Circ J 2006; **70:** 1356–1361

Effects of Ultrasound Energy Application on Cardiac Performance in Open-Chest Guinea Pigs

—— An In Vivo Pilot Study ——

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Background Although ultrasound (US) is widely used in cardiology, little is known about the effects of US energy on cardiac performance. This study aimed to investigate the mechanical effects of high-intensity continuous US energy (1.0 MHz with 3 different intensities) on cardiac performance.

Methods and Results Either left ventricular (LV) pressure or aortic blood flow (ABF) was evaluated in open-chest guinea pigs (n=30) under surface ECG monitoring. LV systolic pressure and ABF increased significantly (ie, maximum percent increases in these parameters were 2.5%, 3.1% and 7.1% for LV systolic pressure and 9.4%, 4.9% and 8.8% for mean ABF at intensities of 0.06, 0.67 and 2.90 W/cm^2, respectively). LV end-diastolic pressure was reduced significantly by US (5.3±0.9 to 4.8±0.8, 5.5±1.3 to 4.8±1.0 and 5.8±2.0 to 5.0±1.2 mmHg, respectively), indicating positive inotropic and lusitropic effects and resultant ABF augmentation. Local temperature was not significantly changed. ECG showed neither chronotropic action nor arrhythmogenesis.

Conclusions Although the basic mechanisms of these phenomena remain unclear, this pilot study of the short-term effects of US energy on cardiac performance suggests the possibility of physical therapy for heart failure. (*Circ J* 2006; **70:** 1356–1361)

Key Words: Cardiac function; Guinea pig; Hemodynamics; Ultrasound energy

The development of the use of ultrasound (US) technology has been remarkable in cardiology. For many years, it has been a diagnostic imaging tool for showing various biological effects, with promising therapeutic implications[1,2] in accelerated thrombolysis for acute coronary syndrome[3,4] plaque dissolution for stable coronary artery disease (ultrasonic angioplasty)[5] efficient cardiac gene delivery[6] and treatment of arrhythmia by ultrasonic defibrillation[7] and ablation[8–10] However, there is still controversy about the mechanical effects of US energy on cardiac performance.

This controversy stems from device problems and experimental inaccuracy. The power distribution created by a high-intensity US generator must be quantified spaciotemporally and reflection of the US waves must be strictly avoided. Moreover, high-intensity US energy is considerably transformed to heat and the biological US effects are ascribed mainly to thermal effects and nonthermal actions, such as acoustic cavitation, and these 2 components need to be discriminated.

In this pilot study, we examined whether brief, continuous and focused applications of high-intensity US energy produced by a fabricated generator can modulate the cardiac function of open-chest anesthetized guinea pigs. For this purpose, the spaciotemporal average US intensity ranged from 0.06 to 2.90 W/cm^2, which is far greater than that used for diagnostic purposes (3–300 mW/cm^2).

(Received March 2, 2006; revised manuscript received July 14, 2006; accepted July 25, 2006)

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Methods

The study was performed according to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Japanese Physiological Society (2003). The experiments were designed to investigate the effects of US energy (1.0 MHz with average intensity up to 2.90 W/cm^2) on left ventricular (LV) pressure and aortic blood flow (ABF). The acoustic field was wedge-shaped, with the tip of the probe as the origin. Guinea pigs were subjected to US of fixed intensity to avoid possible cumulative effects.

Animal Preparation and Protocols

Protocol 1 The first series of experiments was performed on healthy male Hartley guinea pigs weighing 400–450 g (n=30). All animals were anesthetized with ketamine (25 mg/kg ip) and xylazine (0.15 mg/kg ip), supplemented as required. Intratracheal intubation was performed using a blunt 17-gauge polyvinyl outer sheath of a needle via tracheotomy. Artificial ventilation was with a constant-pressure ventilator for small animals (KN-55, Nazme, Tokyo, Japan). A heating pad set at 37°C and US absorption board were placed underneath the animal. A midline thoracotomy was performed to provide an acoustic window for US application to the LV. A 22-gauge needle-guided polyvinyl catheter (0.9 and 0.5 mm in outer and inner diameters, respectively) was inserted through the apex into the LV. Continuous LV pressure was monitored

