# Direct epicardial shock wave therapy improves ventricular function and induces angiogenesis in ischemic heart failure

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**Objectives:** Direct application of low-energy unfocused shock waves induces angiogenesis in ischemic soft tissue. The potential effects of epicardial shock wave therapy applied in direct contact to ischemic myocardium are uncertain.

**Methods:** For induction of ischemic heart failure in a rodent model, a left anterior descending artery ligation was performed in adult Sprague–Dawley rats. After 4 weeks, reoperation with (treatment group, n = 60) or without (control group, n = 60) epicardial shock wave therapy was performed. Low-energy shock waves were applied in direct contact with the infarcted myocardium (300 impulses at 0.38 mJ/m$^2$). Additionally, healthy animals (n = 30) with normal myocardium were studied. Angiogenesis, ventricular function upregulation of growth factors, and brain natriuretic peptide levels were analyzed.

**Results:** Histologic analysis revealed significant angiogenesis 6 weeks (treatment group: 8.2 ± 3.7 vs control group: 2.9 ± 1.9 vessels per field, $P = .016$) and 14 weeks (treatment group: 7.1 ± 3.1 vs control group: 3.2 ± 1.8 vessels per field, $P = .011$) after shock wave treatment. In the treatment group ventricular function improved throughout the follow-up period (6 weeks: 37.4% ± 9% [$P < .001$] and 14 weeks: 39.5% ± 9% [$P < .001$]). No improvement of ventricular function was observed in the control group (6 weeks: 28.6% ± 5% and 14 weeks: 21.4% ± 5%). Rat brain natriuretic peptide 45 levels were lower in the treatment group compared with those in the control group 6 and 14 weeks after treatment. Vascular endothelial growth factor, Fms-related tyrosine kinase 1, and placental growth factor levels were upregulated after 24 and 48 hours and 7 days in the treatment group. No effects on healthy myocardium were observed.

**Conclusion:** Direct epicardial low-energy shock wave therapy induces angiogenesis and improves ventricular function in a rodent model of ischemic heart failure.

The increasing incidence of advanced ischemic heart failure and the availability of traditional therapies to selected patients only has driven the need for alternative myocardial regenerative therapies.[1] Based on the consideration that angiogenesis might reverse the pathophysiologic process that leads to ischemic heart failure, a therapy that induces angiogenesis could be developed as an alternative or adjunctive treatment for patients with advanced ischemic heart failure.

Defocused low-energy shock wave therapy (SWT) applied in direct contact to ischemic tissue induces angiogenesis and, consecutively, tissue regeneration in ischemic limbs, skin flaps, and vascular ulcers.[2-6] However, the effects of epicardial SWT applied in direct contact with ischemic myocardium are uncertain.

To address this issue, we tested a system for direct epicardial SWT and initiated an experimental study in a rodent model of ischemia-induced heart failure. We measured the effect of direct epicardial SWT on angiogenesis and followed the animals by means of echocardiographic analysis to study the efficacy of this treatment in the reversal of impaired left ventricular (LV) function. Upregulation of growth factors and development of brain natriuretic peptide (BNP) levels were analyzed. Furthermore, we studied the effects of direct epicardial SWT on healthy myocardium.

## MATERIALS AND METHODS

The Institutional Animal Care and Use Committee at the Medical University of Vienna approved this study (GZ: 66.009/0176-BrGT/2005, GZ: 66.009/0217-BrGT/2006). Operations and animal care were provided in accordance with the ''Guide for the care and use of laboratory animals'' (National Institutes of Health, volume 25, no. 28, revised 1996).

### Experimental Animals

A total of 210, random, 8- to 10-week-old Sprague–Dawley rats (240–280 g) were obtained from the ''Institut für Labortierkunde und Genetik'' (Himberg, Austria) and inbred in a pathogen-free facility under strict veterinary supervision and maintained in controlled conditions with 12-hour light/dark cycles. The animals received a commercial rat diet and water ad libitum.

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Supported by a research grant provided by CRT/MTS-Europe GmbH, Konstanz, Germany.

