Tohoku J. Exp. Med., 2009, **219**, 1-9

Invited Review for the 90th Anniversary

Extracorporeal Shock Wave Therapy as a New and Non-invasive Angiogenic Strategy

Kenta Ito,[1] Yoshihiro Fukumoto[1] and Hiroaki Shimokawa[1]

[1]Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan

Ischemic heart disease is the leading cause of death and the number of refractory severe patients is increasing. Therefore, it is crucial to develop new therapeutic strategies for severe ischemic heart disease. We found that a low-energy shock wave (SW) (about 10% of energy density that used for urolithiasis) effectively increases the expression of vascular endothelial growth factor (VEGF) in cultured endothelial cells. Based on this *in vitro* study, we have started *in vivo* studies and have demonstrated that extracorporeal cardiac shock wave therapy with a low-energy SW upregulates the expression of VEGF, induces neovascularization, and improves myocardial ischemia in a porcine model of chronic myocardial ischemia without any adverse effects *in vivo*. On the basis of the promising results in animal studies, we have subsequently developed a new, non-invasive angiogenic therapy with low-energy SW for ischemic heart disease. Our extracorporeal cardiac SW therapy improved symptoms and myocardial perfusion evaluated with stress-scintigraphy in patients with severe coronary artery disease without indication of percutaneous coronary intervention or coronary bypass surgery. Importantly, no procedural complications or adverse effects were noted. The SW therapy was also effective to ameliorate LV remodeling after acute myocardial infarction in pigs and to enhance angiogenesis in hindlimb ischemia in rabbits. Based on these animal studies, we are also conducting clinical studies in patients with acute myocardial infarction and those with peripheral artery disease. Thus, our extracorporeal cardiac SW therapy is an effective, safe, and non-invasive angiogenic strategy in cardiovascular medicine and its indication is now rapidly expanding.

———— Shock wave therapy; Angiogenesis; Ischemic heart disease; Growth factors.

Tohoku J. Exp. Med., 2009, **219** (1), 1-9.

Ischemic heart disease is the leading cause of death in developed countries and a major cause of hospital admissions, and the number of patients is increasing worldwide (Jessup and Brozena 2003). Its risk increases with age, diabetes, hypercholesterolaemia, hypertension, smoking, and is more common in men and those who have family history of ischemic heart disease. Since the coronary arteries supply blood to the heart muscle, the 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 and reduced exercise tolerance ("angina pectoris"), permanent heart muscle damage ("acute myocardial infarction"), lethal arrhythmia, and sudden cardiac death. The current management of ischemic heart disease has 3 major therapeutic options, including medication (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 due to the lack of effective treatments to treat the disorder. Thus, it is crucial to develop alternative therapeutic strategies for severe ischemic heart disease. During this decade, a variety of regenerative therapies, such as gene and stem cell therapies, are under development (Kawamoto et al. 2003; Khan et al. 2003; Rutanen et al. 2004; Schächinger et al. 2004; Wollert et al. 2004; Kastrup et al. 2005; Choi et al. 2006; Schächinger et al. 2006; Qian et al. 2006; Kajiguchi et al. 2007; Tatsumi et al. 2007). However, most of these regenerative therapies are invasive in nature. In addition, although many of these therapies have shown to be effective in animal models, their effectiveness and safeness in patients have not yet been established in clinical trials (Epstein et al. 2001; Forrester et al. 2003; Mathur and Martin 2004; Davani et al. 2005; Dimmeler et al. 2005; Choi et al. 2007; Kang et al. 2008; Martin-Rendon et al. 2008).

Extracorporeal SW therapy has been introduced for medical therapy more than 20 years ago to fragmentize kid-

Received July 30, 2009; revision accepted for publication August 5, 2009. doi:10.1620/tjem.219.1

Correspondence: Hiroaki Shimokawa, MD, PhD., 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

ney stones, which has markedly improved the treatment of urolithiasis. Furthermore, the lithotripsy therapy with high-energy SW is indicated for gallstones, pancreatic and salivary stones. We have recently developed a new, non-invasive angiogenic therapy using low-energy shock waves (SW) which level is approximately 10% of that used for urinary lithotripsy treatment (Nishida et al. 2004; Fukumoto et al. 2006; Uwatoku et al. 2007; Oi et al. 2008; Shimokawa and Ito 2009). Based on our reports in animals and humans (Nishida et al. 2004; Fukumoto et al. 2006), Aicher et al employed a low-energy SW therapy to treat hindlimb ischemia in rats in combination with cell-based therapy (Aicher et al. 2006). In this article, we outline our recent works in animals and humans, and then discuss the potential mechanisms for SW-induced neovascularization and advantages of our cardiac SW therapy. All of the animal studies and clinical studies mentioned in this article are approved by the institutional committee.

In vitro study

SW is a longitudinal acoustic wave that propagates through water or soft tissue as ultrasound does. In contrast to ultrasound, SW is a single pressure pulse with a short needle-like positive spike < 1 μs in duration and up to 100 MPa in amplitude, followed by a tensile part of several microseconds with lower amplitude. SW also exerts the "cavitation effect" (a micrometer-sized violent collapse of bubbles inside and outside the cells) (Apfel. 1982) and was shown to induce localized stress on cell membranes that resembles shear stress (Maisonhaute et al. 2002). It is also reported that a low level of SW could up-regulate nitric oxide (NO) production in vitro (Mariotto et al. 2005). 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 umbilical vein 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) (Nishida et al. 2004).

