ingly elderly AMI patients.[17]

Insufficient Control of Coronary Risk Factors

The WHO-MONICA studies, as well as the Japanese epidemiological studies, have previously shown that the risk of cardiovascular diseases increases with clustering of risk factors, such as hypertension, hyperlipidemia and diabetes mellitus.[18–20] The present study demonstrates that the control of major coronary risk factors is still insufficient in Japan (Figure 3), which could largely account for the increasing incidence of AMI. The westernization of lifestyle and the high rate of aging in Japan are apparent causative factors for the trend. Furthermore, the prevalence of smoking still remains high at ~40% in male patients with AMI, although it has been reported that the smoking rate has declined by 20% in the general Japanese population.[21,22]

Higher Risk for Females for In-Hospital Mortality of AMI

One of the important findings in the present study is that the in-hospital mortality still remains relatively higher for female patients than for male patients (Figure 5). A similar trend has been reported from the American Heart Association Heart Disease and Stroke Statistics.[23] Several factors could be involved in the sex difference in in-hospital mortality, including higher age, longer time elapsed from onset to hospitalization, and low prevalence of PCI in female AMI patients. Indeed, in the present study, the average age of the female patients was 10 years older than that of the male patients. The older age of female patients at the time of admission may further limit the use of several therapies,[24] which could have been the case in the present study. In addition, the incidence of death from procedural complications, such as vascular and hemorrhagic complications, is greater in females.[25] Thus, more attention should be paid to these factors when treating female AMI patients.

Unchanged Time of Onset and Infarct Site

It has been repeatedly demonstrated that the onset of AMI peaks early in the morning in both Japan[26] and Western countries.[27,28] The present study not only confirmed this point but also demonstrated that such a tendency has remained unchanged for the past 30 years in Japan (Figure 4). These results suggest that the triggering mechanism(s) for AMI has remained unchanged despite the increasing incidence of the disease.

The present study also demonstrated that the AMI site has unchanged in the last 30 years. Although anterior AMI is associated with worse outcome, as compared with inferior AMI,[29] the present result indicates that the improvement of morality is likely to be related to factors other than the AMI site.

Improvement of Critical Care and In-Hospital Care for AMI

The present study demonstrated the overall in-hospital mortality (age-adjusted) has significantly reduced from ~20% in 1979 to 12.2% in 2008. The duration of hospital stay was also significantly shortened over the past 30 years (Figure 8), during which the paradigm of AMI management has shifted from a conservative strategy to an interventional strategy.[30] In fact, in the present study, use of primary PCI has been increasing from 20% in 1992 to ~80% in 2008 (Figure 6), and in-hospital mortality was lower in patients who underwent primary PCI than in those who did not. The progress in reperfusion therapy, especially that of primary PCI, appears to have contributed to the reduction in in-hospital mortality and hospital stay, as previously reported from this registry.[8,9]

Currently, approximately half of AMI patients in the Western countries are transported to hospital by ambulance.[31,32] The present study demonstrated the ambulance use in Japan has increased to ~70% in the past 10 years (Figure 5). Because the majority of AMI patients in the past 30 years were hospitalized within 6 h (Figure 7), the increased use of ambulances may not have directly contributed to the shortened interval from onset of symptoms to hospitalization. However, the increased use of ambulances should have resulted in increased use of primary PCI with a resultant improvement in the in-hospital mortality.

The increasing incidence of, but decreasing in-hospital mortality from, AMI in Japan may have resulted from the recent increase in the number of patients with ischemic heart failure, as reported in the Chronic Heart Failure Analysis and Registry in the Tohoku District (CHART) registry study.[33] For surviving AMI patients, it is important to understand the underlying risk factors that lead to secondary cardiac events.[34] Indeed, a more effective strategy to improve the management of post-infarction heart failure needs to be developed.[33,34]

Conclusions

Our MIYAGI-AMI Registry Study demonstrates that over the past 30 years in Japan, there has been a steady trend of increasing incidence, but decreasing mortality, for AMI in the Japanese population, although female patients are still at higher risk for in-hospital mortality than male patients, a result in which both positive (eg, increased use of ambulance and primary PCI) and negative factors (eg, insufficient control of coronary risk factors and aging of the whole society) may be involved.

