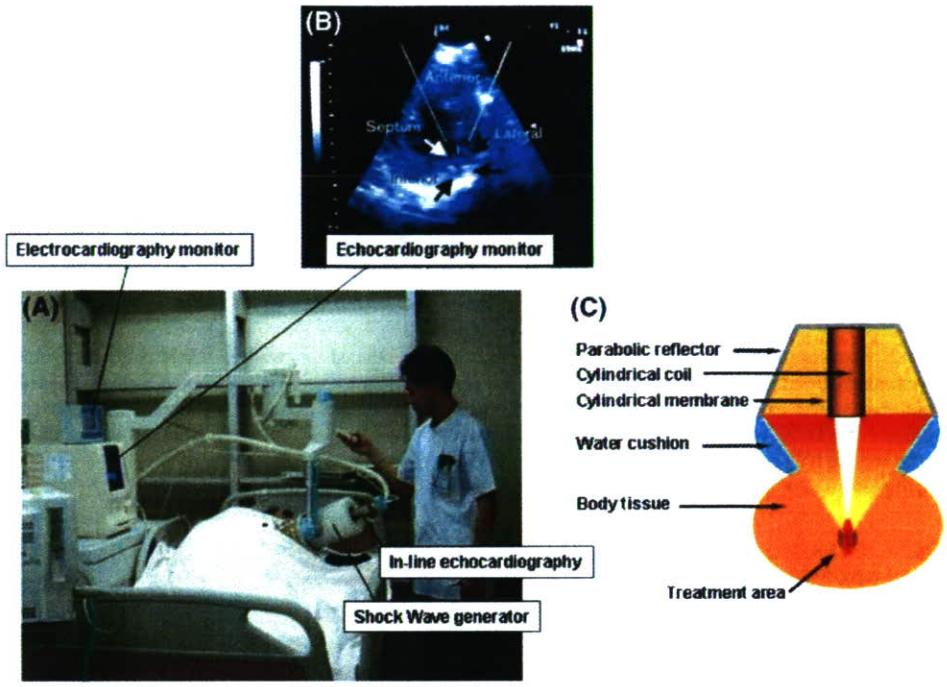


Fig. 4 Extracorporeal cardiac SW therapy in action in a patient with severe coronary artery disease. **a** The machine is equipped with a SW generator and in-line echocardiography. The SW generator is attached to the chest wall when used. **b** The cardiac ultrasound monitor. The SW pulse is easily focused on the ischemic myocardium under the guidance of echocardiography (*black* and *white arrows*). **c** The SW generator is equipped with parabolic reflector, cylindrical coil, and cylindrical membrane with water cushion

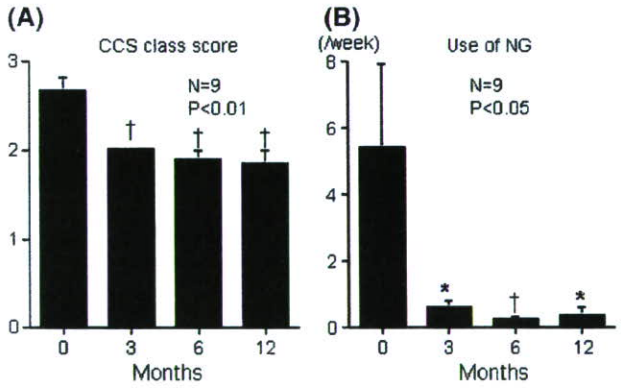


Fig. 5 Extracorporeal cardiac SW therapy significantly improved Canadian Cardiovascular Society (CCS) scores **a** and the use of nitroglycerin (NG) **b**. Results are expressed as mean ± SEM. $*P < 0.05$ and $\dagger P < 0.01$ vs. 0 month (statistically analyzed by post-hoc test after one-way ANOVA) (quoted from Ref. [6] with permission)

the treatment. The SW therapy improved symptoms and reduced nitroglycerin use (Fig. 5) as well as myocardial perfusion as assessed by dipyridamole stress thallium scintigraphy only in the ischemic area treated with the therapy (Fig. 6). These beneficial effects of the SW therapy persisted for at least 12 months (Figs. 5 and 7). No procedural complications or adverse effects were noted. These data indicate that our extracorporeal cardiac SW therapy is an effective and non-invasive treatment for end-stage ischemic heart disease. To further confirm the usefulness and safety of our SW therapy, we are currently conducting the second clinical trial in a randomized and placebo-controlled manner.

3 Extracorporeal cardiac SW therapy for acute myocardial infarction

Acute myocardial infarction (AMI) is associated with a loss of heart muscle. After AMI, the heart is gradually dilated and cardiac ability to pump blood to the rest of the body is impaired. This process is called "LV remodeling" [1]. The development of LV remodeling leads to sudden cardiac death, heart failure, and poor prognosis. If sufficient angiogenesis can be induced in the border zone of infarcted myocardium, the progression of LV remodeling could be suppressed with a resultant improvement of prognosis. Therefore, we examined whether our SW therapy is also effective to ameliorate LV remodeling after acute myocardial infarction in pigs.

