whereas HR at rest in other subtypes of LQTS has not been fully investigated. In clinical practice, we have noted that in some cases of LQT2 that TdP was triggered by HR of <60 b.p.m. and suppressed by pacing at 80 b.p.m., which made us evaluate the importance of HR in arrhythmic events of LQT2 patients. For these reasons, we aimed to analyse whether HR at rest before BBT could be a novel risk factor for cardiac events besides gender, genetic locus, and prolonged QT interval in LQT2. We also evaluated the relationship between HR at rest and arrhythmic events before and after BBT through the analysis of clinical data on patients with LQT2.

Methods

Study population

From September 1996 to July 2009, 587 probands with QT prolongation underwent genetic testing in three institutes in Japan, Shiga University of Medical Science, Kyoto University Graduate School of Medicine, and the National Cardiovascular Center. One hundred and fifty-two probands (26%) were genotyped as LQT2. We also screened mutations in *KCNQ1*, *SCN5A*, *KCNE1–3*, and *KCNJ2* using the standard genetic tests[17–20] and excluded 20 probands with compound mutations and/or modifier single-nucleotide polymorphisms known to affect the QT interval (*KCNH2* K897T and *KCNE1* D85N).[21,22] The remaining 132 probands were found to have a single *KCNH2* mutation, and among them, we excluded from analyses patients under 15 years and those without detailed clinical information or with medication (except for β-blocker) which could influence baseline ECG measurements at the first medical contact and thereafter. Children <15 years old were not studied because they had relatively high basal HR. Family members of the 152 probands were recruited for the analysis if we could obtain necessary clinical information and if they were over 15 years old. As a result, the study population became 110 patients (45 probands and 65 family members) from 74 unrelated Japanese LQT2 families.

Both symptomatic and asymptomatic patients were included in the groups of probands and family members. Regardless of being probands or family members, patients were defined as symptomatic when they had a history of cardiac events (defined as ventricular fibrillation, TdP, or syncope due to ventricular arrhythmia) at the first medical contact or at the time of yearly follow-up. Patients with an apparent history of vasovagal syncope were not included in the study. The protocol for genetic analysis complied with the Declaration of Helsinki and was approved by the institutional ethics committees and performed under their guidelines. All individuals or their guardians gave written informed consent to genetic and clinical data analyses. Follow-up data were obtained from patients' regular hospitals working with the authors in case patients lived far from our institutions or hospitals and were not able to visit us.

Genetic analysis and characterization

Genomic DNA was isolated from venous blood lymphocytes using the QIAamp DNA blood midikit (Qiagen, Hilden, Germany). Established primer settings were used to amplify the entire coding regions of known LQTS genes from genomic DNA.[17–20] Denaturing high performance liquid chromatography (DHPLC) or direct sequencing techniques were employed as described elsewhere.[11] Polymerase chain reaction fragments presenting abnormal signals in DHPLC analysis were subsequently sequenced by the dideoxynucleotide chain termination method with fluorescent dideoxynucleotides on an ABI 3113xl genetic analyzer (PE Applied Biosystems).

The pore region of the *KCNH2* channel was defined as the area extending from S5 to the mid-portion of S6 involving amino acid residues from 550 through 650 according to the previous report.[10] The non-pore region included the N-terminus region, transmembrane domains apart from the pore region and the C-terminus region.

Clinical characterization

Routine demographic data and basal 12-lead ECGs were obtained from all subjects at the first medical contact as well as at yearly follow-up. In 104 patients, ECG parameters were measured before BBT was introduced. The remaining six patients, in whom BBT was started after the first cardiac event by an attending physician in other hospitals, visited a university hospital for further diagnostic confirmation of the symptoms. One of the six patients experienced aborted sudden cardiac death, four had documented TdP, and one had a syncopal attack. After obtaining informed consent, BBT was discontinued for more than five times the half life and examinations were performed, including a blood test, basal ECG, chest X ray, echocardiogram, and treadmill test for the diagnosis of congenital long QT syndrome.

Electrocardiograph parameters measured in the study were HR and QT interval. Rate-dependent QT intervals were corrected for HR using Bazett's method. QT interval was manually measured in lead V_5 using the tangent method[4] with an average of 2 or 3 consecutive beats by three investigators who were completely unaware of the patients' clinical and genetic state. There were no significant differences in the measured data among three investigators. Bifid T waves, but not U waves, were included in the QT measurements. In the presence of bifid T waves, the end of the second T wave was defined as the end of the QT interval. If ECG recordings were obtained during a cardiac event, such as the appearance of frequent ventricular tachycardia, TdP, or cardiac arrest, they were requested to perform another examination after patient's general status had improved.

Data on patients who received BBT after the initial check-up were evaluated, including the dose of each drug, HR under medication, and recurrent arrhythmic episodes. Other treatments, such as implantable cardioverter-defibrillator (ICD) implantation and surgical left cardiac sympathetic denervation, were also evaluated. Follow-up data, including the occurrence of cardiac events and therapeutic changes, were collected retrospectively.

Statistical analysis

Student's *t*-test was employed to compare continuous data. Differences in frequencies were analysed by the χ^2 test or Fisher's exact test. Analysis of variance was used to test differences of variables among more than three groups. Stepwise regression analysis was performed to determine predictors of cardiac events. Variables with $P < 0.05$ on univariate analysis were included in a logistic regression model with cardiac events as dependent variables. To determine the connection of the selected clinical variables with the occurrence of cardiac events, odds ratios for unadjusted data and their 95% confidence intervals were calculated. The cumulative probability of the first cardiac event between 15 and 50 years old was estimated using the Kaplan–Meier method. The Cox proportional-hazards survivorship model was used to investigate whether there were any prognostic factors that could influence the occurrence of cardiac events. Data are reported as the mean ± SD. Two-sided probability values <0.05 were considered significant. Statistical calculations were performed using SPSS software (version 18.0J).

Results

Clinical and genetic characteristics

The study population consisted of 110 consecutive patients from 74 unrelated Japanese LQT2 families (*Table 1*). The baseline ECG showed that the mean HR of probands tended to be lower than that of family members ($P = 0.06$).

All patients were genotyped to be a heterozygous carrier of 70 different *KCNH2* mutations (18 in the N-terminus, 15 in non-pore regions, 13 in pore regions, and 24 in the C-terminus). Forty-three mutations were missense mutations, 15 were deletion/insertions, 9 were frameshifts, and 3 were nonsense mutations.

Table 1 Basal characteristics of the study population

	All ($n = 110$)	Proband ($n = 45$)	Family member ($n = 65$)
Clinical characteristics			
Age (years)	40.8 ± 17.5 (15–87)	31.2 ± 15.6 (15–77)	47.4 ± 15.6 (16–87)**
Sex (male/female)	40/70	10/35	30/35*
Symptomatic patients [*n* (%)]	48 (44)	38 (84)	10 (15)**
Cardiac arrest (*n*)	7	4	3
Syncope (*n*)	46	38	8
Both (*n*)	5	4	1
ECG			
HR (b.p.m.)	62 ± 10	60 ± 9	63 ± 11
QTc (ms)	483 ± 58	508 ± 60	467 ± 50**

*$P < 0.05$ vs. proband.
**$P < 0.001$ vs. proband.

Factors determining cardiac events in LQT2 patients

We first evaluated whether HR and other variables (age at onset of cardiac events, female gender, site of mutation, missense mutation, and QTc) served as risk factors for cardiac events in LQT2 patients. Univariate analysis (*Table 2*) showed that HR of <60 b.p.m. *per se* was a significant risk for cardiac events ($P < 0.01$). In addition, female gender, HR as a continuous variable, a QTc interval of ≥500 ms, and pore site mutation were associated with an increased risk for cardiac events ($P < 0.05$). Other variables such as age at onset of cardiac events, sites of mutation (non-pore transmembrane, N-terminal, and C-terminal), and missense mutation were not statistically significant.

Multivariate analysis (*Table 2*) was subsequently performed using female gender, HR of <60 b.p.m., QTc of ≥500 ms, and pore site mutation. As for HR, we chose HR of <60 b.p.m. for multivariate analysis because we aimed to clarify if low HR of <60 b.p.m. was a significant risk factor for cardiac events. As shown in *Table 2*, female gender, HR <60 b.p.m., and QTc ≥500 ms were revealed to be significant risk factors for cardiac events ($P < 0.05$).

Bradycardia as an arrhythmic risk factor in LQT2 patients

We employed two ECG parameters, HR and QTc, to scrutinize who were more prone to have cardiac events in our LQT2 cohort. Using cutoff values of 60 b.p.m. for HR without β-blockers and 500 ms for QTc, we classified 110 LQT2 patients into four groups (*Figure 1*). Closed and open circles in the figure indicate symptomatic and asymptomatic patients, respectively (including both probands and family members). There were only eight symptomatic patients (23%) in the quadrant area of HR ≥60 b.p.m. and QTc <500 ms. In contrast, in the quadrant area defined as HR <60 b.p.m. and QTc ≥500 ms, 12 subjects (86%) experienced cardiac events ($P < 0.05$, vs. HR ≥60 b.p.m. and QTc <500 ms).