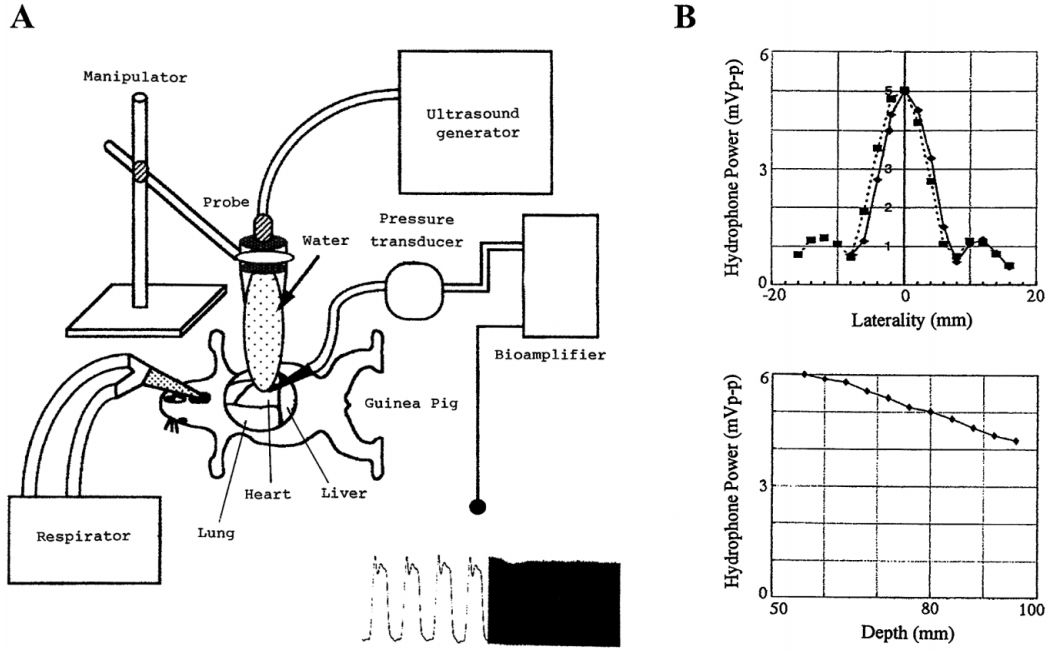

Fig 1. (A) Schematic illustration of the experiment. Ultrasound (US) energy is 1 MHz at a fixed frequency, 2.90 W/cm^2, in the maximum spaciotemporal average intensity and with a 16-mm diameter focus. (B) Distribution of the relative US power, termed hydrophone power (mVp-p in unit). Power distribution is plotted as a function of laterality (X-axis –◆–, Y-axis ···■···) or depth (Z-axis). Symmetrical power distribution is observed at a depth of 80 mm (Upper) and US fading is observed at depths greater than 50 mm (Lower). US intensity is proportional to the square of hydrophone power (Data supplied by Toshiba Medical Systems Corporation (Tokyo, Japan) with permission).

using a strain-gauge type pressure transducer (Baxter Co Ltd, USA). Signals were transferred to the bioamplifier (Nihon Kohden Co Ltd, Tokyo, Japan), and stored automatically on a personal computer (McCintosh, Apple Japan, Tokyo, Japan). Commercially available software (MacLab/8s data-acquisition system, AD Instruments Co Ltd, Japan) was used for A/D conversion, automatic data acquisition and measurement.

Fig 1A illustrates the experiments. The US generator system was specifically fabricated by Toshiba Medical Systems Corporation (Tokyo, Japan) and US energy was produced at a constant frequency of 1.0 MHz and peak intensities up to 12.5 W/cm^2. The US energy showed remarkable lateral scattering and gradual fading by 16.7% at a depth of 80 mm in air-saturated water (Fig 1B). The intensity observed 8 mm laterally was 16% of that obtained at the center. Therefore, the focus was set at 16 mm in diameter at a depth of 80 mm. Applied US peak intensities of 0.28, 0.72, 2.90 6.60 and 12.50 W/cm^2 corresponded to spaciotemporal average intensities of 0.06, 0.17, 0.67, 1.50 and 2.90 W/cm^2, respectively.