Acknowledgments

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Appendix 1

List of Participating Hospitals

Fukaya Hospital, Hiroshi Akiho, MD; Hikarigaoka Spellman Hospital, Tomofumi Mimata, MD; Ishinomaki Municipal Hospital, Kenjirou Akai, MD; Ishinomaki Red-Cross Hospital, Hiroyasu Sukegawa, MD; JR Sendai Hospital, Masao Kuroha, MD; Katta General Hospital, Hiroyuki Kanno, MD; Kesen-numa Hospital, Kazunori Ogata, MD; Kurihara Central Hospital, Seiji Komatsu, MD; Tohoku Rosai Hospital, Tatsuya Komaru, MD; Marumori National Health Insurance Hospital, Masataka Otomo, MD; Miyagi Eastern Cardiovascular Institute, Toru Naganuma, MD; Miyagi Cancer Center, Nobuo Tomisawa, MD; Miyagi Cardiovascular and Respiratory Center, Noboru Osawa, MD; Mori Hospital, Akio Mori, MD; Nagamachi Hospital, Hidetoshi Mitobe, MD; Nishitaga National Hospital, Shigenori Kitaoka, MD; NTT EAST Tohoku Hospital, Aki Yamada, MD; Oizumi Memorial Hospital, Yoshirou Koiwa, MD; Osaki Citizen Hospital, Tetsuya Hiramoto, MD; Saito Hospital, Keiji Otsuka, MD; Saka General Hospital, Atsushi Obata, MD; Sanuma Municipal General Hospital, Hiroshi Ishii, MD; Sendai Cardiovascular Center, Shin-ya Fujii, MD; Sendai City Hospital, Tetsuo Yagi, MD; Sendai Kosei Hospital, Taiichirou Meguro, MD; Sendai Medical Center, Tsuyoshi Shinozaki, MD; Sendai Open Hospital, Masaharu Kanazawa, MD; Sendai Public Health Insurance Hospital, Yoshichika Oikawa, MD; Sendai Red-Cross Hospital, Yuji Konno, MD; Sendai Tokushukai Hospital, Kimihiko Ogata, MD; Sen-en General Hospital, Ryouichi Hashiguchi, MD; Shichigashuku National Health Insurance Clinic, Takahiro Nagashima, MD; Shiogama City Hospital, Jun Goto, MD; South Miyagi Medical Center, Kan-ichi Inoue, MD; Tohoku Kosai Hospital, Mitsumasa Fukuchi, MD; Tohoku University Hospital, Department of Cardiovascular Medicine, Hiroaki Shimokawa, MD; Department of Cardiovascular Surgery, Kouichi Tabayashi, MD; Department of Gastroenterology, Toru Shimosegawa, MD; Tohoku Welfare and Pension Hospital, Yoshiaki Katahira, MD; Tome Public Hospital, Munehiko Ishii, MD.

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RAPID COMMUNICATION

Double-Blind and Placebo-Controlled Study of the Effectiveness and Safety of Extracorporeal Cardiac Shock Wave Therapy for Severe Angina Pectoris

Yoku Kikuchi, MD; Kenta Ito, MD; Yoshitaka Ito, MD; Takashi Shiroto, MD; Ryuji Tsuburaya, MD; Kentaro Aizawa, MD; Kiyotaka Hao, MD; Yoshihiro Fukumoto, MD; Jun Takahashi, MD; Morihiko Takeda, MD; Masaharu Nakayama, MD; Satoshi Yasuda, MD; Shinichi Kuriyama, MD*; Ichiro Tsuji, MD*; Hiroaki Shimokawa, MD

Background: Low-energy shock wave (SW) therapy has improved myocardial ischemia in both a porcine model and in patients with severe angina pectoris.