Table 2 Predictors of cardiac events (syncope, aborted cardiac arrest, or sudden cardiac death) in univariate and multivariate analyses

	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	*P*-value	Odds ratio (95% CI)	*P*-value
Age at onset	1.08 (0.78–1.49)	0.639		
Female gender	3.56 (1.51–8.38)	0.004	4.54 (1.72–12.00)	0.002
HR <60 b.p.m.	2.83 (1.30–6.16)	0.009	4.46 (1.77–11.24)	0.001
HR (continuous variable)	0.95 (0.91–0.99)	0.022		
QTc ≥500 ms	2.65 (1.18–6.00)	0.019	2.93 (1.13–7.59)	0.026
Mutation location				
Pore	2.45 (1.07–5.60)	0.034	1.77 (0.70–4.48)	0.230
Transmembrane, non-pore	0.91 (0.27–3.08)	0.914		
N-terminal	0.83 (0.33–2.04)	0.677		
C-terminal	0.57 (0.26–1.27)	0.169		
Missense mutation	2.10 (0.91–4.85)	0.081		

Table 3 summarizes the baseline characteristics of four groups divided by HR and QTc. The group of HR ≥60 b.p.m. and QTc <500 ms was defined as Group A, the group of HR <60 b.p.m. and QTc <500 ms as Group B, HR ≥60 b.p.m. and QTc ≥500 ms as Group C, and HR <60 b.p.m. and QTc ≥500 ms as Group D. There were no significant differences among four groups regarding age at baseline ECG recording, age at the first event, percentages of female gender, and BBT. In Group A, the number of proband was significantly lower than that in Groups B and D. The incidence of syncope or aborted cardiac arrest in Group A was significantly lower than in the Groups B and C. In groups of HR <60 b.p.m. (B and D), patients with QTc ≥500 ms (Group D) had more arrhythmic events than those with QTc <500 ms (Group B).

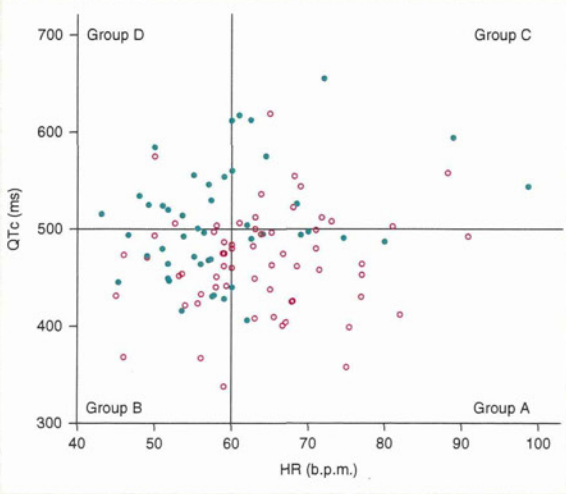

Figure 1 Distribution of KCNH2 mutation carriers according to the resting HR and QTc duration. Closed and open circles indicate symptomatic and asymptomatic patients, respectively. Two solid lines in the graph are drawn using the cutoff values of 60 b.p.m. and 500 ms. QTc was measured in lead V5. Groups A-D in the graph correspond to those in the text, *Table 3* and *Figure 2*.

We then estimated the cumulative probability of the first cardiac event between the age of 15 and 50 in four groups (Groups A–D, *Figure 2*). The Kaplan–Meier analysis of all subjects (*Figure 2A*) showed that cumulative event-free survival was significantly different ($P = 0.007$ by the log-rank test) and when adjusted for multiple comparisons, cumulative event-free survival was higher in Group A than in groups of HR <60 b.p.m. ($P = 0.014$ vs. Group B, $P = 0.001$ vs. Group D). In contrast, the survival rate was not statistically different among Groups B–D.

In *Figure 2B* and *C*, we examined the clinical course of 45 probands and 65 family members separately. The Kaplan–Meier analysis revealed no statistical difference in probands (*Figure 2B*, $P = 0.206$ by the log-rank test), whereas in family members, cumulative event-free survival was significantly different among the subgroups (*Figure 2C*, $P = 0.017$ by the log-rank test, $P = 0.058$ for Group A vs. Group B, $P = 0.002$ for Group A vs. Group D in multiple comparisons). Thus, the statistical difference in overall subjects may result from the prognosis of family members in our study population.

Finally, in order to assess the significance and independence of HR and QTc for cardiac events, we evaluated the parameters with the Cox proportional-hazards survival model (*Table 4*). The values of HR and QTc were centred at 60 b.p.m. and 500 ms for ease of interpretation. Compared with patients in Group A, patients in groups of HR <60 b.p.m. (Groups B and D) showed a higher risk for cardiac events by 2.6–4.4-fold. Although the hazard ratio in Group C was 2.16, there was no statistical difference between Groups A and C.

Table 3 Baseline clinical characteristics of four subgroups defined by QTc and basal HR

	QTc <500 ms		QTc ≥500 ms	
	Group A: HR ≥60 b.p.m. ($n = 35$)	**Group B: HR <60 b.p.m. ($n = 39$)**	**Group C: HR ≥60 b.p.m. ($n = 22$)**	**Group D: HR <60 b.p.m. ($n = 14$)**
Age (years) at ECG (range)	43 ± 18 (16–87)	39 ± 17 (15–71)	42 ± 18 (16–77)	39 ± 17 (15–64)
Age (years) at first event (range, number of patients)	25 ± 10 (13–42, $n = 8$)	27 ± 15 (15–71, $n = 19$)	26 ± 19 (15–77, $n = 10$)	26 ± 15 (13–54, $n = 10$)
Female gender [*n* (%)]	23 (66)	22 (55)	16 (73)	9 (64)
Proband [*n* (%)]	8 (23)*	18 (46)	12 (55)	7 (50)
Pore site mutation [*n* (%)]	6 (17)**	11 (28)	10 (46)	7 (50)
Schwarz score	3.1 ± 2.0§	3.6 ± 1.7§	5.5 ± 1.7	6.2 ± 1.2
Syncope or aborted cardiac arrest [*n* (%)]	8 (23)‡	19 (49)†	10 (46)	11 (79)
β-Blockers [*n* (%)]	7 (20)	13 (33)	9(41)	6 (43)

Values are given as the mean ± SD where indicated. HR = heart rate.
*$P < 0.05$ vs. Groups B and C.
**$P < 0.05$ vs. QTc ≥500 ms (Groups C and D).
§$P < 0.001$ vs. QTc ≥500 ms (Groups C and D).
†$P < 0.05$ vs. Group D.
‡$P < 0.05$ vs. HR <60 b.p.m. (Groups B and D).

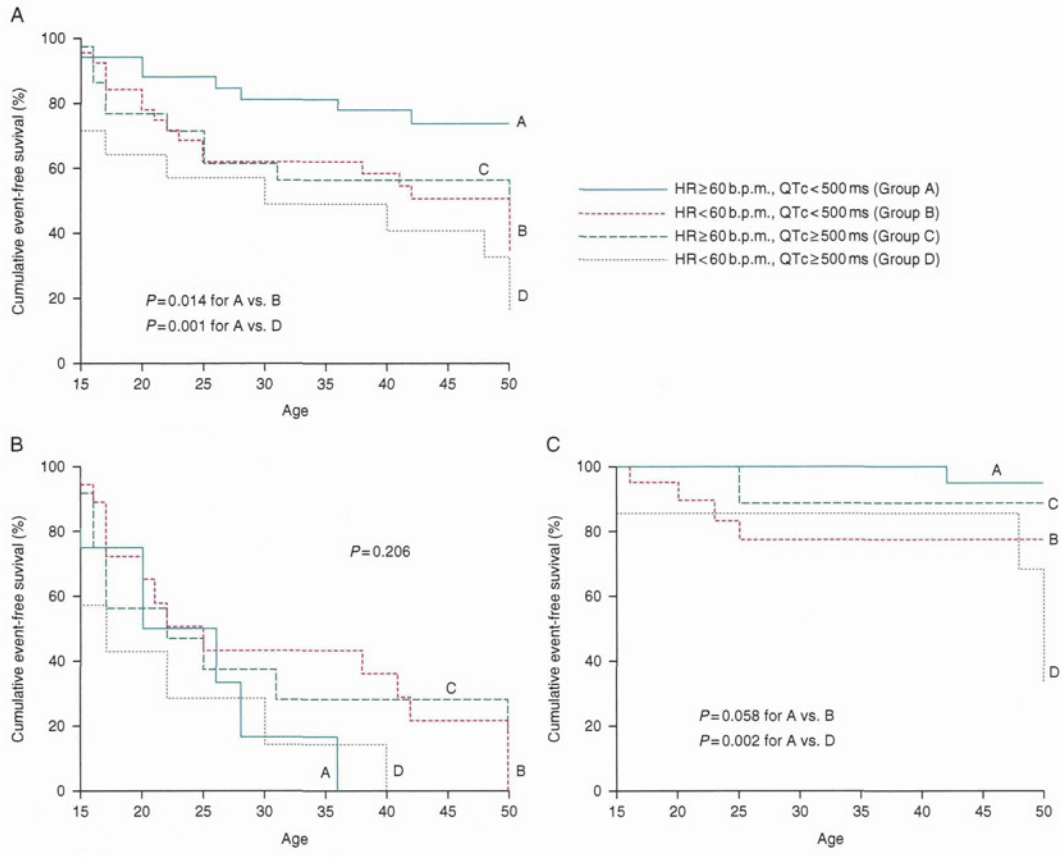

Figure 2 The Kaplan–Meier cumulative cardiac event-free survival curves from the age of 15–50 among each of four subgroups defined by cutoff values for HR of 60 b.p.m. and QTc of 500 ms. *Panels A–C* show the Kaplan–Meier curves of 110 patients, 45 probands and 65 family members, respectively. The group of HR ≥60 b.p.m. and QTc <500 ms was defined as Group A, the group of HR <60 b.p.m. and QTc <500 ms as Group B, HR ≥60 b.p.m. and QTc ≥500 ms as Group C, and HR <60 b.p.m. and QTc ≥500 ms as Group D.

Table 4 Contribution of QTc duration and HR to COX survival model

	Number of patients	Hazard ratio	95% CI	*P*-value
QTc <500 ms				
HR ≥60 b.p.m. (Group A)	35	1	–	–
HR <60 b.p.m. (Group B)	39	2.60	1.14–5.97	0.023
QTc ≥500 ms				
HR ≥60 b.p.m. (Group C)	22	2.16	0.85–5.47	0.105
HR <60 b.p.m. (Group D)	14	4.39	1.76–10.92	0.001

Treatment

β-Blocker therapy was introduced in 35 patients (29 probands) after diagnosis of LQT2 was made. Mean HR on medication was 56 ± 8 b.p.m. Metoprolol was used in 3 patients (90 ± 52 mg, 30–120), carvedilol in 3 (15 ± 9 mg, 5–20), atenolol in 4 (50 ± 0 mg, 50), propranolol in 21 (42 ± 16 mg, 30–80), and bisoprolol in 4 (4 ± 1 mg, 2.5–5).