Received for publication Aug 19, 2008; revisions received Oct 20, 2008; accepted for publication Nov 2, 2008.

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J Thorac Cardiovasc Surg 2009;137:963-70
0022-5223/$36.00



doi:10.1016/j.jtcvs.2008.11.006

**Abbreviations and Acronyms**

BNP = brain natriuretic peptide
Flt-1 = Fms-related tyrosine kinase 1
LAD = left anterior descending artery
LV = left ventricular
PCR = polymerase chain reaction
PlGF = placental growth factor
SWT = shock wave therapy
VEGF = vascular endothelial growth factor
vWF = von Willebrand factor

Induction of myocardial infarction was performed in 180 rats according to a standardized model by means of left anterior descending artery (LAD) ligation.[7] Animals were anesthetized with ketamine (100 mg/kg), xylazine (Rompum; 10 mg/kg), and isoflurane (2%) and ventilated after orotracheal intubation. LAD ligation was performed through a left minithoracotomy, the pericardium was opened, and the proximal LAD was ligated with Prolene 6-0 sutures to induce a sizable LV infarct. A total of 122 rats survived the LAD ligation. Thirty rats underwent thoracotomy without LAD ligation under the same conditions.

Four weeks after LAD ligation, the surviving 120 animals with large, echocardiographically proved LV infarcts were randomized to one of 2 groups: reoperation with (treatment group, n = 60) and reoperation without (control group, n = 60) SWT. For reoperation, animals were anesthetized with ketamine (100 mg/kg), xylazine (10 mg/kg), and isoflurane (2%) and ventilated after orotracheal intubation. Rethoracotomy was performed, and the heart was dissected free from the thoracic wall. Homemade air-filled plastic bags were positioned around the heart to expose the LV anterior wall and to avoid shock wave–induced lung injury.[8] A commercially available ultrasound gel serving as contact medium was applied to the LV anterior wall of the heart (Aquasonic; Parker Laboratories, Inc, Fairfield, NJ; Figure 1, *A*). In the treatment group a total of 300 shock wave impulses were applied to the LV anterior wall by using a CardioGold (CRT/MTS-Europe GmbH, Konstanz, Germany) SWT system and a specially designed applicator (CRT/MTS-Europe GmbH; Figure 1, *B*). An identical procedure without application of SWT was performed in the control group.

To investigate the effects of SWT on healthy myocardium, a third group was studied (SWT only, n = 30): thoracotomy without LAD ligation and, 4 weeks later, rethoracotomy with SWT on healthy noninfarcted myocardium.

Animals were killed 6 and 14 weeks after treatment, hearts were harvested and processed for immunohistochemistry, and serum was processed for enzyme-linked immunosorbent assays. For real-time polymerase chain reaction (PCR) and Western blotting of proangiogenic cytokines, a subpopulation of animals was killed 24 and 48 hours and 7 days after reoperation.

## Shock Wave Therapy

The CardioGold (CRT/MTS-Europe GmbH) SWT system and the used handheld applicator (CRT/MTS-Europe GmbH) were developed for the direct epicardial application of SWT (Figure 1, *A*). In contrast to SWT systems for nephrolithiasis or percutaneous cardiac SWT that produced focused shock waves, the CardioGold uses a parabolic reflector that produces unfocused, nearly parallel shock waves that allow treatment of a target area with a diameter of 0.5 to 0.7 mm and a penetration depth of 1 to 1.5 cm. The used energy flux density (0.38 mJ/mm$^2$) and the cumulative treatment dose are based on our experience with the treatment of acute and chronic wounds, as well as diabetic and vascular ulcers, compared with that used for percutaneous cardiac SWT and represent about 10% of that used for lithotripsy.[6]