The probe was wrapped in a rubber sack containing water (perpendicular length: 80 mm) and positioned on the LV surface. Water left overnight was considered to be air-saturated and filled the rubber sack. Constant pressure on the LV was secured by the weight of water within the rubber sack and not by the weight of the US probe, which was held by a manipulator. After a 5-min equilibration, continuous US energy was applied for 1 min under the monitoring of LV pressure and limb lead (II) of the ECG (Nihon Kohden). The recovery period was at least 5 min.

Protocol 2 Another group of healthy male Hartley guinea pigs (weighing 400–450 g) were anesthetized and ventilated on the heating pad and US absorption board placed as in protocol 1 (n=30). A midline thoracotomy was performed, and the aorta was dissected free from the surrounding tissue and overlying pulmonary artery. A pulsed Doppler flow probe (DBF-30-R, Crystal Biotech, Holliston, MA, USA) with an internal diameter of 2.5–3.0 mm was implanted around the ascending aorta. This Doppler probe was connected directly to a PD-10/20 mainframe (Crystal Biotech) fitted with a 20 MHz pulsed Doppler flow module (Crystal Biotech) for the measurement of continuous ABF. Blood flow velocity (V) was calculated theoretically from the Doppler shift frequency (f: kHz) by the following equation:

$$V = c \cdot f / 2 f_o / \cos\theta$$

where c is the speed of sound in blood (1,500 m/s), f_o is the US frequency of the pulsed Doppler probe (20 MHz), and θ is the angle between the US beam and the blood velocity vector![1] ABF is principally quantified by the following equation:

$$ABF = V \cdot A$$

where the aortic cross sectional area (A) is calculated by defining the internal diameter of ascending aorta as D (mm):

$$A = \pi \cdot (D/2)^2.$$

By making the assumption of θ=45°, V (cm/min) and ABF (ml/min) were calculated by as:

$$V = 161 f$$
$$ABF = 1.25 D^2 f.$$

As in protocol 1, US energy was applied under the monitoring of actual and mean ABF. It was also confirmed that the pulsed Doppler flow probe was not included in the area

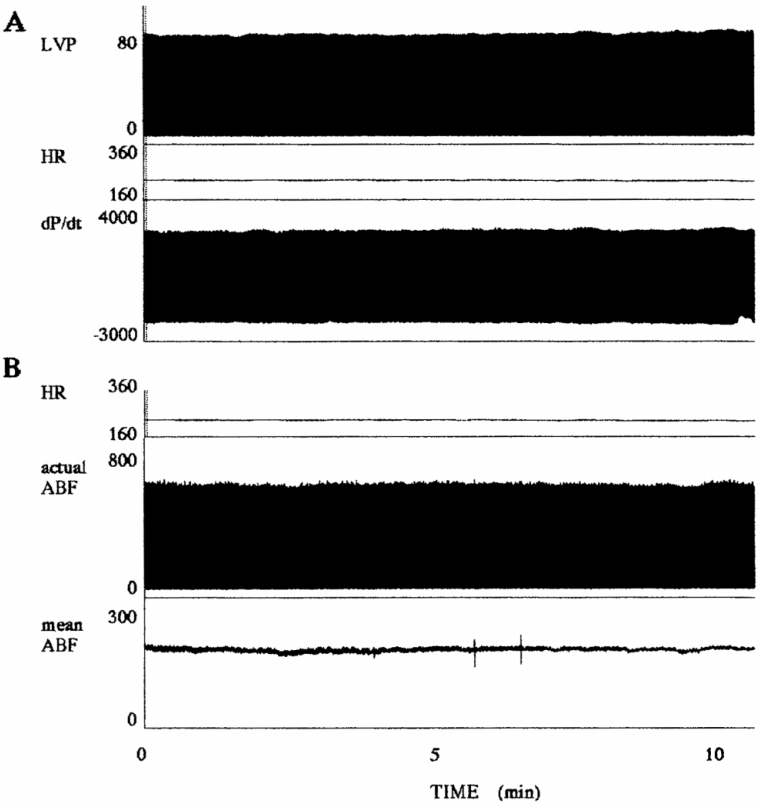

Fig 2. Representative 'placebo' ultrasound (US) application. (A) Actual recordings of left ventricular pressure (LVP in mmHg), heart rate (HR in beats/min) and the first time-derivative of LVP (dP/dt in mmHg/s), and (B) HR, actual aortic blood flow (ABF, ml/min) and mean ABF (ml/min). The recording period was 11 min and 'placebo' US was applied in the time period of 5–6 min. The guinea pig in experiment A was different from that used in B.