Implantable cardioverter-defibrillator was implanted in 12 patients (VF: five patients, syncope: seven patients) during the first hospitalization or follow-up. In seven patients with a history of cardiac arrest due to VF (*Table 1*), two patients were treated with an ICD, three with both ICD and β-blocker, one with a pacemaker, and one with β-blocker alone (because the patient rejected ICD implantation). In a patient with a pacemaker, TdP was

observed repeatedly whenever she fell asleep and sinus rhythm became <60 b.p.m. during her first admission to the hospital. After pacemaker implantation, atrial pacing at 80 b.p.m. completely suppressed TdP. None of the patients received surgical left cardiac sympathetic denervation in our study population.

Recurrence of arrhythmic events during follow-up

For the follow-up data, 36 patients (22 patients on BBT) followed more than 3 months were recruited and 86% of patients (31 patients: 18 patients on BBT, 8 patients with an ICD, 10 patients without treatment) completed the mean follow-up period of 50 ± 39 months (40 ± 35 months for 18 patients on BBT and 63 ± 42 months for 13 patients without BBT).

Eighteen subjects on BBT consisted of 14 symptomatic (due to syncope) and 4 asymptomatic patients. Arrhythmic events during follow-up were observed only in symptomatic patients (seven patients: VF was observed in one patient, syncope in six patients). Analysis of the relationship between HR of <60 b.p.m. and recurrent events was also performed. Cardiac events during follow-up were observed in three of nine patients who showed HR <60 b.p.m. before BBT and four of eight patients with HR <60 b.p.m. after BBT ($P = 0.60$ and 0.06, respectively). Therefore, low HR of <60 b.p.m. at rest before or after β-blockers did not predispose ventricular arrhythmia, although the statistical insignificance could be due to a small number of patients for analysis. Details of treatment after recurrence in each individual were described below.

A 16-year-old male patient with a history of syncope experienced VF and was resuscitated. He underwent ICD implantation and dosage of bisoprolol was increased from 2.5 to 5 mg/day, which prevented any cardiac events for a follow-up period of 34.5 months. Recurrent syncope or documented TdP on BBT were observed in six patients: two patients who took metoprolol (one did not comply with the drug regimen and one with a syncopal episode on medication), one patient with atenolol (syncope twice and electrical storm due to TdP twice on medication), and three patients with propranolol (one did not comply with the drug regimen, two experienced a recurrent syncopal episode on medication). In those who did not comply with medication, syncope or TdP was suppressed by resuming BBT. Recurrent episodes of syncope in one patient on metoprolol (120 mg/day) have been suppressed by changing BBT to bisoprolol (2.5 mg/day) for 20 months. Implantable cardioverter-defibrillator implantation was also performed in this patient. Episodes of one patient on atenolol (50 mg/day) were not suppressed with additional prescription of mexiletine (400 mg/day), and ICD was implanted. He experienced an electrical storm after ICD implantation. While adjusting BBT, he was diagnosed with oesophageal cancer and died after 19.8 months follow-up. Syncope in one patient on propranolol (60 mg/day) was suppressed with combined medication of propranolol and diazepam. The other patient on propranolol (30 mg/day) was implanted with an ICD after recurrent episodes of syncope. Atrial pacing of 84 b.p.m. prevented arrhythmic events.

In 13 patients without BBT, 5 were symptomatic (1 VF and 4 syncope) at the first medical contact. In these patients, only one patient with a history of VF experienced an appropriate ICD shock following recurrent VF. To note, pacing using ICD leads was introduced during the first hospitalization in three of five symptomatic patients in whom TdP was repeatedly observed under HR of 60 b.p.m. In these patients, pacing prevented recurrent cardiac events during follow-up.

Discussion

The present study demonstrated that basal HR of <60 b.p.m. was an apparent risk factor for cardiac events in LQT2 patients. Corrected QT ≥500 ms and female gender were also useful for risk stratification in LQT2. The Kaplan–Meier analysis in total study population revealed that cumulative event-free survival was significantly higher in the subgroup with HR ≥60 b.p.m. and QTc <500 ms than in the two groups with HR <60 b.p.m. ($P < 0.05$). The same trend was observed in the analysis of family members. On the other hand, there was no significant difference in basal HR irrespective of cardiac events in probands. Because, first, the number of probands ($n = 45$) was relatively smaller than that of family members ($n = 65$), and second, there was an entry bias: 84% of probands were referred for genetic testing as they were symptomatic, which influenced the evaluation of basal HR and cardiac events. Our examination of family members therefore suggested that *KCNH2* mutation carriers associated with more severe bradycardia may show a stronger penetrance.

Mutations in *KCNH2* are causative of LQT2, and *KCNH2* encodes for the rapid component of the delayed rectifier K-current (I_{Kr}). In electrophysiological studies, I_{Kr} was shown to be present in rabbit[23] and mouse[24] sinoatrial node cells. Pharmacological inhibition of I_{Kr} by E-4031 markedly suppressed the spontaneous activity of sinoatrial node cells, suggesting that I_{Kr} activation plays a key role in maintaining an adequate HR. In other experimental models,[25] I_{Kr} blockade has also been shown to cause bradycardia. In clinical studies, bradycardia is more frequently observed in LQT2.[3,26] However, no previous studies have demonstrated the validity of bradycardia as a predictor of prognosis.

As for pore site mutations of *KCNH2*, known as a risk factor for cardiac events in LQT2, they were correlated with cardiac events in univariate but not multivariate analysis in our study cohort (*Table 2*). This contrasts with the previous report of Moss *et al.*[10] and is probably due to the difference in the number of studied mutations as well as the exclusion of patients who had their first cardiac events before 15 years old.

β-Blockers are first line therapy for prevention of TdP in LQT2 because it suppresses early afterdepolarizations carried by L-type Ca^{-} channels or Ca^{2+} channels.[27–29] The result of our study, however, may cause concerns that BBT-induced HR-reduction could lead to recurrence of ventricular arrhythmias. To answer the question, we analysed the patient group on BBT during follow-up, but low HR of <60 b.p.m. at rest before or after β-blockers did not predict recurrence of cardiac events ($P =$ 0.60 and 0.06, respectively). Our study cohort may be too small to clarify this issue and therefore, further clinical evaluation with a large number of patients will be required to conclude the significance of low HR on/off β-blockers in LQT2. On the basis of our

findings, however, it is reasonable to hypothesize that pacing could be used as an adjunctive therapy in LQT2 patients showing HR <60 b.p.m. irrespective of QTc values. Our combined risk-evaluating scales (*Figure 1*) would help physicians estimate long-term therapy in asymptomatic *KCNH2* mutation carriers, both probands and family members.

Limitations

In some symptomatic patients, there was a long period between the average age at onset of symptoms and the average age at ECG recording. Regarding this issue, the risk evaluation should be carefully considered. In addition, it was difficult to gather ECG recordings of the first event, because many patients suffered syncope without a doctor witnessing the first event. However, among the four subgroups, there was no significant difference in age at ECG recording and age at the first event (*Table 3*). Therefore, we evaluated cardiac risk using the HR recorded by ECG at the first medical contact. As for the effect of BBT on HR as a risk factor for cardiac events, our cohort was too small to lead a relevant conclusion because follow-up of patients was insufficient. Hence, it awaits a further study with a larger number of genotyped LQT2 patients.

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Conflict of interest: none declared.

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Cardiac shock wave therapy ameliorates left ventricular remodeling after myocardial ischemia–reperfusion injury in pigs *in vivo*

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Objectives Left ventricular (LV) remodeling after acute myocardial infarction (AMI) is associated with a poor prognosis and an impaired quality of life. We have shown earlier that low-energy extracorporeal cardiac shock wave (SW) therapy improves chronic myocardial ischemia in pigs and humans and also ameliorates LV remodeling in a pig model of AMI induced by permanent coronary ligation. However, in the current clinical setting, most of the patients with AMI receive reperfusion therapy. Thus, in this study we examined whether our SW therapy also ameliorates LV remodeling after myocardial ischemia–reperfusion (I/R) injury in pigs *in vivo*.

Methods Pigs were subjected to a 90-min ischemia and reperfusion using a balloon catheter and were randomly assigned to two groups with or without SW therapy to the ischemic border zone (0.09 mJ/mm^2, 200 pulses/spot, 9 spots/animal, three times in the first week) ($n=15$ each).

Results Four weeks after I/R, compared with the control group, the SW group showed significantly ameliorated LV remodeling in terms of LV enlargement (131 ± 9 vs. 100 ± 7 ml), reduced LV ejection fraction (28 ± 2 vs. 36 ± 3%), and elevated left ventricular end-diastolic pressure (11 ± 2 vs. 4 ± 1 mmHg) (all $P<0.05$, $n=8$ each). The SW group also showed significantly increased regional myocardial blood flow (−0.06 ± 0.11 vs. 0.36 ± 0.13 ml/min/g, $P<0.05$), capillary density (1.233 ± 31 vs. 1.560 ± 60/mm^2, $P<0.001$), and endothelial nitric oxide synthase activity (0.24 ± 0.03 vs. 0.41 ± 0.05, $P<0.05$) in the ischemic border zone compared with the control group ($n=7$ each).