Extracorporeal SW therapy for angina pectoris

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. Therefore, we performed in vivo animal experiments with pigs. A porcine model of chronic 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 (Nishida et al. 2004). At 4 weeks after the implantation of an ameroid constrictor, we performed extracorporeal SW therapy to the ischemic myocardial region 3 times during the first week (n = 8), whereas animals in the control group (n = 8) received the same anesthesia procedures 3 times a week but without the SW treatment. On the basis of our *in vitro* experiments, we applied low-energy SW (0.09 mJ/mm^2) to 9 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). In order to treat the targeted ischemic myocardium without inducing ventricular arrhythmia, we applied SW at end-diastole during the cardiac cycle with a R wave-triggered system. We evaluated cardiac function before 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

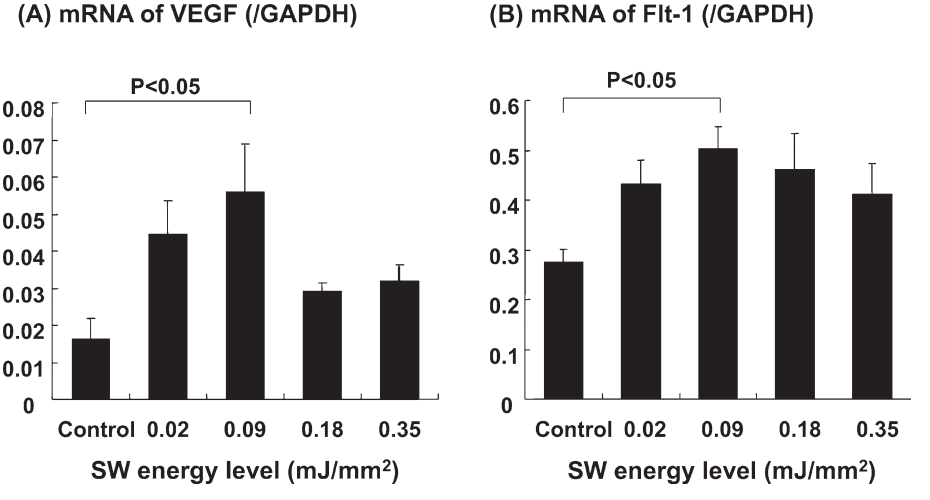

Fig. 1. Effects of SW on mRNA expression in HUVECs in vitro. SW treatment up-regulates mRNA expression of VEGF (A) and Flt-1 (B) with a maximum effect noted at 0.09 mJ/mm^2. Results are expressed as mean ± SEM (n = 10 each). Quoted from Nishida et al. 2004 with permission.

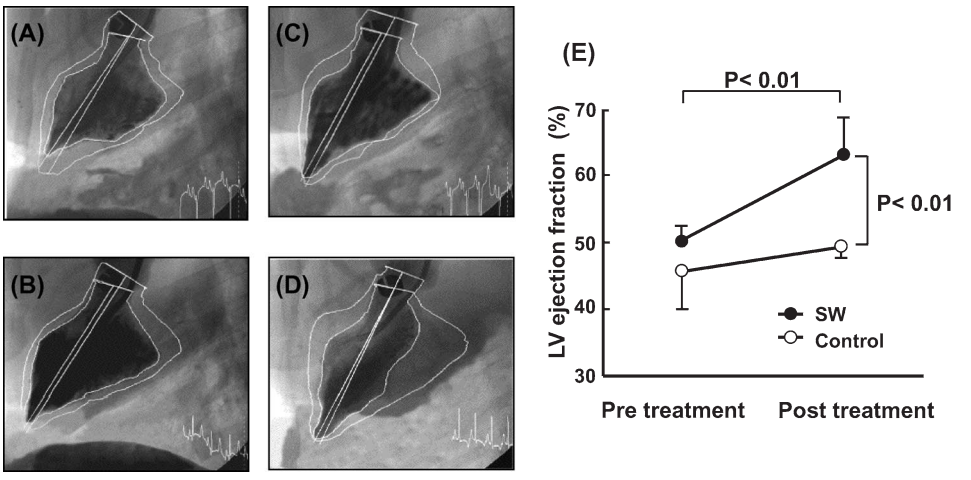

Fig. 2. Effects of SW therapy on LV function in pigs in vivo. Extracorporeal cardiac SW therapy improves ischemia-induced myocardial dysfunction in vivo as evaluated with left ventriculography. Four weeks after the implantation of an ameroid constrictor, LV wall motion of the LCX (posterolateral) region 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 Nishida et al. 2004 with permission.