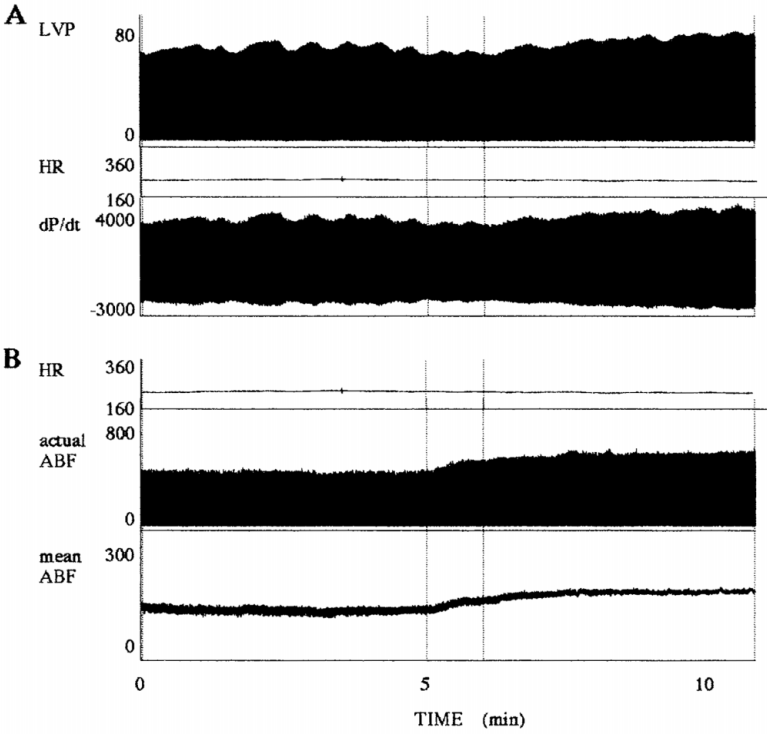

Fig 3. Representative 'active' ultrasound (US) application. The hemodynamic parameters in A and B are the same as those described in Fig 2. 'Active' US with its average intensity of 2.9 W/cm^2 was applied in the time period 5–6 min. The guinea pig in experiment A was different from that used in B. As compared with the 'placebo' experiment (Fig 2), the hemodynamic effects of 'active' US are evident during and after US application: increases in left ventricular (LV) systolic pressure, maximum of the first time-derivative of LV pressure (dP/dt_{max}) and aortic blood flow (ABF), as well as a decrease in minimum of the first time-derivative of LV pressure (dP/dt_{min}). HR, heart rate.

of the US beam. The protocol for US application was the same as in protocol 1.

'Placebo' US application was defined as no US energy delivery by the US generator system when all the other conditions remained the same as those during 'active' US application. This 'placebo' US application was performed prior to the 'active' US application in both protocols. Temperature was monitored using the tip of a thermister (PI-S1301, Shibaura Electronics Co Ltd, Tokyo, Japan) in the water within the rubber sack during the entire experimental period. All experiments were performed at room temperature of 22±3°C.