Conclusion These results indicate that our SW therapy is also effective in ameliorating LV remodeling after myocardial I/R injury in pigs *in vivo*. *Coron Artery Dis* 21:304–311 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Keywords: angiogenesis, reperfusion, shock wave, ventricular remodeling

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Introduction

Ischemic heart disease is the leading cause of death in western countries. The development of left ventricular (LV) remodeling after acute myocardial infarction (AMI) leads to sudden cardiac death, heart failure, and poor prognosis. Thus, it is important to improve LV remodeling after AMI to improve prognosis and the quality of life. Several regenerative therapies, such as gene [1–3] and cell therapies [4–8], are currently under development; however, most of these are invasive in nature and their effectiveness and safety have not yet been fully established. Thus, more effective and less invasive therapies need to be developed.

We have shown earlier that low-energy extracorporeal cardiac shock wave (SW) therapy effectively induces angiogenesis and improves cardiac functions in a porcine model of chronic myocardial ischemia [9], and that SW therapy improves symptoms, reduces the use of nitroglycerin, and improves myocardial perfusion in patients with end-stage coronary artery disease [10,11]. Furthermore, we have recently shown that SW therapy improves LV remodeling in a porcine model of AMI with permanent coronary ligation [12]. However, in the current clinical setting, most patients with AMI receive emergency reperfusion therapy with either percutaneous coronary intervention or thrombolytic agents. It remains to be determined whether our extracorporeal cardiac SW therapy also ameliorates myocardial ischemia–reperfusion (I/R) injury *in vivo*. Thus, in this study we examined whether our SW therapy also ameliorates LV remodeling after myocardial I/R injury in pigs *in vivo*, and if so, what mechanism(s) might be involved.

Methods

The investigation conforms with the Guide for the Care and Use of Laboratory Animals established by the US National Institutes of Health (Publication No. 85-23, revised 1996). All procedures were performed according to the protocols approved by the Institutional Committee for Use and Care of Laboratory Animals at Tohoku University (20-Idou-151 and 21-Idou-156).

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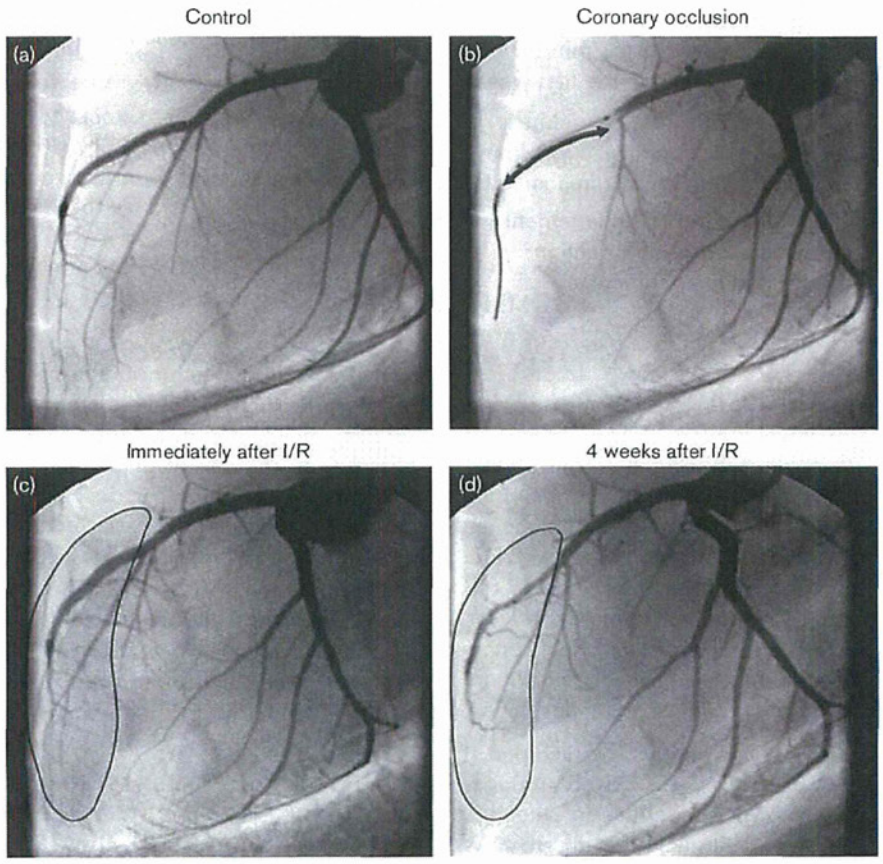
Porcine model of myocardial I/R

A total of 30 domestic male pigs (25–30 kg in body weight) were used in this study. They underwent myocardial I/R injury with and without SW therapy. They were subjected to cardiac catheterization and histology study at 4 weeks after I/R ($n = 8$ each) and to western blotting study at 1 week after I/R ($n = 7$ each). The animals were anesthetized with ketamine hydrochloride (15 mg/kg, intramuscular), and after intubation, they were kept anesthetized with an inhalation of 2.0% sevoflurane for cardiac catheterization and euthanization. We inserted a 7F sheath into the left carotid artery for cardiac catheterization. A 5000-IU bolus of heparin was administered intravenously and 2000-IU was injected every hour. We performed a left ventriculography (LVG) and coronary angiography (CAG) in a left oblique view with the use of a cineangiography system (Toshiba Medical, Tochigi, Japan) [9,12]. LV volume and LV ejection fraction (LVEF) were calculated using Simpson's method. A coronary angioplasty balloon (2.5–3.5 mm in diameter depending on the vessel size) was then introduced into the left anterior descending coronary artery (LAD) and inflated just distal to the first diagonal branch for 90 min, which has been shown earlier to effectively induce myocardial infarction [13,14], at the lowest pressure that completely occluded distal flow (Fig. 1). After 90 min of ischemia, the balloon was deflated and both CAG and LVG were reperformed to confirm the patency of distal LAD and the reduced LV wall motion, respectively. Cardiac catheterization was performed before ischemia, immediately after reperfusion, and 4 weeks after I/R. After the study, 4 weeks after I/R, the animals were euthanized by an overdose of pentobarbital.

Extracorporeal cardiac SW therapy

On the basis of our earlier studies [9–12,15,16], we applied a low-energy SW (0.09 mJ/mm^2, approximately 10% of the energy used for the lithotripsy treatment, 200 shots/spot for 27 spots) to the border zone around the infarcted myocardium with the guidance of an echocardiogram equipped within the specially designed SW generator (Storz Medical AG, Kreuzlingen, Switzerland) in an R-wave-triggered manner to avoid ventricular

Fig. 1



Porcine model of myocardial ischemia–reperfusion (I/R). Coronary angiograms at baseline (a), during balloon inflation in the left anterior descending coronary artery (b), immediately after reperfusion (c), and 4 weeks after the I/R in the same pig. The inflated balloon is shown by an arrowed line (b) and the ischemic myocardial area by the shaded area (c and d).

arrhythmias. In a preliminary study, we confirmed that no adverse effects, such as cardiac rupture or tamponade, were noted even if we applied a SW to the infracted myocardium (data not shown). We examined LV wall motion by echocardiography during I/R and defined the border zone as the edge of the area where the LV wall motion was severely depressed after I/R. We were able to accurately focus a SW to any part of the heart under the guidance of echocardiography with a focus of approximately 2 mm [9–12]. We performed the SW treatment three times in the first week (day 1, 3, and 5), whereas the animals in the control group received the same procedures three times but without the SW treatment.

Cardiac enzymes

We measured serum concentrations of cardiac troponin T and creatinine kinase myocardial blood isoform (CK-MB) using an electrochemiluminescence immunoassay and a chemiluminescence immunoassay, respectively. Blood samples were serially collected before and 5, 12, 24, 48, and 72 h after the I/R injury, and the extent of myocardial infarction was expressed as the area under the curves of troponin T and CK-MB [12].

Echocardiography

We performed a transthoracic echocardiographic study (Aplio 80, Toshiba Medical). We calculated the wall thickening fraction (WTF, %) by using the following formula: WTF = 100 × (end-systolic wall thickness − end-diastolic wall thickness)/end-diastolic wall thickness [9]. We measured the WTF in the infarcted area and the border zone when the animals were sedated.

Regional myocardial blood flow

We evaluated regional myocardial blood flow (RMBF) with colored microspheres (Dye-Trak VII+, Triton Technology, San Diego, USA) ($n = 4$ each) [9,12]. We injected 6 million microspheres (diameter 15 μm) into the left atrium before the induction of myocardial ischemia and 4 weeks after I/R. We drew a reference arterial blood sample from the descending aorta at a constant rate of 12 ml/min for 90 s using a withdrawal pump. We extracted microspheres from the LV wall and blood samples by potassium hydroxide digestion, extracted the dyes from the microspheres with ethylene glycol monoethyl ether acetate (70 μl), and determined their concentrations by spectrophotometry. We calculated the change of myocardial blood flow (ml/min/g) in the infarcted region and border zone.

Myocardial capillary density

The heart was removed and 10% formaldehyde was injected into the left coronary artery with a pressure of 100–120 mmHg. After fixation, tissue specimens were obtained from the border zone of each animal. We treated the paraffin-embedded sections with a rabbit anti-factor VIII antibody (N1505, Dako, Copenhagen, Denmark), and counted the number of factor VIII-positive cells in 10 random fields of the border zone and the remote area in each heart at × 400 magnification, and calculated capillary density [9,12]. Ten random fields of each sample were examined in a blinded manner. Each field covered 0.036 mm^2.

Myocardial fibrosis

Masson-trichrome staining was performed using the paraffin-embedded sections. We evaluated the fibrosis area in 10 random fields of the border zone in each heart at × 200 magnification. A digital image processing software AxioVision 4.5.0.0 (Carl Zeiss, Göttingen, Germany) was used to detect the myocardial fibrosis area, and the ratio of the fibrosis area to the myocardial area was calculated.