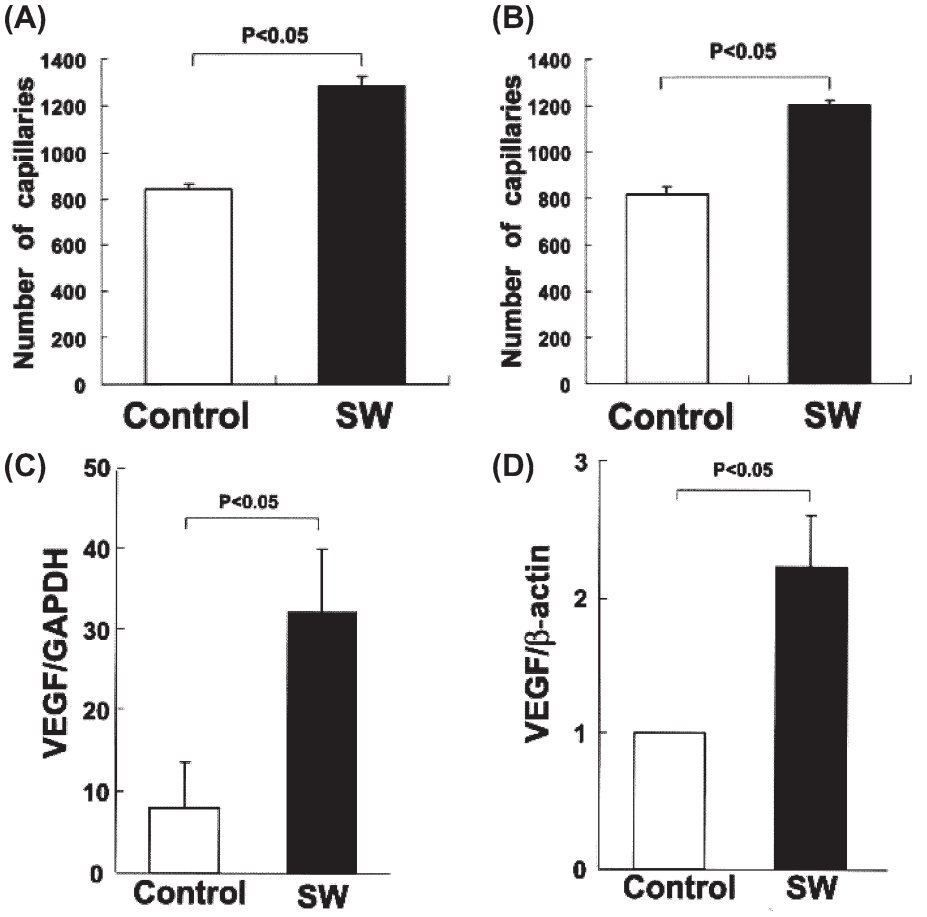

Fig. 3. Effects of SW therapy on capillary density and VEGF expression in the ischemic myocardium. Extracorporeal cardiac SW therapy increases the density of factor VIII-positive capillaries and VEGF expression in the ischemic myocardium. Capillary density was significantly greater in the SW group (SW) than in the control group (Control) in both the endocardium (A) and the epicardium (B). The mRNA expression (C) and the protein levels (D) of VEGF were significantly higher in the SW group than in the control group. Results are expressed as mean ± SEM (n = 6 each). Quoted from Nishida et al. 2004 with permission.

Table 1. Baseline characteristics and outcome of the patients.

Patient	Age	Sex	CAD	Previous treatment	OMI	ASO	HT	DM	HL	HD	SW therapy				CCS class score		NG use (per week)	
											0	1	3	6 months	0	12 months	0	12 months
1	82	M	3VD	CABG	+	+	+	–	–	–	+	–	–	–	3	2	1	0
2	66	M	3VD	None	–	–	+	+	–	–	+	+	+	–	3	2	0.5	0.2
3	64	F	3VD	PCI, CABG	+	–	+	+	+	–	+	+	–	+	3	2	15	0
4	56	F	3VD	PCI, CABG	+	–	–	+	+	+	+	–	+	–	2	2	0	0
5	70	M	3VD	CABG	+	–	–	+	–	–	+	+	+	–	2	2	0	0
6	76	M	1VD	None	+	+	+	–	–	–	+	+	–	–	3	1	3	0
7	62	M	3VD	None	+	–	–	–	+	+	+	+	+	–	3	2	21	2
8	70	F	3VD	PCI	+	+	+	+	–	–	+	+	–	–	3	2	7	0.25
9	55	F	3VD	None	–	–	–	+	+	–	+	+	+	–	2	2	1	0.5

M, male; F, female; CAD, coronary artery disease; VD, vessel disease; PCI, percutaneous coronary intervention; CABG, coronary artery bypass graft surgery; OMI, old myocardial infarction; ASO, arteriosclerosis obliterans; HT, hypertension; DM, diabetes mellitus; HL, hyperlipidemia; HD, hemodialysis; CCS, Canadian Cardiovascular Society; NG, Nitroglycerin.

Quoted from Fukumoto et al. (2006) with permission.

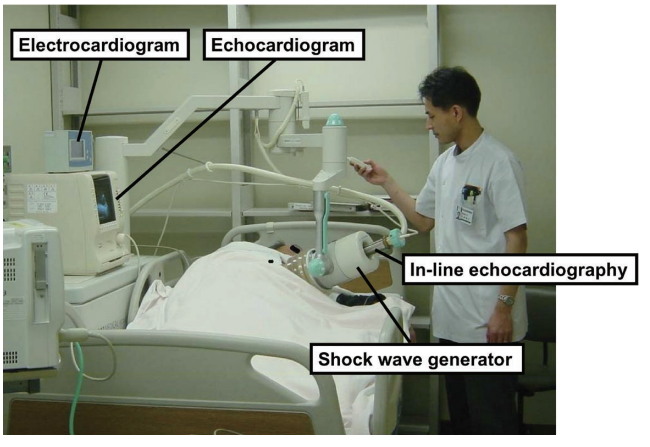

Fig. 4. Extracorporeal cardiac SW therapy in action in a patient with severe coronary artery disease. The machine is equipped with a SW generator and in-line echocardiography. The SW generator is attached to the chest wall of the patient when used. The SW pulse is easily focused on the ischemic myocardium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

in the SW group (Fig. 2B, 2D). 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 therapy increased capillary density and up-regulated VEGF expression in the ischemic myocardium in vivo (Fig. 3). Importantly, no procedural complications or adverse effects, such as tissue injury, hemorrhage, or arrhythmia, were noted during or after the SW therapy. These data suggest that our low-energy SW therapy activated the endogenous angiogenic pathways in pigs in vivo. This was the first report that demonstrates the potential usefulness of extracorporeal cardiac SW therapy as a non-invasive treatment of chronic myocardial ischemia, suggesting its usefulness for the treatment of ischemic heart disease in humans.

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 9 patients with end-stage ischemic heart disease with no indication of PCI or CABG (55-82 years old, 5 men and 4 women; Table 1) with our cardiac SW therapy (200 shots/spot at 0.09 mJ/mm^2 for 20-40 spots, 3 times a week/series) (Fukumoto et al. 2006). As shown in Fig. 4, a patient just lied down on the bed without anesthesia during the therapy. The SW therapy improved symptoms and reduced nitroglycerin use (Fig. 5, Table 1) as well as myocardial perfusion as assessed by dipyridamole stress thallium scintigraphy only in the ischemic area treated with the SW (Fig. 6). These beneficial effects of the SW therapy persisted for at least 12 months. No procedural complications or adverse effects were noted. These data indicate that our extracorporeal cardiac SW therapy is a safe, effective, and non-invasive therapeutic strategy for severe 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.