Acute myocardial infarction was created by surgically excising the proximal segment of the LCX [7]. In the early treatment protocol, the SW therapy was started 3 days after AMI, whereas in the late treatment protocol, the SW therapy was started 4 weeks after AMI ($n = 5$ each). The remaining animals were treated in the same manner but without the SW treatment in each protocol ($n = 5$ each). In the early treatment protocol in which the treatment was started at 3 days after AMI, LV ejection fraction and LV end-diastolic volume were significantly improved in the SW group compared with the control group at 4 weeks after the treatment (Fig. 8). Furthermore, regional myocardial blood flow and number of capillaries in the border zone were significantly improved in the SW group compared with the control group. By contrast, in the late treatment group in which the SW treatment was started 4 weeks after AMI, no such beneficial

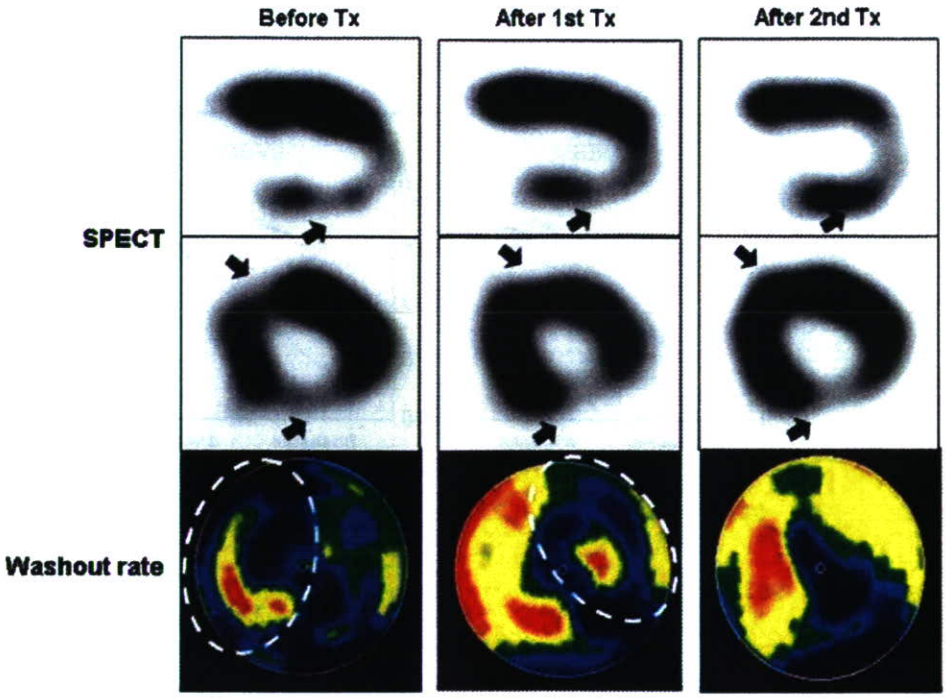


Fig. 6 Dipyridamole stress thallium-201 single photon emission computed tomography (SPECT) imaging and polar map demonstrated that the SW treatment ameliorated myocardial perfusion only where SW was applied; in the anteroseptal wall after the first treatment (Tx) and in the lateral wall after the second treatment (*arrows*). The areas where shock waves were shot were indicated with *dotted lines*. (quoted from Ref. [6] with permission)

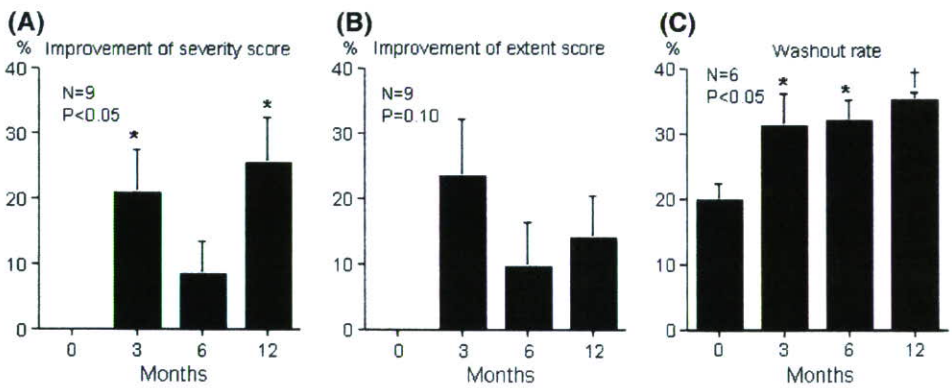


Fig. 7 The SW therapy significantly improved severity score **a**, tended to improve extent score **b**, and significantly improved local washout rate in patients with initial low washout rate (<30%) **c** in the dipyridamole stress thallium scintigraphy. Results are expressed as mean ± SEM. $*P < 0.05$ and $\dagger P < 0.01$ vs. 0 month (statistically analyzed by post-hoc test after one-way ANOVA) (quoted from Ref. [6] with permission)

effects of the SW therapy were noted. These results suggest that our extracorporeal cardiac SW therapy is an effective and non-invasive therapy to ameliorate LV remodeling after AMI when started in the early phase of the disorder. We are currently conducting the first clinical trial in patients with AMI who are successfully treated with PCI, in order to examine whether our SW therapy combined with PCI ameliorates LV remodeling and dysfunction after AMI in humans.