Western blot analysis

To examine the mechanisms of the inhibitory effects of SW therapy on LV remodeling, another set of animals with I/R injury, with and without SW therapy, were made and they were euthanized at 1 week after the procedure. We performed western blot analysis for phosphorylated endothelial nitric oxide synthase (phospho-eNOS) and vascular endothelial growth factor (VEGF). Samples from the border zone were used and the extracted samples (50 μg of protein) were subjected to SDS-PAGE/immunoblot analysis by using the specific antibody for phospho-eNOS at Ser1177 (No. 9571, Cell Signaling Technology, Danvers, Massachusetts, USA), total-eNOS (No. 610296, Becton Dickinson, Franklin Lakes, New Jersey, USA), and VEGF (sc-152, Santa Cruz Biotechnology, Santa Cruz, California, USA). The regions containing proteins were visualized by an electrochemiluminescence western blotting luminal reagent (RPN2132, GE Healthcare Bioscience, Waukesha, Wisconsin, USA). The extents of eNOS phosphorylation and VEGF expression were normalized by that of total-eNOS and β-actin, respectively [9,17].

Statistical analysis

Results were expressed as mean ± SEM. We determined the statistical significance by an analysis of variance for multiple comparisons and the unpaired Student's t-test. Values of P less than 0.05 were considered to be statistically significant.

Results

Extent of myocardial infarction

The extent of myocardial infarction, when evaluated by the area under the curve of troponin T or CK-MB, was comparable between the control and the SW groups (troponin T, 296 ± 32 vs. 319 ± 30 ng/ml*h, $P = 0.61$; CK-MB, 1.641 ± 301 vs. 1.993 ± 353 ng/ml*h, $P = 0.46$), indicating that the extent of myocardial infarction was comparable between the two groups.

Safety of SW therapy

No procedural complications or adverse effects related to SW therapy were noted throughout the experiments.

Cardiac catheterization: CAG and LVG

At 4 weeks after the I/R injury, CAG confirmed the patency of reperfused LAD in all pigs (Fig. 1). Before and immediately after I/R (before the SW treatment), LV end-diastolic volume (LVEDV), LV end-systolic volume (LVESV), and LVEF were all comparable between the two groups (Fig. 2). Four weeks after I/R, LVG showed marked LV enlargement and reduced LVEF in the control group (Fig. 2). In contrast, LV enlargement and reduced LVEF were significantly ameliorated in the SW group. (LVEDV, 100 ± 7 vs. 131 ± 9 ml, $P < 0.05$; LVESV, 65 ± 8 vs. 95 ± 7 ml, $P < 0.05$; LVEF, 36 ± 3 vs. $28 \pm 2\%$, $P < 0.05$) (Fig. 2). Although LV end-diastolic pressure was comparable between the two groups before and immediately after I/R (before the SW treatment), it remained elevated in the control group but was normalized in the SW group 4 weeks after I/R (11 ± 2 vs. 4 ± 1 mmHg, $P < 0.05$) (Fig. 2).

Echocardiography

We measured the WTF of the infarcted region and the border zone by transthoracic echocardiography. The WTF in the infarcted region was significantly decreased to the same extent after I/R and was comparable between the control and the SW groups throughout the experimental period (before I/R, 22 ± 2 vs. $20 \pm 1\%$; immediately after I/R, 2 ± 1 vs. $1 \pm 0.3\%$; 4 weeks, 4 ± 2 vs. $5 \pm 2\%$) (Fig. 3a). In contrast, 4 weeks after I/R, the WTF was significantly improved at the border zone in the SW group as compared with the control group (before I/R, 24 ± 2 vs. $22 \pm 2\%$, $P = 0.54$; immediately after I/R, 16 ± 1 vs. $15 \pm 2\%$, $P = 0.59$; and 4 weeks, 15 ± 2 vs. $24 \pm 4\%$, $P < 0.05$) (Fig. 3b).

Regional myocardial blood flow

In the infarcted region, RMBF was equally decreased in the control and the SW groups at 4 weeks after I/R as compared with before I/R (-0.52 ± 0.22 vs. -0.49 ± 0.08 ml/min/g, $P = 0.89$), whereas RMBF at the border zone was significantly increased only in the SW group (control: -0.06 ± 0.11 vs. SW: 0.36 ± 0.13 ml/min/g, $P < 0.05$) (Fig. 4).

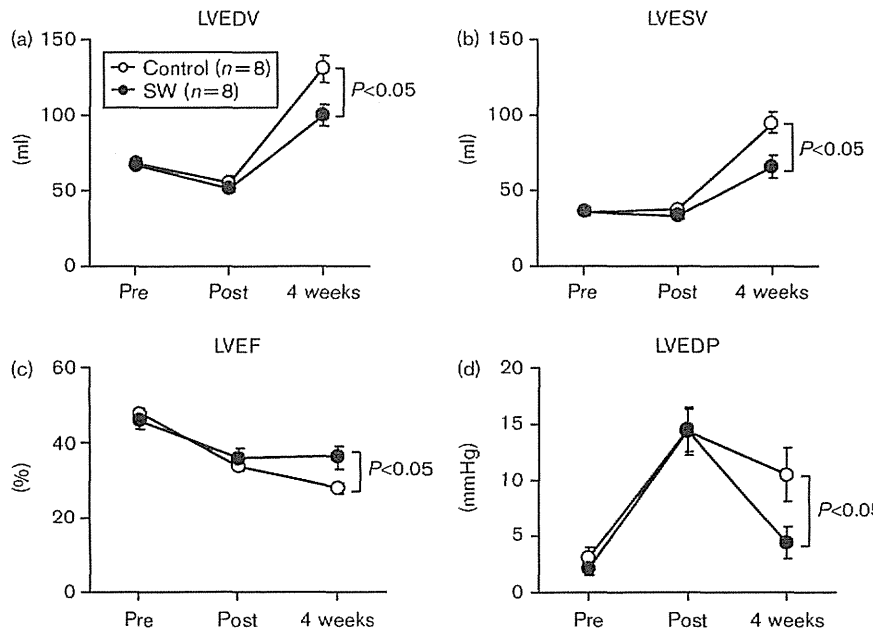
Histopathology

Factor VIII staining showed that 4 weeks after I/R the number of factor VIII-positive blood vessels at the border zone was significantly higher in the SW group than in the control group (1.560 ± 60 vs. $1.233 \pm 31/mm^2$, $P < 0.001$) (Fig. 5a–c). In the remote area, the number of vessels was comparable between the two groups (Fig. 5d). Masson-trichrome staining showed that there was no difference in the extent of myocardial fibrosis at the border zone between the two groups (control, 0.15 ± 0.02 vs. SW, 0.13 ± 0.03, $P = 0.72$).

Western blot analysis

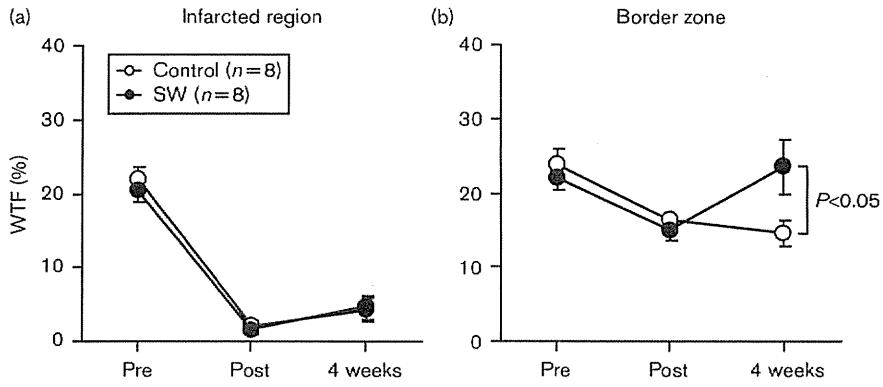
Western blot analysis showed that the ratio of phospho-eNOS to total-eNOS, a marker of eNOS activation, was

Fig. 2



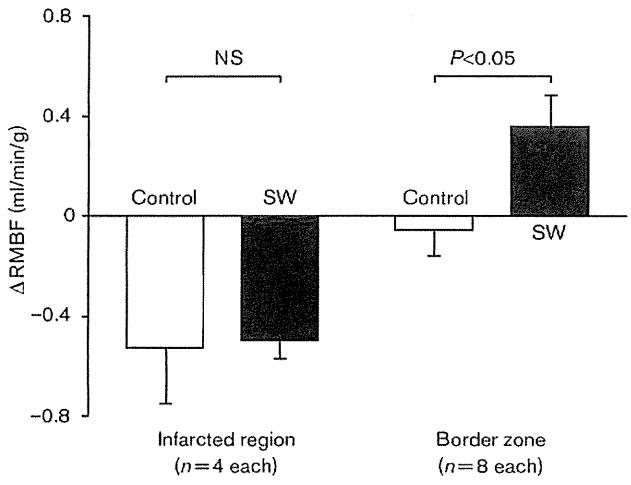
The shock wave (SW) therapy ameliorates left ventricular (LV) remodeling after myocardial ischemia–reperfusion (I/R). The SW therapy significantly ameliorated LV remodeling as evaluated by LV end-diastolic volume (LVEDV) (a), LV end-systolic volume (LVESV) (b) and LV ejection fraction (LVEF) (c) and also normalized LV end-diastolic pressure (LVEDP) (d). Pre, before I/R; Post, immediately after I/R; 4 weeks, 4 weeks after I/R.

Fig. 3



The shock wave (SW) therapy ameliorates left ventricular (LV) systolic function. An echocardiographic study showed that the wall thickening fraction (WTF) in the infarcted region was comparable between the two groups throughout the study period (a), whereas the WTF in the border zone was normalized by the SW therapy at 4 weeks after the ischemia–reperfusion (I/R) injury (b). Pre, before I/R; Post, immediately after I/R; 4 weeks, 4 weeks after I/R.

Fig. 4



The shock wave (SW) therapy ameliorates myocardial blood flow. At 4 weeks after ischemia–reperfusion, although regional myocardial blood flow (RMBF) in the infarcted area was equally reduced in the control and SW groups, the flow in the border zone was significantly increased only in the SW group.

significantly increased in the SW group than in the control group 1 week after I/R (0.41 ± 0.05 vs. 0.24 ± 0.03, $P < 0.05$) (Fig. 6a). The protein expression of VEGF also tended to be increased in the SW group compared with the control group 1 week after I/R (0.78 ± 0.26 vs. 0.40 ± 0.12, $P = 0.22$) (Fig. 6b).