## Immunohistochemistry and Quantitative Histology

Tissue was processed for paraffin embedding, and serial sections were stained with hematoxylin and eosin, Goldner, and a polyclonal anti-von Willebrand factor (vWF) antibody (Abcam, Cambridge, United Kingdom). Briefly, deparaffinized sections were preincubated with 3% $H_2O_2$ and horse serum (Jackson ImmunoResearch, West Grove, Pa) to block endogenous peroxidase and antigen activity before incubation with the primary antibody (vWF, 1:200 dilution). Biotinylated anti-mouse/rabbit IgG (H+L) secondary antibody (Vector Laboratories, Inc, Burlingame, Calif) was used to detect positive staining, followed by streptavidin–biotin peroxidase complex (LSAB®2 streptavidin–horseradish peroxidase; DakoCytomation, Glostrup, Denmark). For quantitative histology, digitalized images were made with a Nikon inverted microscope Eclipse TE2000-U (Nikon Instruments Europe B.V., Badhoevedorp, The Netherlands) at 40× magnification. Quantification was performed with Lucia G image analysis software. Six fields of the myocardial wall in the region of the LAD were evaluated in each heart. Angiogenesis and microvascular density were evaluated according to established procedures.[9] The number of vessels was counted, and vessel lumen areas, vessel wall thickness, and vessel width were measured. Vessels were divided into 3 sizes (small, 0–9 $\mu$m; medium, 10–19 $\mu$m; large, >20 $\mu$m). Additionally, the number of vWF$^+$ cells was obtained. A single observer in an observer-blinded fashion performed all analyses.

## Echocardiographic Analysis

Before LAD ligation, before SWT or sham operation, and before termination (6 and 14 weeks after SWT), echocardiographic analysis was performed (Philips iE33; Philips Medical Systems, Bothell, Wash; Transducer: S12-1). Rats were anesthetized with isoflurane. Standardized views of the heart were obtained at the papillary muscle level. Ejection fraction and LV diameters and volumes were obtained. Examinations were digitalized and evaluated by an independent experienced investigator.

## Real-Time PCR Analysis

Total RNA was isolated from rat heart tissue (anterior wall) by using a TRIzol extraction (Invitrogen, Lofer, Austria), according to the manufacturer's protocol. cDNA was synthesized with M-MuLV Reverse Transcriptase (Fermentas, Burlington, Ontario, Canada) and 2 $\mu$g of total RNA primed with oligo dT-primer. After reverse transcription of RNA into cDNA, real-time PCR was used to monitor gene expression with the FastStart DNA Master SYBR Green kit and a LightCycler instrument (Roche Applied Science, Vienna, Austria), according to the standard procedure. The primer sequences (sense/antisense) were vascular endothelial growth factor (VEGF): 5′-TCCTGCAGCATAGCAGATGT-3′/5′-GCGAGTCTGTGTTTTTGCAG-3′; placental growth factor (PlGF): 5′-ACTGTGTGGCGCTAAAGACA-3′/5′-TTCCTCAGTCTGTGGGGTTT-3′; and Fms-related tyrosine kinase 1 (Flt-1): 5′-GGAGGCGAGGATTACAGTGA-3′/5′-GGAGGCGAGGATTACAGTGA-3′. LCDA Version 3.5.28 was used for PCR data analysis (Roche Applied Science). The specificity of the amplification product was determined by performing a melting curve analysis. Standard curves for expression of each gene were generated by means of serial dilution of known quantities of the respective cDNA gene template. Relative quantification of the signals was done by normalizing the signals of the different genes with the $\beta_2$-microglobulin signal. Measurements were done in triplicate.

## Western Blotting

Rat heart tissues were lysed in solubilization buffer (10 mmol/L Tris–HCl, 50 mmol/L NaCl, 1% Triton X-100, 30 mmol/L sodium pyrophosphate, 100 $\mu$mol/L $Na_3VO_4$, 1 mmol/L phenylmethylsulfonyl fluoride, and 1× Complete ethylenediamine tetraacetic acid–free Protease Inhibitor Cocktail (Roche Applied Science). Insoluble material was removed by means of centrifugation (15,000 rpm for 15 minutes at 4°C). Tissue lysates (50 $\mu$g per lane) were separated by means of sodium dodecylsulfate–polyacrylamide gel electrophoresis before electrophoretic transfer onto 0.2-$\mu$m