Extracorporeal SW therapy for acute myocardial infarction

Animal studies

Acute loss of myocardial cells following acute myo-

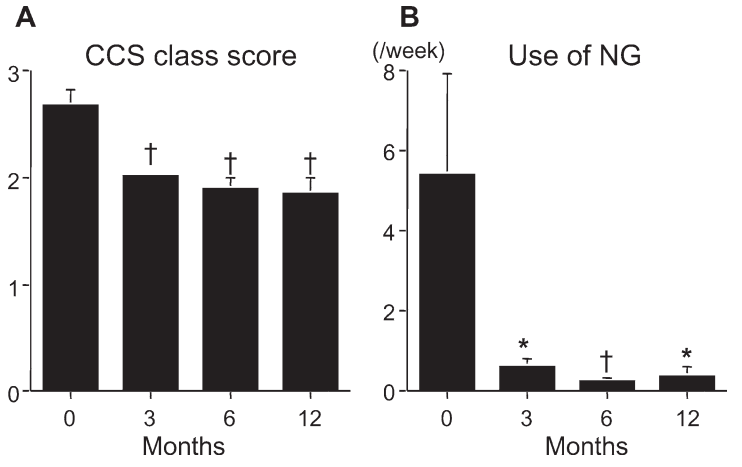

Fig. 5. Effects of SW therapy on symptom and the use of nitroglycerin in patients with severe coronary artery disease. Extracorporeal cardiac SW therapy significantly improved Canadian Cardiovascular Society (CCS) class scores for angina (A) and the use of nitroglycerin (NG) (B). Results are expressed 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 Fukumoto et al. 2006 with permission.

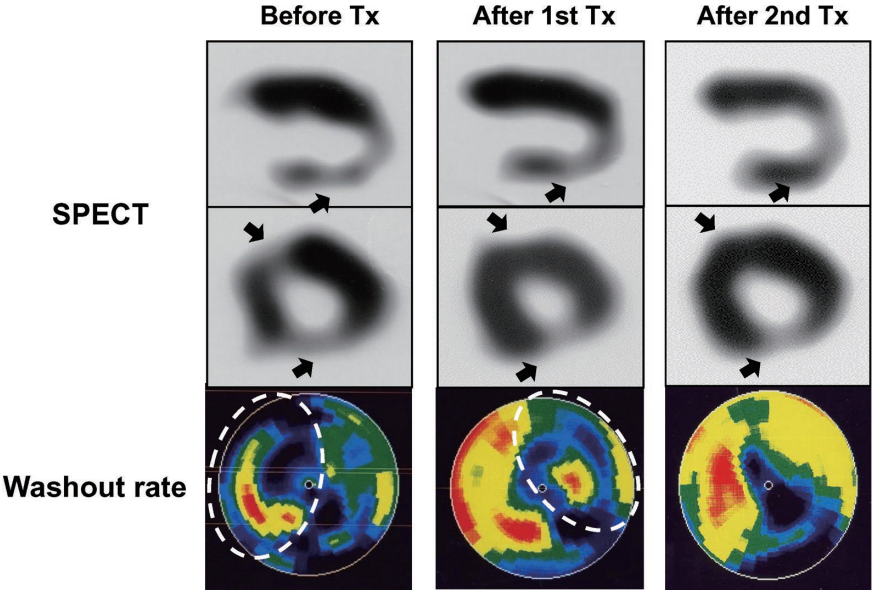

Fig. 6. Effects of SW therapy on myocardial perfusion in a patient with severe coronary artery disease. Dipyridamole stress thallium-201 single photon emission computed tomography (SPECT) imaging and polar map in a patient with severe 3-vessel coronary artery disease before and after the extracorporeal cardiac SW therapy. The results clearly demonstrated that the SW therapy ameliorated myocardial perfusion only where SW was applied; in the anteroseptal wall after the first treatment (1st Tx) and in the lateral wall after the second treatment (2nd Tx) (arrows). The areas where shock waves were shot were indicated with dotted lines. Quoted from Fukumoto et al. 2006 with permission.

cardial infarction (AMI) causes abnormal loading conditions, which may induce LV dilatation and gradual decline in LV contractility during chronic phase. This process is called "LV remodeling" (Jessup and Brozena 2003). The development of LV remodeling leads to heart failure, sudden cardiac death, and poor prognosis (Volpi et al. 1993). It is reported that capillary density in the border zone is negatively correlated with infarct size one month after AMI, suggesting the importance of the adequate growth of the capillary microvasculature (Olivetti et al. 1986). Therefore, it is expected that enhancing neovascularization in the border zone adjacent to infarcted myocardium ameliorate the progression of LV remodeling in patients with AMI, resulting in an improvement of prognosis. Therefore, we examined whether our SW therapy is also effective to ameliorate LV remodeling after AMI in pigs in vivo.

AMI was created by surgically excising the proximal segment of the LCX (Uwatoku et al. 2007). The SW therapy was started at 3 days after AMI ($n = 5$). The remaining animals were treated in the same manner but without the SW treatment ($n = 5$). At 4 weeks after the treatment, LV ejection fraction and LV end-diastolic volume were significantly improved in the SW group compared with the control group (Fig. 7). Furthermore, regional myocardial blood

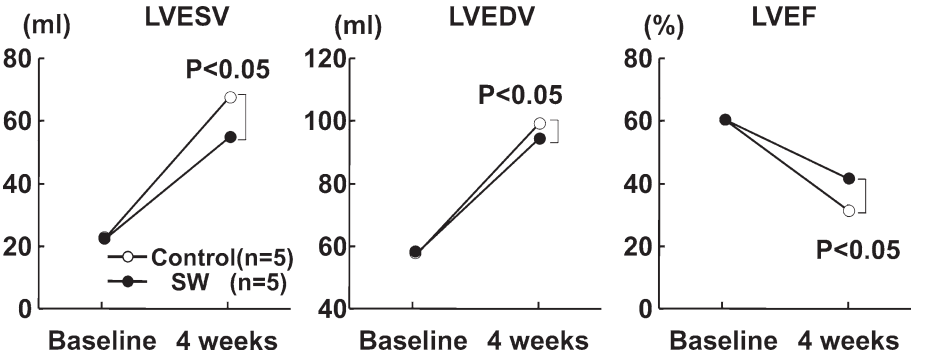

Fig. 7. Effects of SW therapy on LV remodeling in pigs in vivo. The SW therapy significantly ameliorated LV remodeling characterized by the increase in LV end-systolic volume (LVESV) and end-diastolic volume (LVEDV) and reduced LV ejection fraction (LVEF) in a porcine model of AMI. Quoted from Uwatoku et al. 2007 with permission.

flow and number of capillaries in the border zone were significantly improved in the SW group compared with the control group. As in the previous studies, no procedural complications or adverse effects were noted. These results suggest that our extracorporeal cardiac SW therapy is an effective and non-invasive therapy to ameliorate LV remodeling after AMI. This was the first report that demonstrates the usefulness and safety of extracorporeal cardiac SW therapy as a noninvasive treatment of AMI.