Discussion

The novel finding of this study is that our extracorporeal cardiac SW therapy ameliorates LV remodeling after myocardial I/R injury in pigs *in vivo*. Importantly, no procedural complications or adverse effects with SW therapy were noted in this study, a consistent finding with our earlier studies for chronic myocardial ischemia in pigs and humans, AMI in pigs, and hind limb ischemia in rabbits [9–12,15,16,18].

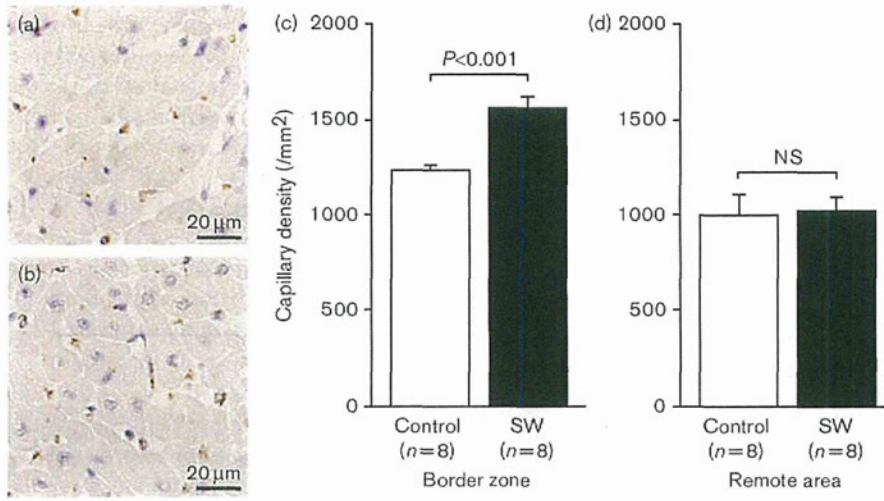
Inhibitory effects of the SW therapy on LV remodeling after I/R

Although short-term and long-term outcomes of patients with AMI have improved during the last decades as reperfusion therapy became widely available in emergency care [19–22], LV remodeling after AMI still remains one of its major complications [23]. We have recently shown that our SW therapy ameliorates LV remodeling after AMI with permanent coronary ligation in pigs *in vivo* [12]. However, in the current clinical setting, most of the AMI patients are treated with emergency reperfusion therapy. In this study, to simulate the current situation with reperfusion therapy, we examined the possible beneficial effects of our SW therapy in a porcine model of myocardial I/R *in vivo*. In this model, severe LV remodeling characterized by marked LV enlargement and reduced LVEF was noted 4 weeks after I/R in the control group, which, on the other hand, was effectively ameliorated by SW therapy. Echocardiographic study also showed that regional LV wall motion was normalized at the border zone accompanied with increased RMBF and capillary density. These results suggest that SW-induced angiogenesis at the border zone substantially contributes to the suppression of LV remodeling *in vivo*.

Mechanisms for the inhibitory effects of the SW therapy on LV remodeling after I/R

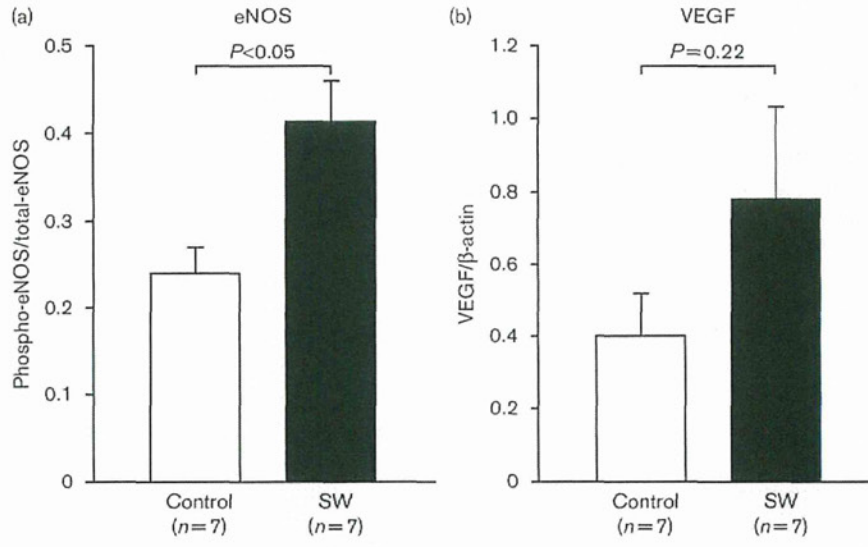
The precise mechanisms of SW-mediated suppression of LV remodeling after myocardial I/R remain to be fully elucidated. However, it is conceivable that multiple

Fig. 5



The shock wave (SW) therapy increases capillary density in the border zone. Representative factor VIII staining in the control group (a) and the SW group (b), and quantitative analysis of the vessel number in the border zone (c) and in the remote area (d). The SW therapy significantly increased the density of factor VIII-positive capillaries in the border zone, whereas the capillary density was comparable between two groups in the remote area.

Fig. 6



The shock wave (SW) enhances endothelial nitric oxide synthase (eNOS) activity. The SW therapy significantly enhanced eNOS phosphorylation, a marker of eNOS activation, in the border zone (a) and tended to do so for vascular endothelial growth factor (VEGF) protein expression (b) at 1 week after myocardial ischemia–reperfusion.

mechanisms are involved in the inhibitory effects of our SW therapy on LV remodeling after I/R. When a SW hits a tissue, the SW induces cavitation (a micrometer-sized violent collapse of bubbles) by the first compression by the positive pressure component and expansion with the tensile component of SW [24]. As the physical forces generated by cavitation are highly localized, the SW could induce localized stress on cell membranes, leading to a variety of biochemical effects including shear stress, hyperpolarization, and Ras activation [25], and the induction of stress fibers and intercellular gaps [26]. In addition, the SW induces nonenzymatic NO synthesis from L-arginine and hydrogen peroxide [27], upregulates eNOS, and suppresses nuclear factor-κB activation in the cultured human umbilical venous endothelial cells [28]. NO exerts a wide variety of biological effects including the regulation of vascular tone and angiogenesis [29–31]. In this study, we confirmed that SW therapy increases

eNOS activity and capillary density at the border zone, associated with an improvement of LV remodeling and dysfunction. These results suggest that our SW therapy improves LV remodeling after I/R, at least in part, by enhancing NO production.

Enhanced expression of multiple angiogenic factors, such as VEGF and stromal-derived factor 1, is crucial for the recruitment and incorporation of endothelial progenitor cells [32–34]. We have shown earlier that our SW therapy upregulates myocardial VEGF/Flt-1 expression in pigs *in vivo* [9]. A SW has also been reported to promote mobilization and differentiation of bone marrow-derived cells in a rat model of chronic hindlimb ischemia [35] and in rat bone marrow-derived mononuclear cells *in vitro* [36]. In this study, we confirmed that SW therapy increases RMBF, capillary density, and eNOS activity, and tended to increase the VEGF expression at the border zone. Although the renin–angiotensin system plays an important role in the pathogenesis of LV remodeling after AMI, partly because of enhanced myocardial fibrosis [37–40], the extent of the fibrosis at the border zone was comparable between the two groups in this study.

Advantage of the non-invasive SW therapy

The gene [1–3] and cell therapies [4–8], although worthy of development, require invasive procedures such as general anesthesia, cardiac catheterizations, and open chest surgery [4–8,41,42] to deliver the genes or cells to the ischemic myocardium, which may limit the usefulness of these therapies in the clinical setting. Our extracorporeal cardiac SW therapy is quite noninvasive and safe without any adverse effects, which is a major advantage of our SW therapy. This is an important point in determining the clinical usefulness of angiogenic therapies, especially in elderly patients with severe ischemic heart disease.

Limitations of the study

Several limitations of this study should be mentioned. First, we observed a trend for but not a significant increase in the VEGF level in the SW group compared with the control group, although we have earlier shown a significant increase in the VEGF expression by SW therapy in a porcine model of chronic myocardial ischemia [9]. This discrepancy may be partly because of the different stage of myocardial ischemia examined. In our earlier study, the VEGF protein level was evaluated 8 weeks after creating a chronic myocardial ischemia, when the VEGF level might have returned to the normal level, and therefore the SW-induced enhancement of the VEGF expression was clearly detected [9]. On the other hand, in this study, the VEGF level was studied 1 week after creating a myocardial I/R, when the expression of VEGF was still strongly enhanced even in the control group. Importantly, however, we were able to show the significant upregulation of eNOS by the SW therapy in this study, which we did not examine in the earlier study [9]. Second, although we were able to show that our SW therapy enhances angiogenesis at the border zone of the LV, the effects of the SW therapy on each component of the myocardial tissue, including vascular endothelial cells, vascular smooth muscle cells, cardiomyocytes, extracellular matrix, and inflammatory cells, remain to be clarified in future studies. Third, although we studied the expression of eNOS and VEGF in this study, there are many other growth factors and chemokines that could enhance angiogenesis such as stromal-derived factor 1/CXCR4 system and angiopoietin/Tie-2 system. This point also remains to be examined in future studies.

Conclusion

We were able to show that our low-energy extracorporeal cardiac SW therapy effectively induces angiogenesis and ameliorates LV remodeling after I/R in pigs *in vivo* without any adverse effects. Thus, our SW therapy could be a novel and safe strategy for the prevention of LV remodeling after AMI in humans.

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There is no conflict of interest.