4 Mechanisms for SW-induced angiogenesis

When a SW hits tissue, cavitation (a micrometer-sized violent collapse of bubbles) is induced by the first compression by the positive pressure part and the expansion with the tensile part of a SW [12]. Because the physical forces generated by cavitation are highly localized, SW could induce localized stress on cell membranes, as altered shear stress affects endothelial cells [13]. Recent reports have demonstrated the biochemical effects of SW, including hyperpolarization and Ras activation [14], non-enzymatic nitric oxide synthesis [15], and induction of stress fibers and intercellular gaps [16].

Enhanced expression of multiple angiogenic factors, such as VEGF and stromal-derived factor 1 (SDF-1), is crucial for the recruitment and incorporation of endothelial progenitor cells (EPCs) [17–22]. VEGF is known to induce angiogenesis by activating mobilization and homing of EPCs from the bone marrow to ischemic tissue [17–20]. We have previously demonstrated that our SW therapy up-regulates the expression of both VEGF and its receptor Flt-1 in cultured human endothelial cells and increases capillary density and regional myocardial blood flow in a porcine model of myocardial

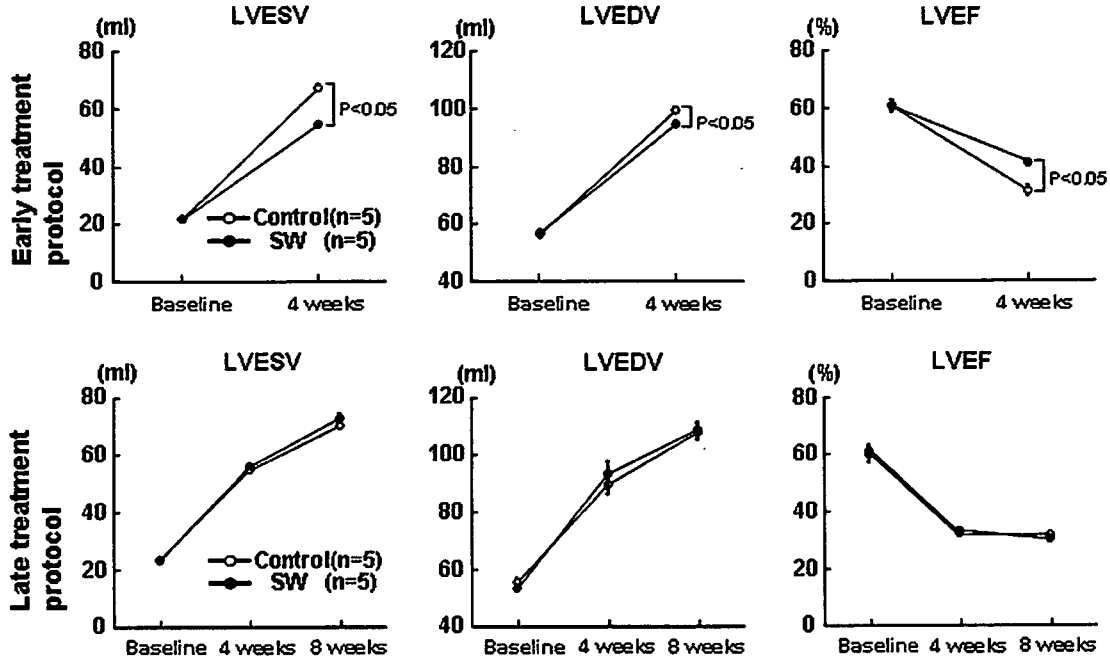


Fig. 8 Results of left ventriculography for the inhibitory effects of the cardiac SW therapy on the development of LV remodeling after AMI. The inhibitory effects of the SW therapy were noted in the early treatment protocol (*upper panel*) but not in the late treatment protocol (lower panel). *LVESV* left ventricular end-systolic volume, *LVEDV* left ventricular end-diastolic volume, *LVEF* left ventricular ejection fraction. Results are expressed as mean ± SEM ($n = 5$ each) (quoted from Ref. [7] with permission)

ischemia [5]. Recently, it was reported that SDF-1 is essential for the retention of pro-angiogenic stem cells in peripheral organs, although the up-regulation of VEGF is sufficient to mobilize stem or progenitor cells from the bone marrow to the systemic circulation [21,22]. Therefore, it is possible that our SW therapy enhances the incorporation of circulating EPCs by up-regulating the expression of SDF-1 in ischemic myocardium. This notion has been supported by the recent study by Aicher et al. [23] that combination of SW therapy enhances the efficacy of the cell-based angiogenic therapy. Further studies are needed to elucidate the precise molecular mechanisms involved in the beneficial effects of SW in the treatment of ischemic heart disease.