Clinical studies

In the clinical setting, most of patients with AMI receive an emergent reperfusion therapy with either PCI or thrombolytic drugs. Therefore, we also confirmed the beneficial effects of our SW therapy in another AMI model in pigs where AMI was induced by 90-min ischemia and reperfusion using a coronary angioplasty balloon catheter (Ito et al. 2008, 2009). 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.

Extracorporeal SW therapy for peripheral artery disease

Peripheral artery disease (PAD) is often associated with systemic atherosclerosis and cardiovascular diseases, and its associated mortality is rapidly increasing worldwide (Sumpio 2000; Regensteiner and Stewart 2006; Al Mheid and Quyyumi 2009). Therefore, we aimed to apply our cardiac SW therapy to PAD. We studied the effects of our SW therapy on hindlimb ischemia in rabbits (Oi et al. 2008). Hindlimb ischemia was induced by surgical excision of the entire unilateral femoral artery. One week after the operation, we started SW therapy to the ischemic region 3 times a week for 3 weeks. Four weeks after the operation, blood flow, blood pressure, and capillary density were all significantly increased in the SW group compared with the control group (Fig. 8). On the basis of this study, we are conducting a clinical study in patients with arteriosclerosis obliterans (ASO) with intermittent claudication (Fontaine stage II) and those with critical limb ischemia (Fontaine stage III and IV).

SW therapy for other disorders

Recently, several studies are reported that showed the beneficial effects of low-energy SW therapy in other disorders, including skin flap model in rodents (Stojadinovic et al. 2008; Yan et al. 2008) and patients with refractory chronic skin ulcers (Saggini et al. 2008; Moretti et al. 2009). Also, low to high energy levels of SW is widely used for the treatment of certain orthopedic conditions, such as nonunions, tendinosis calcarea, epicondylitis and calcaneal spur (Birnbaum et al. 2002; Wang et al. 2003).

Mechanisms for SW-induced neovascularization

When a SW hits tissue, cavitation is induced by the first compression by the positive pressure part and the expansion with the tensile part of a SW (Apfel 1982). Because the physical forces generated by cavitation are highly localized, SW could induce localized stress and subsequent shear stress on cell surface membranes (Fisher et al. 2001). Several biochemical effects of SW were reported including hyperpolarization, Ras activation, non-enzymatic NO synthesis, and induction of stress fibers and intercellular gaps (Seidl et al. 1994; Wang et al. 2001; Gotte et al. 2002). Also, SW treatment is reported to affect the expression of several chemokines and matrix metalloproteinases and therefore bring anti-inflamatory effects (Ciampa et al. 2005; Mariotto et al. 2005; Stojadinovic et al. 2008; Mariotto et al. 2009) in addition to up-regulation of VEGF and its receptor Flt-1 (Nishida et al. 2004), implying that the multiple angiogenic pathways are involved in the beneficial effects of the SW therapy. Recently, in addition to migration and proliferation of endothelial cells in situ ("angiogenesis"), bone marrow-derived endothelial progenitor cells (EPCs) contribute to neovascularization in ischemic tissue by forming capillary vasculature ("vasculogenesis") or by secreting a variety of angiogenic factors ("paracrine effect") (Urbich et al. 2005). Enhanced expression of multiple angiogenic factors, such as VEGF and stromal-derived factor 1 (SDF-1), is crucial for the recruitment and incorpora-

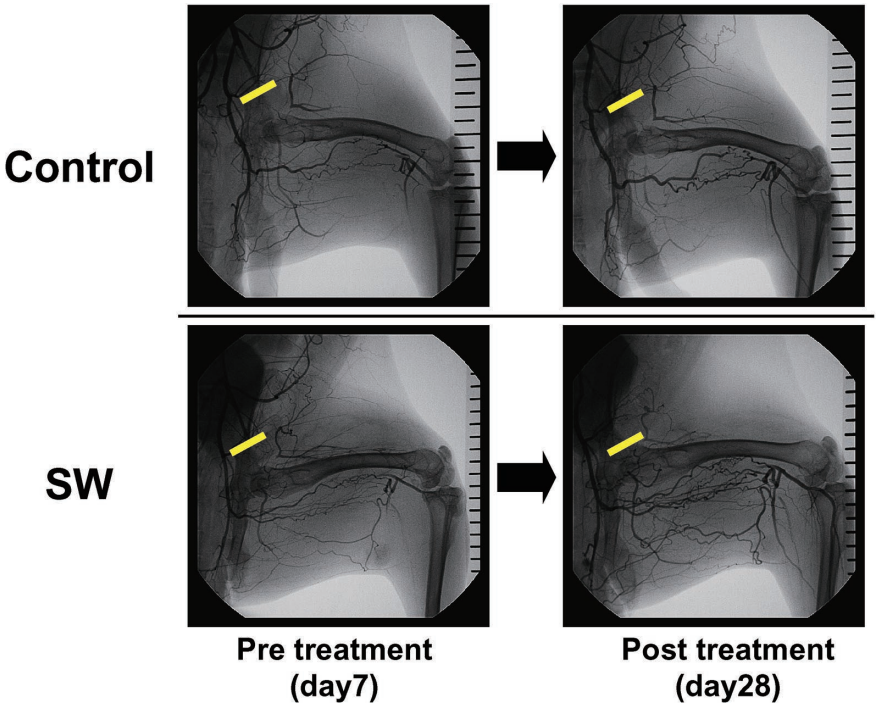

Fig. 8. Effects of SW therapy on angiogenesis in hindlimb ischemia. Representative selective internal iliac angiography of control rabbit and SW-treated rabbit at day 7 (1 week after surgery without the SW therapy) and day 28 (3 weeks after the SW therapy). The development of collateral arteries was enhanced in the SW-treated rabbit compared with the control animals. Quoted from Oi et al. 2008 with permission.

tion of EPCs (Asahara et al. 1997; Askari et al. 2003; Rafii and Lyden 2003; Millauer et al. 1993; Grunewald et al. 2006; Ceradini et al. 2004; Satoh et al. 2009). Also, it was reported that the activation of the SDF-1/CXCR4 axis 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 (Askari et al. 2003; Ceradini et al. 2004). 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 report showing that combination of SW therapy enhances the effectives of the cell-based angiogenic therapy (Aicher et al. 2006). Continuous turnover of cardiac myocytes and myocyte regeneration in response to injury have also been reported (Hsieh et al. 2007). However, it is not known yet whether our SW therapy affects this regenerative potential of cardiac myocytes. Further studies are required to elucidate the precise molecular mechanisms responsible for the beneficial effects of SW in the treatment of various disorders.