Table 1 Effects of Ultrasound Stimulation With Intensity of 0.06 W/cm² on Guinea Pig Cardiac Performance In Vivo

	Time (min)						
	Pre-application	0	1	2	3	4	5
HR	208±5	207±5	208±5	207±5	207±5	207±5	206±5
LVSP	71.5±5.7	72.1±5.5	73.3±5.4†	73.3±5.5*	72.9±5.4	72.9±5.4	73.3±5.4
LVEDP	5.3±0.3	5.0±0.4	4.8±0.2	5.0±0.3	4.9±0.2	4.9±0.3	5.2±0.2
dP/dt_{max}	3,270±330	3,330±310	3,370±310*	3,410±330*	3,400±360	3,370±360	3,380±340
dP/dt_{min}	2,120±250	2,170±260†	2,220±280†	2,230±290*	2,230±290	2,160±280	2,110±230
ABF	17.1±2.0	17.8±1.9*	18.7±1.9†	18.2±1.7	17.7±1.7	17.1±1.7	17.0±1.7

Data are mean±SE (n=10).
Pre-application, immediately before 1 min ultrasound application. 0–5, time (min) elapsed after the termination of ultrasound application.
HR, heart rate (beats/min); LVSP, left ventricular systolic pressure (mmHg); LVEDP, left ventricular end-diastolic pressure (mmHg); dP/dt_{max}, maximum of the first time-derivative of left ventricular pressure (dP/dt, mmHg/s); dP/dt_{min}, minimum of the dP/dt (mmHg/s); ABF, aortic blood flow (ml/min).
**p<0.05 and †p<0.01 vs pre-application.*

Table 2 Effects of Ultrasound Stimulation With Intensity of 0.67 W/cm² on Guinea Pig Cardiac Performance In Vivo

	Time (min)						
	Pre-application	0	1	2	3	4	5
HR	208±5	208±5	207±5	208±5	208±5	207±5	207±5
LVSP	67.7±6.5	67.9±5.9	68.9±6.2	68.7±5.7	68.9±5.7	69.8±5.8	69.0±5.9
LVEDP	5.5±0.4	5.3±0.3	5.3±0.3	5.2±0.4	5.1±0.3	4.8±0.3†	5.1±0.4
dP/dt_{max}	3,160±340	3,170±300	3,230±300	3,230±280	3,210±260	3,300±280	3,250±290
dP/dt_{min}	2,110±230	2,160±200	2,180±210	2,190±190	2,190±180	2,200±200	2,190±200
ABF	18.7±2.1	19.1±1.9	19.5±1.9	19.6±1.8	18.8±1.9	18.7±1.8	18.3±1.9

Abbreviations as in Table 1 (n=10).
†p<0.01 vs pre-application.

Table 3 Effects of Ultrasound Stimulation With Intensity of 2.90 W/cm² on Guinea Pig Cardiac Performance In Vivo

	Time (min)						
	Pre-application	0	1	2	3	4	5
HR	203±6	202±6	202±7	202±6	202±7	202±6	201±6
LVSP	67.7±3.5	68.8±3.7	69.8±4.1	70.3±4.6	70.6±4.8	72.5±5.2	70.9±5.4
LVEDP	5.8±0.6	5.5±0.6	5.6±0.6	5.2±0.5*	5.1±0.6*	5.2±0.5*	5.0±0.4†
dP/dt_{max}	2,880±210	2,960±210	3,020±250*	3,070±280	3,100±290	3,110±310	3,090±350
dP/dt_{min}	2,000±170	2,060±160	2,080±160*	2,110±180*	2,120±190*	2,130±210	2,170±230
ABF	18.4±1.5	19.4±1.4	19.9±1.4*	20.1±1.4*	19.9±1.5	19.5±1.5	19.1±1.5

Abbreviations as in Table 1 (n=10).
**p<0.05 and †p<0.01 vs pre-application.*

Data Analysis

The following variables were acquired every minute during the course of the experiment: peak LV systolic pressure (LVSP: mmHg), LV end-diastolic pressure (LVEDP: mmHg), the maximum and minimum of the first time-derivative of LV pressure (dP/dt_{max} and dP/dt_{min} in mmHg/s), heart rate (HR in beats/min) and ABF (ml/min). LVSP and dP/dt_{max} were evaluated as parameters of LV systolic function, whereas LVEDP and dP/dt_{min} were those of diastolic function. LVEDP corresponded to the R wave on the surface ECG. The averaged value of 5 consecutive beats was adopted for data sampling. All measurements were performed by a data-acquisition system (MacLab/8s), and all digitized data, except for the first time-derivative of LV pressure (dP/dt), were acquired at a sampling rate of 1 kHz; data for the dP/dt were acquired at 2 kHz.