5 Advantages of extracorporeal cardiac SW therapy

Recent attempts to enhance angiogenesis in ischemic organs include gene therapy and bone marrow cell transplantation therapy. However, the need of invasive procedure to deliver those cells to the ischemic myocardium severely limits the usefulness of those therapies in clinical situations. A major advantage of our extracorporeal cardiac SW therapy over those strategies is shown by the fact that it is quite non-invasive and safe without any adverse effects. If necessary, we are able to repeatedly treat patients (even outpatients) with our SW therapy because no surgery, anesthesia, or even catheter intervention is required for the treatment. This is an important factor in determining the clinical usefulness of angiogenic therapies in elderly patients with severe coronary artery disease. Indeed, the SW treatment itself already has been clinically established as an effective and safe treatment for lithotripsy and chronic plantar fasciitis [24,25]. Thus, our extracorporeal cardiac SW therapy appears to be an applicable and non-invasive treatment for ischemic heart disease in humans.

6 Conclusions

We have successfully developed an extracorporeal cardiac SW therapy with a low energy SW, which may be an effective, safe, and non-invasive therapy for the treatment of severe ischemic heart disease in humans. Also, the cardiac SW therapy had no procedural complications or adverse effects.

Acknowledgments We thank Dr. Ernest H. Marlinghaus, Storz Medical AG, Switzerland, for valuable comments on our study. This study was supported in part by the 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.

References

1. Jessup, M., Brozena, S.: Heart failure. N. Engl. J. Med. **348**, 2007–2018 (2003)
2. Dimmeler, S., Zeiher, A.M., Schneider, M.D.: Unchain my heart: the scientific foundations of cardiac repair. J. Clin. Invest. **115**, 572–583 (2005)

3. Forrester, J.S., Price, M.J., Makkar, R.R.: Stem cell repair of infarcted myocardium: an overview for clinicians. Circulation **108**, 1139–1145 (2003)
4. Mathur, A., Martin, J.F.: Stem cells and repair of the heart. Lancet **364**, 183–192 (2004)
5. Nishida, T., Shimokawa, H., Oi, K., Tatewaki, H., Uwatoku, T., Abe, K., Matsumoto, Y., Kajihara, N., Eto, M., Matsuda, T., Yasui, H., Takeshita, A., Sunagawa, K.: Extracorporeal cardiac shock wave therapy markedly ameliorates ischemia-induced myocardial dysfunction in pigs in vivo. Circulation **110**, 3055–3061 (2004)
6. Fukumoto, Y., Ito, A., Uwatoku, T., Matoba, T., Kishi, T., Tanaka, H., Takeshita, A., Sunagawa, K., Shimokawa, H.: Extracorporeal cardiac shock wave therapy ameliorates myocardial ischemia in patients with severe coronary artery disease. Coron. Artery Dis. **17**, 63–70 (2006)
7. Uwatoku, T., Ito, K., Abe, K., Oi, K., Hizume, T., Sunagawa, K., Shimokawa, H.: Extracorporeal cardiac shock wave therapy improves left ventricular remodeling after acute myocardial infarction in pigs. Coron. Artery Dis. **18**, 397–404 (2007)
8. Haupt, G., Haupt, A., Ekkernkamp, A., Gerety, B., Chvapil, M.: Influence of shock waves on fracture healing. Urology **39**, 529–532 (1992)
9. Rompe, J.D., Rumler, F., Hopf, C., Nafe, B., Heine, J.: Extracorporal shock wave therapy for calcifying tendinitis of the shoulder. Clin. Orthop. Relat. Res. **321**, 196–201 (1995)
10. Mariotto, S., Cavalieri, E., Amelio, E., Ciampa, A.R., de Prati, A.C., Marlinghaus, E., Russo, S., Suzuki, H.: Extracorporeal shock waves: from lithotripsy to anti-inflammatory action by NO production. Nitric. Oxide. **12**, 89–96 (2005)
11. Maisonhaute, E., Prado, C., White, P.C., Compton, R.G.: Surface acoustic cavitation understood via nanosecond electrochemistry, part III: shear stress in ultrasonic cleaning. Ultrason Sonochem. **9**, 297–303 (2002)
12. Apfel, R.E.: Acoustic cavitation: a possible consequence of biomedical uses of ultrasound. Br. J. Cancer. **45**(suppl), 140–146 (1982)
13. Fisher, A.B., Chien, S., Barakat, A.I., Nerem, R.M.: Endothelial cellular response to altered shear stress. Am. J. Physiol. **281**, L529–L533 (2001)
14. Wang, F.S., Wang, C.J., Huang, H.J., Chung, H., Chen, R.F., Yang, K.D.: Physical shock wave mediates membrane hyperpolarization and Ras activation for osteogenesis in human bone marrow stromal cells. Biochem. Biophys. Res. Commun. **287**, 648–655 (2001)
15. 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. **520**, 153–155 (2002)
16. Seidl, M., Steinbach, P., Wörle, K., Hofstädter, F.: Induction of stress fibres and intercellular gaps in human vascular endothelium by shock-waves. Ultrasonics **32**, 397–400 (1994)
17. Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witzenbichler, B., Schatteman, G., Isner, J.M.: Isolation of putative progenitor endothelial cells for angiogenesis. Science **275**, 964–967 (1997)
18. Rafii, S., Lyden, D.: Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat. Med. **9**, 702–712 (2003)
19. Millauer, B., Wizigmann-Voos, S., Schnürch, H., Martinez, R., Møller, N.P., Risau, W., Ullrich, A.: High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. Cell **72**, 835–846 (1993)
20. Grunewald, M., Avraham, I., Dor, Y., Bachar-Lustig, E., Itin, A., Jung, S., Chimenti, S., Landsman, L., Abramovitch, R., Keshet, E.: VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell **124**, 175–189 (2006)
21. Ceradini, D.J., Kulkarni, A.R., Callaghan, M.J., Tepper, O.M., Bastidas, N., Kleinman, M.E., Capla, J.M., Galiano, R.D., Levine, J.P., Gurtner, G.C.: Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat. Med. **10**, 858–864 (2004)
22. Askari, A.T., Unzek, S., Popovic, Z.B., Goldman, C.K., Forudi, F., Kiedrowski, M., Rovner, A., Ellis, S.G., Thomas, J.D., DiCorleto, P.E., Topol, E.J., Penn, M.S.: Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. Lancet **362**, 697–703 (2003)
23. Aicher, A., Heeschen, C., Sasaki, K., Urbich, C., Zeiher, A.M., 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 **114**, 2823–2830 (2006)
24. Auge, B.K., Preminger, G.M.: Update on shock wave lithotripsy technology. Curr. Opin. Urol. **12**, 287–290 (2002)
25. Weil, L.S. Jr., Roukis, T.S., Weil, L.S., Borrelli, A.H.: Extracorporeal shock wave therapy for the treatment of chronic plantar fasciitis: indications, protocol, intermediate results, and a comparison of results to fasciotomy. J. Foot Ankle Surg. **41**, 166–172 (2002)