Advantages of Extracorporeal Cardiac SW Therapy

Recent attempts to enhance angiogenesis in ischemic organs include gene therapy and cell-based therapy. However, the need of invasive procedure to deliver those cells to the ischemic tissue severely limits the usefulness of those therapies in clinical situations, especially in elderly patients. A major advantage of our extracorporeal low-energy 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 or anesthesia is required for the treatment. This is an important factor in determining the clinical usefulness of angiogenic therapies especially in elderly patients. In addition, our extracorporeal cardiac SW therapy is quite inexpensive.

Conclusions

We have successfully developed an effective, safe, and non-invasive extracorporeal SW therapy for the treatment of severe angina pectoris. The indication of our SW therapy could be extended to AMI, PAD, and other ischemic disorders in the near future. The angiogenic effects of SW may be mediated by the enhancement of several intrinsic angiogenic systems, although the precise mechanisms remain to be elucidated in future studies.

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, Labour, and Welfare, Tokyo, Japan.

References

Aicher, A., Heeschen, C., Sasaki, K., Urbich, C., Zeiher, A.M. & Dimmeler, S. (2006) 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.

Al Mheid, I. & Quyyumi, A.A. (2009) Cell therapy in peripheral arterial disease. *Angiology*, **59**, 705-716.

Apfel, R.E. (1982) Acoustic cavitation: a possible consequence of biomedical uses of ultrasound. *Br. J. Cancer.*, **45 (suppl)**, 140-146.

Asahara, T., Murohara, T., Sullivan, A., Silver, M., van der Zee, R., Li, T., Witzenbichler, B., Schatteman, G. & Isner, J.M. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science*, **275**, 964-967.

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. (2003) Effect of stromal-cell-derived factor 1 on stem-cell homing and tissue regeneration in ischaemic cardiomyopathy. *Lancet*, **362**, 697-703.

Birnbaum, K., Wirtz, D.C., Siebert, C.H. & Heller, K.D. (2002) Use of extracorporeal shock-wave therapy (ESWT) in the treatment of non-unions. A review of the literature. *Arch. Orthop. Trauma. Surg.*, **122**, 324-330.

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. (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat. Med.*, **10**, 858-864.

Choi, J.H., Choi, J., Lee, W.S., Rhee, I., Lee, S.C., Gwon, H.C., Lee, S.H., Choe, Y.H., Kim, D.W., Suh, W., Kim, D.K. & Jeon, E.S. (2007) Lack of additional benefit of intracoronary transplantation of autologous peripheral blood stem cell in patients with acute myocardial infarction. *Circ. J.*, **71**, 486-494.

Choi, J.S., Kim, K.B., Han, W., Kim, D.S., Park, J.S., Lee, J.J. & Lee, D.S. (2006) Efficacy of therapeutic angiogenesis by intramyocardial injection of pCK-VEGF165 in pigs. *Ann. Thorac. Surg.*, **82**, 679-686.

Ciampa, A.R., de Prati, A.C., Amelio, E., Cavalieri, E., Persichini, T., Colasanti, M., Musci, G., Marlinghaus, E., Suzuki, H. & Mariotto, S. (2005) Nitric oxide mediates anti-inflammatory action of extracorporeal shock waves. *FEBS. Lett.*, **579**, 6839.

Davani, S., Deschaseaux, F., Chalmers, D., Tiberghien, P. & Kantelip, J.P. (2005) Can stem cells mend a broken heart? *Cardiovasc. Res.*, **65**, 305-316.

Dimmeler, S., Zeiher, A.M. & Schneider, M.D. (2005) Unchain my heart: the scientific foundations of cardiac repair. *J. Clin. Invest.*, **115**, 572-583.

Epstein, S.E., Fuchs, S., Zhou, Y.F., Baffour, R. & Kornowski, R. (2001) Therapeutic interventions for enhancing collateral development by administration of growth factors: basic principles, early results and potential hazards. *Cardiovasc. Res.*, **49**, 532-542.

Fisher, A.B., Chien, S., Barakat, A.I. & Nerem, R.M. (2001) Endothelial cellular response to altered shear stress. *Am. J. Physiol.*, **281**, L529-L533.

Forrester, J.S., Price, M.J. & Makkar, R.R. (2003) Stem cell repair of infarcted myocardium: an overview for clinicians. *Circulation,* **108**, 1139-1145.

Fukumoto, Y., Ito, A., Uwatoku, T., Matoba, T., Kishi, T., Tanaka, H., Takeshita, A., Sunagawa, K. & Shimokawa, H. (2006) Extracorporeal cardiac shock wave therapy ameliorates myocardial ischemia in patients with severe coronary artery disease. *Coron. Artery. Dis.*, **17**, 63-70.

Gotte, G., Amelio, E., Russo, S., Marlinghaus, E., Musci, G. & Suzuki, H. (2002) Short-time non-enzymatic nitric oxide synthesis from L-arginine and hydrogen peroxide induced by shock waves treatment. *FEBS. Lett.*, **520**, 153-155.

Grunewald, M., Avraham, I., Dor, Y., Bachar-Lustig, E., Itin, A., Jung, S., Chimenti, S., Landsman, L., Abramovitch, R. & Keshet, E. (2006) VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. *Cell*, **124**, 175-189.