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体外衝撃波を用いた非侵襲性血管新生治療

伊藤　健太　　下川　宏明

要　旨

我が国では，人口の高齢化や生活習慣の欧米化に伴い，虚血性心疾患や閉塞性動脈硬化症といった動脈硬化性疾患患者が増加してきている．我々は，基礎研究の結果を基に，低出力の衝撃波を用いた血管新生療法(「低出力体外衝撃波治療」)を開発し，①重症狭心症，②急性心筋梗塞，③下肢閉塞性動脈硬化症を対象に臨床試験を行っている．重症狭心症に対しては，第1次臨床試験（オープン試験）と第2次臨床試験（二重盲検プラセボ対照試験）を行い，本治療法の有効性と安全性を確認し，論文報告している．本治療法は，麻酔や侵襲的な処置を伴わずに，体外から治療を行うことができる非侵襲的な治療法であり，繰り返し行うことも可能である．今後幅広い疾患への応用が期待される．

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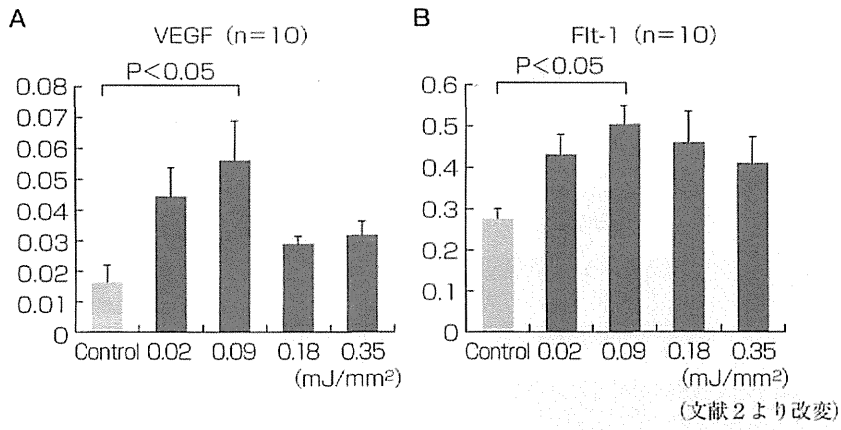
Key words：虚血性心疾患，閉塞性動脈硬化症，血管新生，衝撃波治療

はじめに

我が国では，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，虚血性心疾患や閉塞性動脈硬化症といった動脈硬化性疾患の患者数が増加の一途をたどっている．動脈硬化性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カテーテル治療，③バイパス手術の3本柱から成るが，近年これら従来の治療法では十分な治療効果を得られない重症例(例えば，びまん性狭窄病変を持つ症例)が増加してきている．このような症例は，胸痛や下肢痛・潰瘍形成のため生活の質（QOL）が低下するのみならず，予後も不良である．

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，骨髄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．また，有効性・安全性が認められたとしても，費用が高額になることは避けられない．一方，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．そのため，低侵襲で，かつ有効性の期待できる新しい治療法の開発が望まれている．我々は，培養細胞や大型動物（ブタ）を用いた検討の結果を基に，低出力の衝撃波を用いた血管新生療法(「低出力体外衝撃波治療」)を開発し，現在，①重症狭心症，②急性心筋梗塞，③下肢閉塞性動脈硬化症を対象に臨床試験を行っている[1]．

いとう　けんた，しもかわ　ひろあき：東北大学大学院循環器病態学分野



(文献2より改変)

図1. 衝撃波による血管増殖因子の発現亢進：(A) VEGF, (B) Flt-1
HUVECに衝撃波を照射すると，VEGFとFlt-1の発現が亢進した．発現の亢進は，結石破砕に用いる出力の約10%という弱い出力（0.09 mJ/mm²）の時に最大となった．

1. 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持つ媒体内を直線的に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おり，既に20年以上前から標準的治療の1つとして保険適応にもなっている．我々は培養細胞を用いた基礎実験により，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（0.09 mJ/mm²）をピークに，主要な血管新生因子の1つ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)[2]．そこで，ブタ慢性心筋虚血モデルにおいて体外衝撃波治療の効果を検討した．慢性虚血心筋に1日おきに3回衝撃波を照射し，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，虚血心筋組織におけるVEGFの発現が遺伝子レベル・蛋白レベルのいずれにおいても亢進していた[2]．さらに，毛細血管数の増加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[2]．衝撃波治療中および治療後3日間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示唆された．

2. 狭心症に対する低出力体外衝撃波治療

上記の基礎的検討に基づき，我々は，重症狭心症に対して低出力体外衝撃波治療の臨床試験を行っている．対象は以下の4条件を満たす狭心症症例とした．①20歳以上，②最大量の薬物治療に抵抗性で，かつ経皮的冠動脈形成術(percutaneous coronary intervention：PCI）や冠動脈バイパス手術（coronary artery bypass grafting：CABG）による完全な血行再建が不可能，③カナダ心臓協会（Canadian cardiovascular society：CCS）分類でClass II～IV，④負荷心筋シンチグラムや負荷心エコーで明らかな虚血領域

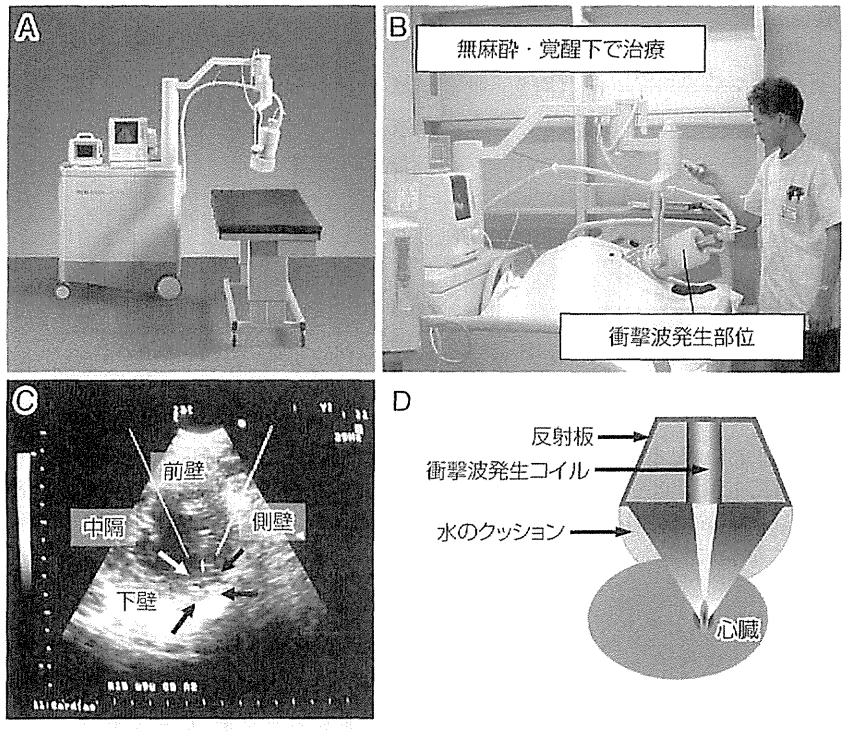


図2. 体外衝撃波治療装置と治療風景：(A) 体外衝撃波治療装置，(B) 治療風景，(C) 心臓超音波検査画面，(D) 概念図

を認める．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ている（図2）．衝撃波発生ヘッドを患者の前胸壁に当て，装置に内蔵された超音波プローブで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カ所につき200発の衝撃波を，虚血領域の広さに応じて20～120カ所照射する．1回の治療時間は約3時間で，1～2日おきに計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．2003年から重症狭心症患者9名を対象に実施した第1次臨床試験では，個人差はあるものの全例で狭心症症状が軽減し，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た（図3）[3]．また，負荷心筋シンチグラムで評価した心筋血流も，衝撃波を照射した部位においてのみ改善を認めた（図3）[3]．この結果から，衝撃波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全く認めなかった．さらに，2005年から，低出力体外衝撃波治療とプラセボ治療を比較する第2次臨床試験を実施し，その結果，低出力体外衝撃波治療後には，CCS分類による狭心症の重症度，ニトログリセリンの使用頻度，6分間歩行距離が有意に改善し，MRIで測定した左室一回拍出量，左室駆出率も有意に増加した（図4）[4]．これらの効果はプラセボ治療後では認められなかった．本年7月，狭心症に対する低出力体外衝撃波治療は，厚生労働省の高度医療に承認された．