循環器科，61(4)：372-377，2007

話　題

体外衝撃波治療*

伊藤健太**　下川宏明**

Key Words：shock wave therapy, angiogenesis, ischemic heart disease, angina pectoris, myocardial infarction

はじめに

虚血性心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カテーテルを用いた冠動脈インターベンション(percutaneous coronary intervention：PCI)，③冠動脈バイパス手術(coronary arterial bypass grafting：CABG)の3本柱から成る．近年わが国では，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，PCIやCABGを施行できないようなびまん性狭窄病変をもつ症例など，従来の治療法では十分な治療効果を得られない重症の虚血性心疾患症例が増加してきている．このような症例では，胸痛のため生活の質(QOL)が低下するのみならず，慢性心筋虚血による心機能低下のため，重症の心不全へと進行し予後も不良である．そのため，新たな治療法の開発が望まれている．

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未分化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，未分化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．さらに，有効性・安全性が認められたとしても，費用が高額にな

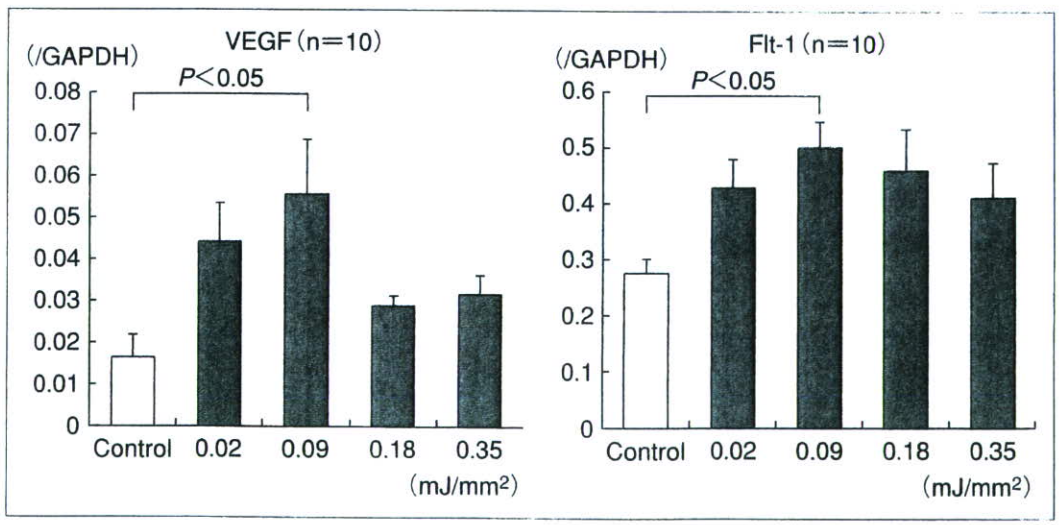


図1　衝撃波によるヒト培養内皮細胞(HUVEC)における血管新生因子の発現亢進
(文献[6]より改変)

* Extracorporeal cardiac shock wave therapy.