Hsieh, P.C., Segers, V.F., Davis, M.E., MacGillivray, C., Gannon, J., Molkentin, J.D., Robbins, J. & Lee, R.T. (2007) Evidence from a genetic fate-mapping study that stem cells refresh adult mammalian cardiomyocytes after injury. *Nat. Med.*, **13**, 970-974.

Ito, Y., Ito, K., Shiroto, T., Tsuburaya, R., Gao, J.Y., Kikuchi, Y., Aizawa, K., Takeda, M., Yasuda, S. & Shimokawa, H. (2008) Extracorporeal cardiac shock wave therapy ameliorates left ventricular remodeling after myocardial ischemia-reperfusion injury in pigs. *Circulation*, **118 (Suppl)**, 291.

Ito, Y., Ito, K., Shiroto, T., Tsuburaya, R., Gao, J.Y., Kikuchi, Y., Aizawa, K., Takeda, M., Yasuda, S. & Shimokawa, H. (2009) Extracorporeal cardiac shock wave therapy ameliorates left ventricular remodeling after myocardial ischemia-reperfusion injury in pigs in vivo. *Circ. J.*, **73 (Suppl. I)**, 513.

Jessup, M. & Brozena, S. (2003) Heart failure. *N. Engl. J. Med.*, **348**, 2007-2018.

Kajiguchi, M., Kondo, T., Izawa, H., Kobayashi, M., Yamamoto, K., Shintani, S., Numaguchi, Y., Naoe, T., Takamatsu, J., Komori, K. & Murohara, T. (2007) Safety and efficacy of autologous progenitor cell transplantation for therapeutic angiogenesis in patients with critical limb ischemia. *Circ. J.*, **71**, 196-201.

Kang, S., Yang, Y.J., Li, C.J. & Gao, R.L. (2008) Effects of intracoronary autologous bone marrow cells on left ventricular function in acute myocardial infarction: a systematic review and meta-analysis for randomized controlled trials. *Coron. Artery. Dis.*, **19**, 327-335.

Kastrup, J., Jørgensen, E., Rück, A., Tägil, K., Glogar, D., Ruzyllo, W., Bøtker, H.E., Dudek, D., Drvota, V., Hesse, B., Thuesen, L., Blomberg, P., Gyöngyösi, M. & Sylvén, C. (2005) 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.*, **45**, 982-988.

Kawamoto, A., Tkebuchava, T., Yamaguchi, J., Nishimura, H., Yoon, Y.S., Milliken, C., Uchida, S., Masuo, O., Iwaguro, H., Ma, H., Hanley, A., Silver, M., Kearney, M., Losordo, D.W., Isner, J.M. & Asahara, T. (2003) Intramyocardial transplantation of autologous endothelial progenitor cells for therapeutic neovascularization of myocardial ischemia. *Circulation*, **107**, 461-468.

Khan, T.A., Sellke, F.W. & Laham, R.J. (2003) Gene therapy progress and prospects: therapeutic angiogenesis for limb and myocardial ischemia. *Gene. Ther.*, **10**, 285-291.

Maisonhaute, E., Prado, C., White, P.C. & Compton, R.G. (2002) Surface acoustic cavitation understood via nanosecond electrochemistry, part III: shear stress in ultrasonic cleaning. *Ultrason. Sonochem.,* **9**, 297-303.

Mariotto, S., Cavalieri, E., Amelio, E., Ciampa, A.R., de Prati, A.C., Marlinghaus, E., Russo, S. & Suzuki, H. (2005) Extracorporeal shock waves: from lithotripsy to anti-inflammatory action by NO production. *Nitric Oxide*, **12**, 89-96.

Mariotto, S., de Prati, A.C., Cavalieri, E., Amelio, E., Marlinghaus, E. & Suzuki, H. (2009) Extracorporeal Shock Wave Therapy in Inflammatory Diseases: Molecular Mechanism that Triggers Anti-Inflammatory Action. *Curr. Med. Chem.*, **16**, 2366-2372.

Martin-Rendon, E., Brunskill, S.J., Hyde, C.J., Stanworth, S.J., Mathur, A. & Watt, S.M. (2008) Autologous bone marrow stem cells to treat acute myocardial infarction: a systematic review. *Eur. Heart J.*, **29**, 1807-1818.

Mathur, A. & Martin, J.F. (2004) Stem cells and repair of the heart. *Lancet*, **364**, 183-192.

Moretti, B., Notarnicola, A., Maggio, G., Moretti, L., Pascone, M.,

Tafuri, S. & Patella, V. (2009) The management of neuropathic ulcers of the foot in diabetes by shock wave therapy. *BMC Musculoskelet. Disord.*, **10**, 54.

Millauer, B., Wizigmann-Voos, S., Schnürch, H., Martinez, R., Møller, N.P., Risau, W. & Ullrich, A. (1993) High affinity VEGF binding and developmental expression suggest Flk-1 as a major regulator of vasculogenesis and angiogenesis. *Cell*, **72**, 835-846.

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. (2004) Extracorporeal cardiac shock wave therapy markedly ameliorates ischemia-induced myocardial dysfunction in pigs in vivo. *Circulation*, **110**, 3055-3061.

Oi, K., Fukumoto, Y., Ito, K., Uwatoku, T., Abe, K., Hizume, T. & Shimokawa, H. (2008) Extracorporeal shock wave therapy ameliorates hindlimb ischemia in rabbits. *Tohoku J. Exp. Med.*, **214**, 151-158.

Olivetti, G., Ricci, R., Beghi, C., Guideri, G. & Anversa, P. (1986) Response of the border zone to myocardial infarction in rats. *Am. J. Pathol.*, **125**, 476-483.

Qian, H.S., Liu, P., Huw, L.Y., Orme, A., Halks-Miller, M., Hill, S.M., Jin, F., Kretschmer, P., Blasko, E., Cashion, L., Szymanski, P., Vergona, R., Harkins, R., Yu, J., Sessa, W.C., Dole, W.P., Rubanyi, G.M. & Kauser, K. (2006) Effective treatment of vascular endothelial growth factor refractory hindlimb ischemia by a mutant endothelial nitric oxide synthase gene. *Gene Ther.*, **13**, 1342-1350.

Rafii, S. & Lyden, D. (2003) Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat. Med.*, **9**, 702-712.