Statistical Analysis

All values are expressed as the mean±SE. Three groups of animals (n=10 in each) were used for the 3 different US intensities (0.06, 0.67 and 2.90 W/cm²). Comparisons of baseline data in different groups were performed by the non-paired, two-tailed Student t-test. Effects of US application within a group were analyzed by analysis of variance. These computations were conducted automatically by StatView 5.0 software (SAS Institute Inc, Cary, NC, USA) and $p<0.05$ was considered statistically significant.

Results

There was no significant difference in the body weight of the guinea pigs in the 2 protocols or among those used for the 3 different US intensities. Coefficient of variance in all the variables was less than 5% over the entire period of longer than 11 min of the 'placebo' US applications (n=30), in which US energy was not applied after the fulfillment of the US application systems (Fig 2).

A representative experiment from protocol 1 is presented in Fig 3A. During a 1-min application of US energy (2.90 W/cm²), LVSP and dP/dt_{max} showed no remarkable changes. However, LVSP and dP/dt_{max} increased, whereas dP/dt_{min} decreased gradually after the termination of US

application. HR showed no discernible change during or after application. Likewise, a representative experiment from protocol 2 is presented in Fig 3B. During US application, peak ABF gradually increased and this increase was sustained after termination of the application. Mean ABF increased during and after the application under stable HR.

Tables 1–3 summarize the data for cardiac performance altered by 1 min US application with 3 different intensities. There were no significant differences in baseline values within the 3 groups. With respect to LVSP, the absolute value showed a significant ($p<0.01$) increase with US at an intensity of 0.06 W/cm^2. However, the maximum percent increases caused by US application were 2.5%, 3.15 and 7.1% at the respective intensities of 0.06, 0.67 and 2.90 W/cm^2, showing dependency on US energy. Likewise, a significant increase in absolute dP/dt_{max} was obtained with US at intensities of 0.06 W/cm^2 ($p<0.05$) and 2.90 W/cm^2 ($p<0.05$). The maximum percent increases of dP/dt_{max} were 4.3%, 4.4% and 7.8%, induced by US at the respective intensities. Therefore, the dependency of cardiac contractility on US energy was confirmed by both LVSP and dP/dt_{max}.

With respect to LVEDP, the maximum decreases by US at the 3 different intensities (ie, 0.06, 0.67 and 2.90 W/cm^2) were 5.3±0.9 to 4.8±0.8, 5.5±1.3 to 4.8±1.0 and 5.8±2.0 to 5.0±1.2 mmHg, respectively. Significance was obtained by the application of US energy at 0.67 ($p<0.01$) and 2.90 W/cm^2 ($p<0.05$). The maximum percent decreases in dP/dt_{min} induced by US at these intensities were 5.6%, 4.0% and 8.5%, respectively. A significant decrease in absolute dP/dt_{min} occurred with US at intensities of 0.06 W/cm^2 ($p<0.01$) and 2.90 W/cm^2 ($p<0.05$).

The percent increases in mean ABF was 9.4%, 4.9% and 8.8% with respective US intensities of 0.06, 0.67 and 2.90 W/cm^2. The absolute value of mean ABF was significantly augmented by US at intensities of 0.06 ($p<0.01$) and 2.90 W/cm^2 ($p<0.05$). This increase was observed immediately and 1 min after the termination of US (0.06 W/cm^2) or 1–2 min after termination (2.90 W/cm^2).

HR estimated by monitoring ECG showed no significant changes by US at any intensity. No arrhythmia was observed during the entire experimental period. There were no significant changes in water temperature caused by the application of US energy at a wide range of average intensities (0.06–2.90 W/cm^2). Respective pre- and post application water temperatures were 31.23±1.09°C and 31.27±1.31°C for 0.06 W/cm^2, and 30.78±1.26°C and 30.89±1.11°C for 2.90 W/cm^2 (p=NS).

Discussion

The major findings of the present study are that brief, continuous and focused US energy application in open-chest guinea pigs demonstrated positive inotropic and lusitropic actions and resultant ABF augmentation without any influences on cardiac rhythm.