** Kenta ITO, M.D., Ph.D. & Hiroaki SHIMOKAWA, M.D., Ph.D.: 東北大学大学院循環器病態学分野〔〒980-8574 仙台市青葉区星陵町1-1〕; Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai 980-8574, JAPAN

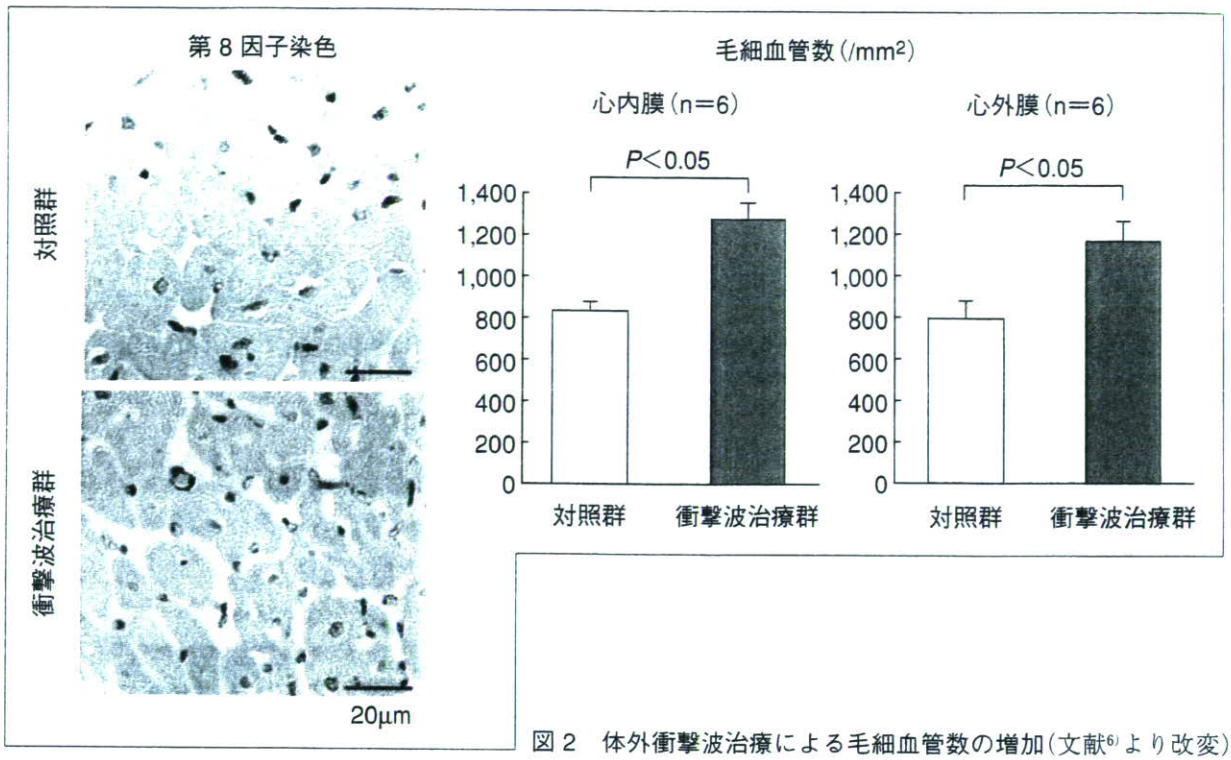


図2　体外衝撃波治療による毛細血管数の増加(文献[6]より改変)

る．一方，最近では，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[1)~5)]．そのため，低侵襲で有効性の期待できる治療法の開発が期待されている．

われわれは虚血性心疾患患者を対象に，低出力の衝撃波を用いた血管新生療法を開発し，現在，重症狭心症患者に対する臨床試験と急性心筋梗塞患者に対する臨床試験を行っている．

低出力の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もつ媒体内を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いる．われわれは基礎実験により，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(0.09mJ/mm²)をピークに，主要な血管新生因子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)[6)]．そこで，ブタ慢性心筋虚血モデルを作成して体外衝撃波治療の効果を検討した．慢性虚血心筋に1か所あたり200発の衝撃波を9か所照射し，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，虚血心筋におけるVEGFの発現が遺伝子レベル・蛋白レベルのいずれでも亢進していた．さらに，毛細血管数の増加(図2)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[6)]．衝撃波治療中および治療後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られなかった．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確認された．

重症狭心症に対する体外衝撃波治療

上記の基礎的検討に基づき，われわれは，重症狭心症に対して体外衝撃波治療の臨床試験を行っている．対象は，①20歳以上，②最大量の薬物治療に抵抗性で，かつPCIやCABGで完全な血行再建が不可能，③カナダ心臓病学課分類(CCS)