Regensteiner, J.G. & Stewart, K.J. (2006) Established and evolving medical therapies for claudication in patients with peripheral arterial disease. *Nat. Clin. Pract. Cardiovasc. Med.*, **3**, 604-610.

Rutanen, J., Rissanen, T.T., Markkanen, J.E., Gruchala, M., Silvennoinen, P., Kivelä, A., Hedman, A., Hedman, M., Heikura, T., Ordén, M.R., Stacker, S.A., Achen, M.G., Hartikainen, J. & Ylä-Herttuala, S. (2004) Adenoviral catheter-mediated intramyocardial gene transfer using the mature form of vascular endothelial growth factor-D induces transmural angiogenesis in porcine heart. *Circulation*, **109**, 1029-1035.

Saggini, R., Figus, A., Troccola, A., Cocco, V., Saggini, A. & Scuderi, N. (2008) Extracorporeal shock wave therapy for management of chronic ulcers in the lower extremities. *Ultrasound Med. Biol.*, **34**, 1261-1271.

Satoh, K., Fukumoto, Y., Nakano, M., Sugimura, K., Nawata, J., Demachi, J., Karibe, A., Kagaya, Y., Ishii, N., Sugamura, K. & Shimokawa, H. (2009) Statin ameliorates hypoxia-induced pulmonary hypertension associated with down-regulated stromal cell-derived factor-1. *Cardiovasc. Res.*, **81**, 226-234.

Schächinger, V., Assmus, B., Britten, M.B., Honold, J., Lehmann, R., Teupe, C., Abolmaali, N.D., Vogl, T.J., Hofmann, W.K., Martin, H., Dimmeler, S. & Zeiher, A.M. (2004) Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J. Am. Coll. Cardiol.*, **44**, 1690-1699.

Schächinger, V., Erbs, S., Elsässer, A., Haberbosch, W., Hambrecht, R., Hölschermann, H., Yu, J., Corti, R., Mathey, D.G., Hamm, C.W., Süselbeck, T., Werner, N., Haase, J., Neuzner, J., Germing, A., Mark, B., Assmus, B., Tonn, T., Dimmeler, S. & Zeiher, A.M. (2006) 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.*, **27**, 2775-2783.

Seidl, M., Steinbach, P., Wörle, K. & Hofstädter, F. (1994) Induction of stress fibres and intercellular gaps in human vascular endothelium by shock-waves. *Ultrasonics*, **32**, 397-400.

Shimokawa, H. & Ito, K. (2009) Extracorporeal Cardiac Shock Wave Therapy for Ischemic Heart Disease. In *New Trends in Shock Wave Applications to Medicine and Biotechnology.*, edited by Loske, A.M. Research Signpost Publishers, Trivandrum, India, in press.

Stojadinovic, A., Elster, E.A., Anam, K., Tadaki, D., Amare, M., Zins, S. & Davis, T.A. (2008) Angiogenic response to extracorporeal shock wave treatment in murine skin isografts. *Angiogenesis*, **11**, 369-380.

Sumpio, B.E. (2000) Foot ulcers. *N. Engl. J. Med.*, **343**, 787-793.

Tatsumi, T., Ashihara, E., Yasui, T., Matsunaga, S., Kido, A., Sasada, Y., Nishikawa, S., Hadase, M., Koide, M., Nakamura, R., Irie, H., Ito, K., Matsui, A., Matsui, H., Katamura, M., Kusuoka, S., Matoba, S., Okayama, S., Horii, M., Uemura, S., Shimazaki, C., Tsuji, H., Saito, Y. & Matsubara, H. (2007) Intracoronary transplantation of non-expanded peripheral blood-derived mononuclear cells promotes improvement of cardiac function in patients with acute myocardial infarction. *Circ. J.*, **71**, 1199-1207.

Urbich, C., Aicher, A., Heeschen, C., Dernbach, E., Hofmann, W.K., Zeiher, A.M. & Dimmeler, S. (2005) Soluble factors released by endothelial progenitor cells promote migration of endothelial cells and cardiac resident progenitor cells. *J. Mol. Cell. Cardiol.*, **39**, 733-742.

Uwatoku, T., Ito, K., Abe, K., Oi, K., Hizume, T., Sunagawa, K. & Shimokawa, H. (2007) Extracorporeal cardiac shock wave therapy improves left ventricular remodeling after acute myocardial infarction in pigs. *Coron. Artery Dis.*, **18**, 397-404.

Volpi, A., De Vita, C., Franzosi, M.G., Geraci, E., Maggioni, A.P., Mauri, F., Negri, E., Santoro, E., Tavazzi, L. & Tognoni, G. (1993) Determinants of 6-month mortality in survivors of myocardial infarction after thrombolysis. Results of the GISSI-2 data base. The Ad hoc Working Group of the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico (GISSI)-2 Data Base. *Circulation*, **88**, 416-429.

Wang, C.J., Wang, F.S., Yang, K.D., Weng, L.H., Hsu, C.C., Huang, C.S. & Yang, L.C. (2003) Shock wave therapy induces neovascularization at the tendon-bone junction. A study in rabbits. *J. Orthop. Res.*, **21**, 84-89.

Wang, F.S., Wang, C.J., Huang, H.J., Chung, H., Chen, R.F. & Yang, K.D. (2001) Physical shock wave mediates membrane hyperpolarization and Ras activation for osteogenesis in human bone marrow stromal cells. *Biochem. Biophys. Res. Commun.*, **287**, 648-655.

Wollert, K.C., Meyer, G.P., Lotz, J., Ringes-Lichtenberg, S., Lippolt, P., Breidenbach, C., Fichtner, S., Korte, T., Hornig, B., Messinger, D., Arseniev, L., Hertenstein, B., Ganser, A. & Drexler, H. (2004) Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet*, **364**, 141-148.

Yan, X., Zeng, B., Chai, Y., Luo, C. & Li, X. (2008) Improvement of blood flow, expression of nitric oxide, and vascular endothelial growth factor by low-energy shockwave therapy in random-pattern skin flap model. *Ann. Plast. Surg.*, **61**, 646-653.