No attempt has been made to investigate the hemodynamic US effects in in vivo for various reasons, although several investigations report positive inotropic effects of US application on isolated rat papillary muscles. Forester et al[12] reported augmented contraction and improved relaxation during US application (963 kHz) within the intensity range of 0.25–2.0 W/cm^2 and Zakharov et al[13] also revealed inotropic US effects (543 kHz) with intensities up to 3.0 W/cm^2. Although the US frequency in their studies (<1.0 MHz) was lower than that used in the present study (1.0 MHz), the results of our in vivo and their in vitro studies both show US-induced positive inotropic and lusitropic effects. Moreover, the present study demonstrated that US energy augmented the mean ABF, leading to improved hemodynamics. ABF reflects global cardiac performance, based on the interplay of LV contractility and compliance. Therefore, this parameter was more sensitive to US energy application than the other parameters (Fig 3). The reason for the limited dependency of US energy on systolic but not diastolic function is unknown (Tables 1–3) and hence optimization of US energy was not accomplished in our study.

Air-saturated water within the rubber sack attached to the US probe effectively focused the US energy at a point exactly 8.0 cm away from the US probe. It was also effective in compressing the heart gently under its own weight, stabilizing the vigorous heart beats and enabling monitoring of temperature. US-induced heat generation is a confounding factor, because cardiac performance is highly temperature-dependent. Water between the US probe and the tissue preparation theoretically prevents heat transfer and in fact no significant temperature change was confirmed during the 1-min US energy application. Improved cardiac performance and favorable hemodynamics mediated by thermal effects are unlikely therefore, although microscopic thermal effects can not be ruled out. Rather, the effects are ascribed mainly to nonthermal, acoustic effects of US generating cavitation and subsequent streaming. These phenomena induce the oscillation and agitation of many cellular constituents, affect myocardial cross-bridge kinetics and modulate intracellular Ca^{2+} availability[13]

Intracellular Ca^{2+} plays a pivotal role in cardiac performance and biological US effects are Ca^{2+}-dependent[14] Subcellular mechanisms of US-altered cardiac performance remain to be elucidated, but may be related to US-induced cavitation, which modulates various intracellular metabolic and ionic environments[15] The availability of activator Ca^{2+} for contractile protein may be improved during systole, whereas the efficacy of Ca^{2+} removal may be improved for diastole by cavitation[13] The role of the Ca^{2+}-handling mechanism on cardiac performance in the rat is considerably different from that in other mammals[16,17] including the guinea pig[18] The present results from guinea pigs and the data from rats in the literature[12,13] consistently support the positive inotropic and lusitropic effects of US energy, which are considered to be species-independent.

In clinical practice, cardiologists are managing heart failure with various therapeutic strategies including pharmacologic and nonpharmacologic options. The importance of diastolic impairment is currently noted, but a promising pharmacologic strategy for this problem is currently unavailable. Physical therapy has been attempted in diastolic failure; that is, small-amplitude mechanical vibration is reported to accelerate the impaired relaxation[19] and improve the subsequent systolic function[20] in ischemic;[21] hypertrophic and failing hearts;[22] probably by mechanically modulating the cross-bridge kinetics. Clinical implication of our pilot study using high-energy US is limited because of the differences between the normal guinea pig hearts and diseased human hearts and between extracorporeal and direct US applications. Moreover, the effects of continuous US application should be distinguished from those of phased application. Therefore, long-term effects of transthoracic and ECG-triggered phased US applications should

be investigated clinically as a therapeutic option for refractory heart failure.

In conclusion, this pilot study demonstrated acute mechanical and hemodynamic effects of brief, continuous and focused US application in open-chest guinea pigs. The positive inotropic and lusitropic effects and resultant ABF augmentation observed in this study suggests the possibility of nonpharmacologic therapy for heart failure.

Acknowledgments

We thank Mr Mitsuru Himeno (Nihon Kohden Co Ltd, Tokyo, Japan) for technical assistance and Mr Satoshi Aida (Toshiba Medical Systems Corporation, Tokyo, Japan) for thoughtful discussion.

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