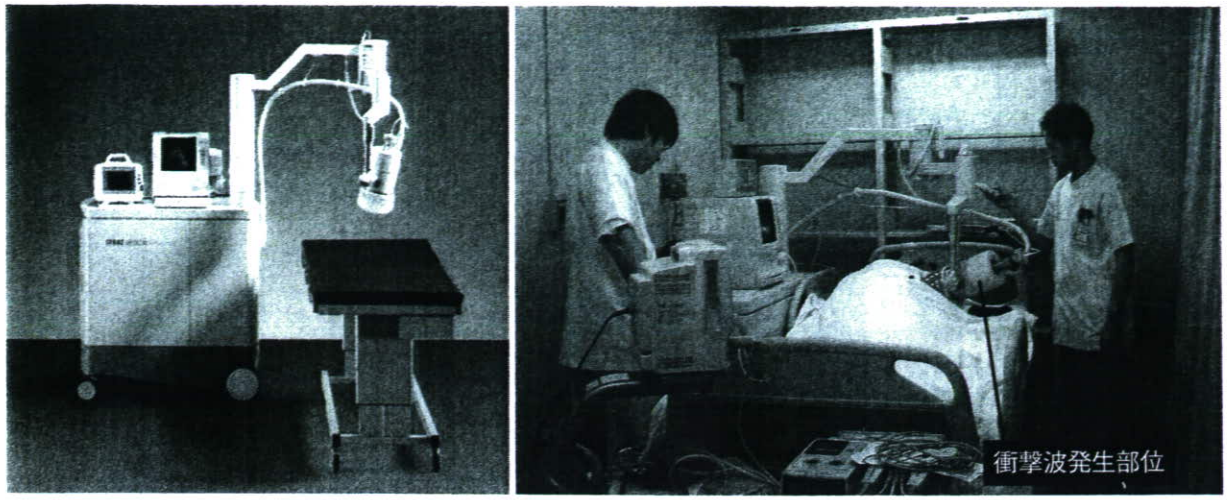


図3　非侵襲性体外衝撃波治療装置(左)と治療風景(右)

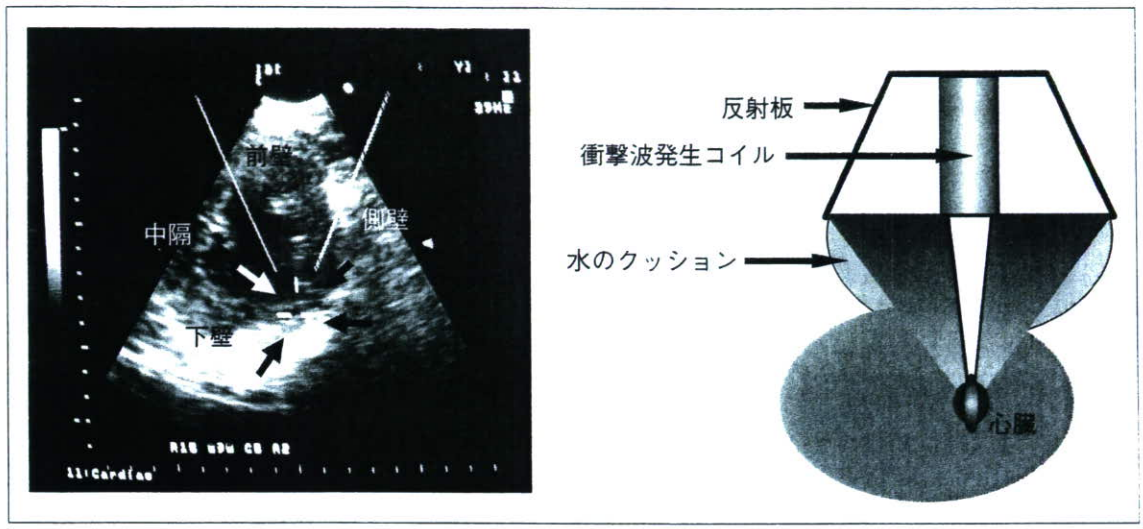


図4　衝撃波治療の方法
心臓超音波検査画面(左)と概念図(右)．(文献[7]より改変)

でClass II～IV，④負荷心筋シンチや負荷心エコーで明らかな虚血領域を認める重症狭心症とした．実際の治療には，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る(図3)．装置に内蔵した超音波プローブを患者の前胸壁にあてて，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か所につき200発の衝撃波を，虚血領域の広さに応じて20～120か所照射する(図4)．1回の治療時間は約3時間で，1日おきに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．ただし，衝撃波は空気があると破裂する性質があるため，慎重に肺を避ける必要がある．2003年1月から重症狭心症患者9名を対象に九州大学で実施した第一次臨床試験では，個人差はあるものの全例で胸痛の自覚症状が軽減し，また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ている(図5)[7]．自覚症状のみならず，負荷心筋シンチグラムで評価した心筋灌流も，低出力の衝撃波を照射した部位において改善を認めた[7]．図6にあげた例では，1回目の治療で左室前壁中隔に対して体外衝撃波治療を行ったところ，治療部位においてのみ心筋灌流が改善した．さらに2回目の治療で左室側壁に対して体外衝撃波治療を行ったところ，今度は側壁領域の心筋灌流が改善した．この結果から，衝撃波治療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まったく認めなかった．