3. 急性心筋梗塞に対する低出力体外衝撃波治療

我が国では，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

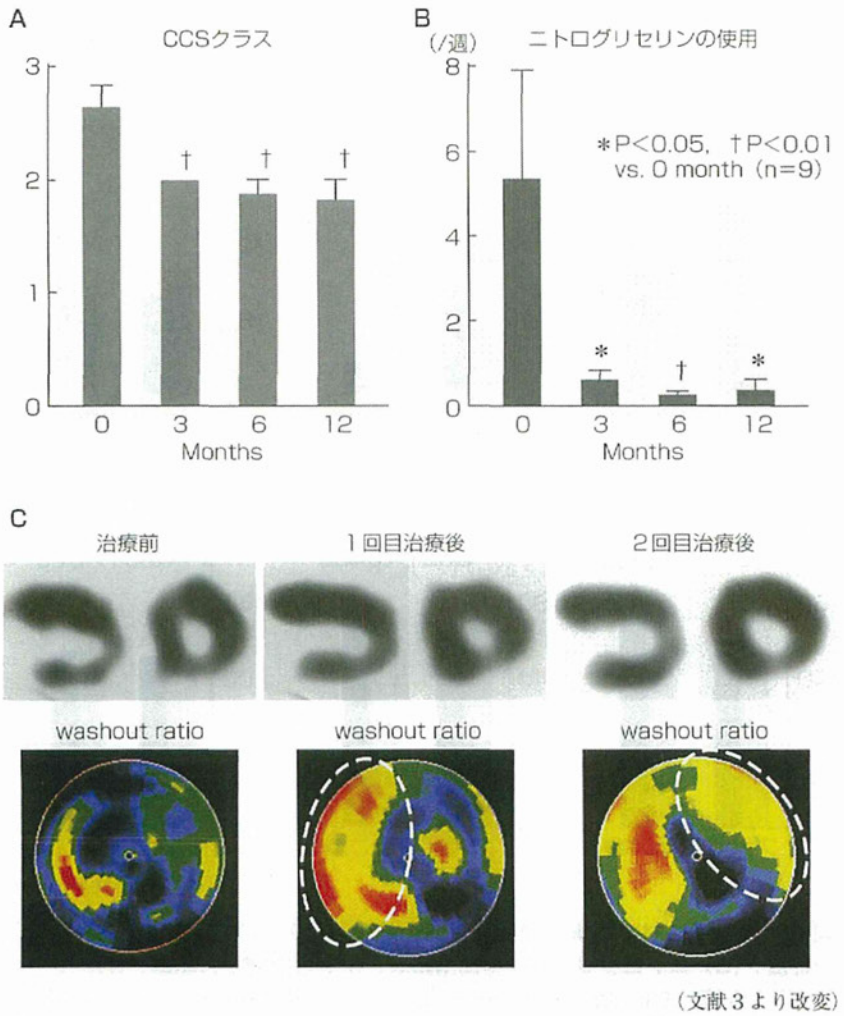


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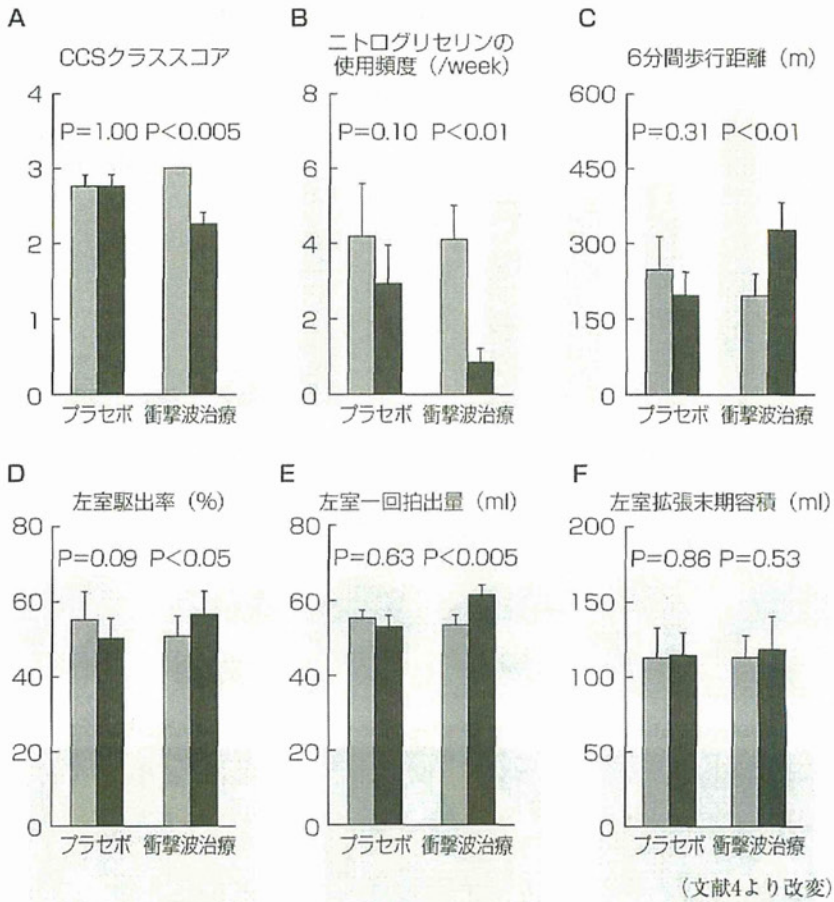
図3. 狭心症に対する第1次臨床試験の結果：(A) CCS分類による狭心症の重症度，(B) ニトログリセリンの使用頻度，(C) 負荷心筋シンチグラムによる心筋血流（青が虚血，黄～赤が血流良好を意味する）
自覚症状の改善を認めた（A，B）．また，衝撃波治療を受けた領域（破線で示された領域）でのみ血流の改善を認めた（C）．

の死亡率低下に貢献している．しかし十分な薬物治療を受けていても，慢性期には左室リモデリング（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や炎症により微小循環障害が遷延しており，これが左室リモデリング進行の一因と考えられている．そこで我々は，ブタ急性心筋梗塞モデルを用いて，低出力体外衝撃波治療が，慢性期の左室リモデリングを抑制するか検討した．急性心筋梗塞作成急性期に梗塞境界領域に対して低出力の衝撃波を照射し，4週間後に評価を行ったところ，左室リモデリングが軽減されることが確認された[5,6]．さらに，梗塞巣周囲の毛細血管数の増加していたことから，急性期の低出力体外衝撃波治療により境界領域での血管新生が促進されて梗塞サイズの拡大が抑制されたと考えられた．この結果を基に，2007年から急性心筋梗塞に対



（文献4より改変）

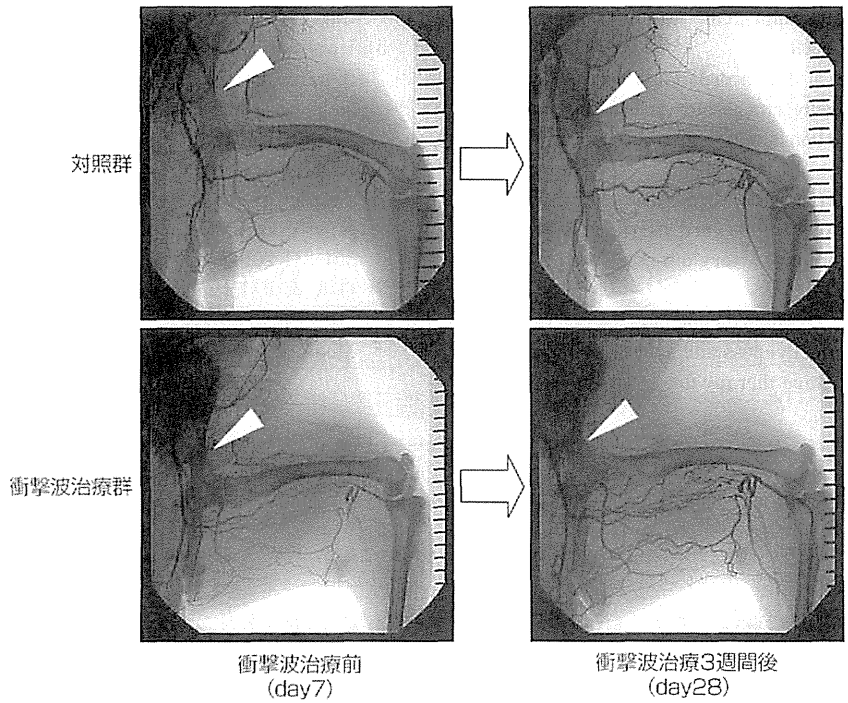
図4. 狭心症に対する第2次臨床試験の結果：(A) CCS分類による狭心症の重症度，(B) ニトログリセリンの使用頻度，(C) 6分間歩行距離，(D) MRIで測定した左室一回拍出量，(E) MRIで測定した左室駆出率，(F) MRIで測定した左室拡張末期容積

自覚症状の改善（A, B），運動耐用能の改善（C），心機能の改善（D, E）を認めた．

する体外衝撃波治療の臨床試験を行っている．この試験では，PCIによる再灌流療法が成功した症例に対して，心筋梗塞発症72時間以内に衝撃波治療を開始し，1〜2日おきに計3回行い，慢性期の左室リモデリングや心不全発症の抑制効果を評価する．

4. 下肢閉塞性動脈硬化症に対する低出力体外衝撃波治療

我々は，ウサギの大腿動脈〜膝窩動脈を摘出することにより下肢虚血を作成し，下肢虚血作成1週間後に低出力体外衝撃波治療を行った．治療3週間後に評価を行った結果，対照群に比して，低出力体外衝撃波治療群では虚血肢における側副血行路の発達が促進され，血管造影上の血流スコアや下肢血圧の改善効果を認めた（図5）[7]．これらの結果を基に，2007年から，間歇性跛行症状を有する慢性閉塞性動脈硬化症症例を対象に，低出力体外衝撃波治療の臨床試験を行っている．



(文献5より改変)

図5. 下肢虚血に対する体外衝撃波治療の効果
体外衝撃波治療群（下段）では，対照群（上段）に比して，虚血肢における側副血行路の発達が促進された．

おわりに

我々が開発している低出力体外衝撃波治療は極めて低侵襲であり，血管新生を促進することにより組織灌流を改善することが期待される新しい治療法である．本治療法の着想や至適治療条件の決定，動物実験および臨床試験などの先駆的な研究は，すべて我々がトップランナーとして，世界で最初に論文発表を行ってきた．狭心症に対する低出力体外衝撃波治療については，欧州などで医療機器として認可され，既に世界10カ国以上で500名以上の狭心症症例の治療に用いられている．我が国においては，厚生労働省の高度医療評価制度へ申請中である．近年，整形外科領域でも肘や肩，足底等の疼痛治療や難治性の骨折の治療にも低出力の衝撃波を用いた治療の有効性が報告されており，今後，幅広い疾患への応用が期待される．

本治療法の開発は，厚生労働科学研究費補助金を得て行っており，平成21年度には，研究課題「低侵襲性体外衝撃波治療法の実用化を目指したエビデンス確立のための拠点形成」が厚生労働省の先端医療開発特区（スーパー特区）に採択された．現在，幅広い疾患への適応拡大を目指して，様々な専門領域の研究者との共同研究による基礎研究および臨床試験を進めている．治療法の詳細並びに最新情報に関しては，以下のホームページを参照されたい（http://www.cardio.med.tohoku.ac.jp/shockwave/index.html）．

文　献

1) Ito K, et al: Extracorporeal shock wave therapy as a new