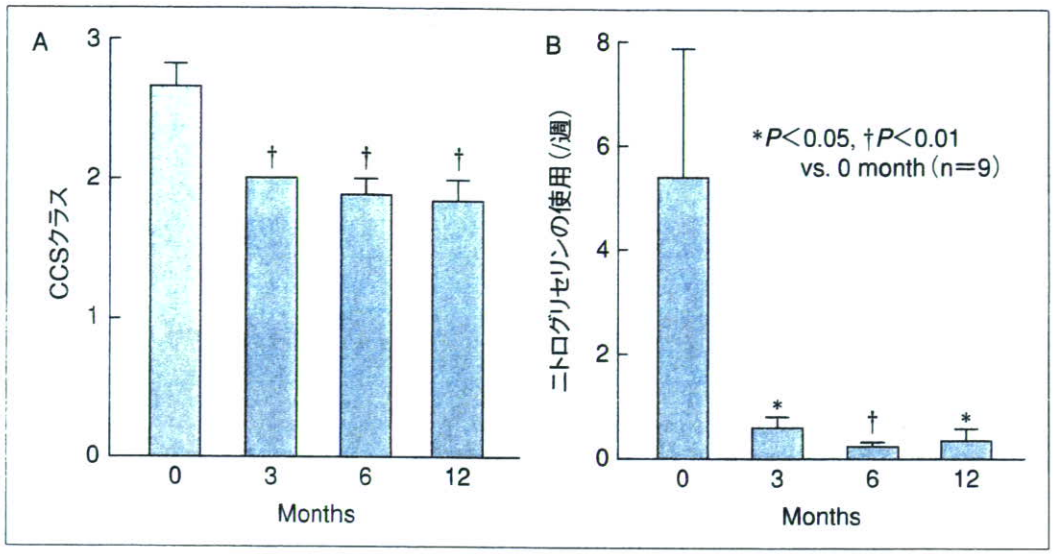


図5　体外衝撃波治療による自覚症状の改善（文献[7]より改変）

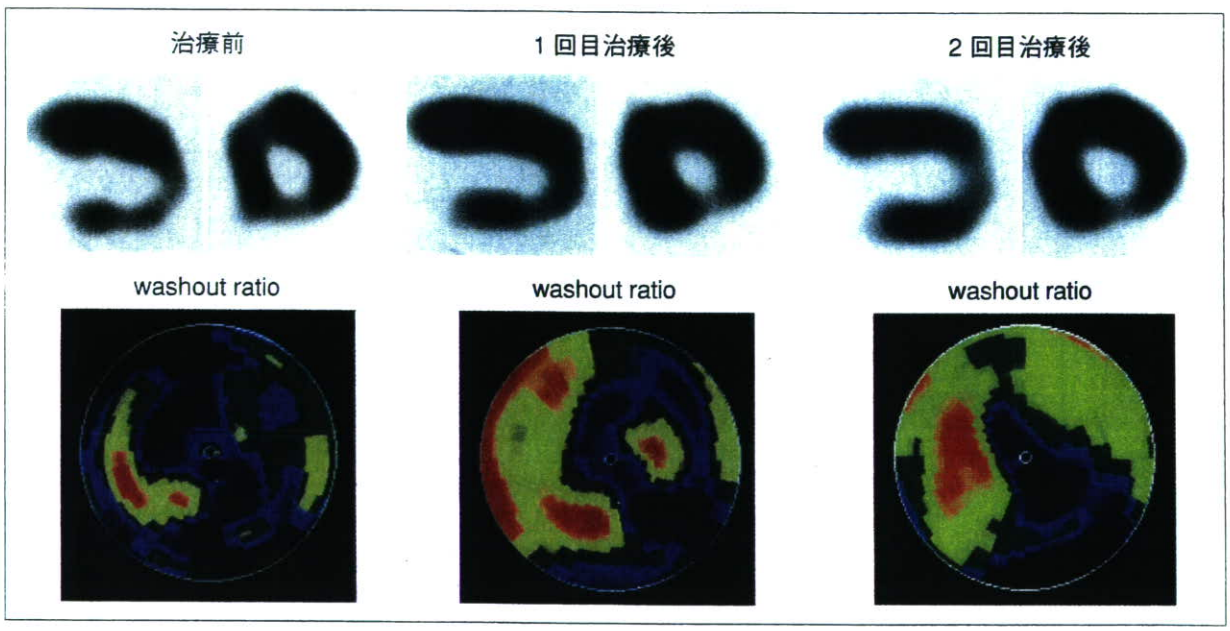


図6　体外衝撃波治療による心筋灌流の改善（文献[7]より改変）

有効性をさらに科学的に評価するために，2005年11月から東北大学病院において，衝撃波治療とプラセボ治療を比較する第二次臨床試験が進行中である．3か月間隔で衝撃波治療とプラセボ治療を一度ずつ受けるプロトコールとなっており，初回に衝撃波治療を受けた症例は3か月後にプラセボ治療を受け，初回プラセボ治療を受けた症例は3か月後に衝撃波治療を受けることになる．

急性心筋梗塞に対する体外衝撃波治療

わが国では，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，心筋梗塞発症早期の死亡率低下に貢献している．しかし，慢性期には十分な薬物治療を受けていても左室リモデリング（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などにより微小血管の血流が完全には改善せず心筋は虚血にさらされており，これが左室リモデリング進行の一因と考えられている．そこでわれわれは，ブタ急性心筋梗塞モデルを用いて，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3, 6, 9日目に体外