Methods and Results: To further confirm the effectiveness and safety of SW therapy, 8 patients with severe angina pectoris were treated with SW therapy in a double-blind, placebo-controlled and cross-over manner. SW therapy, but not placebo, significantly improved chest pain symptoms and cardiac function without any complications or adverse effects.

Conclusions: Extracorporeal cardiac SW therapy is an effective, safe and non-invasive therapeutic option for severe angina pectoris. (*Circ J* 2010; **74:** 589–591)

Key Words: Angina pectoris; Angiogenesis; Myocardial ischemia; Shock wave

The number of patients with severe angina pectoris without indications for coronary artery bypass grafting (CABG) or percutaneous coronary intervention (PCI) is rapidly increasing worldwide and their prognosis still remains poor.[1,2] Thus, it is crucial to develop new therapeutic strategies for these patients. We have previously demonstrated that extracorporeal cardiac shock wave (SW) therapy with low-energy SW (≈10% of the energy density used for urolithiasis) ameliorates myocardial ischemia and dysfunction in a porcine model of chronic myocardial ischemia in vivo.[3,4] We subsequently demonstrated in an open trial that our SW therapy effectively improved chest pain symptoms and exercise tolerance without any adverse effects in 9 patients with severe angina pectoris.[3,5] In the present study, to further confirm the effectiveness and safety of our SW therapy, we performed a double-blind placebo-controlled trial in patients with severe angina pectoris.

Methods

We enrolled 8 consecutive patients with severe angina pectoris who already had undergone CABG or PCI, but who no longer had further indications for these therapies even though they still suffered from stable effort angina under intensive medication (M/F, 5/3; age, 70±3 years) (Table).

The patients were treated with one series of placebo and the SW therapy in a double-blind and cross-over manner with an interval of 3 months. One series of therapy comprised 3 sessions per week. Throughout the study, the patient and the doctor in charge were not informed of the type of therapy. We performed the SW therapy (200 shoots/spot at 0.09 mJ/mm^2 for 40–60 spots per session; Modulith SLC, Storz Medical, Kreuzlingen, Switzerland) as described previously.[3,5] As placebo, the patients underwent the procedure of SW therapy but without irradiation. The patients were followed-up for 3 months after completion of the therapy. We evaluated symptoms using the Canadian Cardiovascular Society (CCS) class score, the patient's requirement for nitroglycerin,[5] exercise tolerance in a 6-min walk, and a cardiopulmonary exercise test, and cardiac function assessed by MRI (Achieva 1.5 T, Philips, Eindhoven, Netherlands). The left ventricular ejection fraction (LVEF) was measured using contiguous short-axis slices obtained by cine MRI; end-diastolic and end-systolic endocardial traces were used to determine end-diastolic and end-systolic left ventricular (LV) volumes, respectively. We also evaluated the number of circulating progenitor cells in peripheral blood by FACS analysis 2 days before the 1st session and 1 h after the 3rd session in 7 of the 8 patients

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Table. Baseline Characteristics of the 8 Patients With Severe Angina Pectoris

Patient no.	Age (years)	Gender	CAD	Previous treatment	OMI	ASO	HT	DM	HL
1	69	M	3VD	CABG, PCI	+	+	+	+	+
2	61	M	3VD	CABG, PCI	+	−	+	+	+
3	70	M	3VD	CABG	+	+	+	−	+
4	78	F	3VD	CABG, PCI	+	−	+	−	+
5	80	M	3VD	CABG, PCI	+	−	+	+	+
6	60	F	3VD	CABG, PCI	+	−	+	+	+
7	72	F	3VD	CABG, PCI	+	−	−	−	+
8	70	M	3VD	CABG, PCI	+	+	+	+	+

CAD, coronary artery disease; OMI, old myocardial infarction; ASO, arteriosclerosis obliterans; HT, hypertension; DM, diabetes mellitus; HL, hyperlipidemia; VD, vessel disease; CABG, coronary artery bypass grafting; PCI, percutaneous coronary intervention.

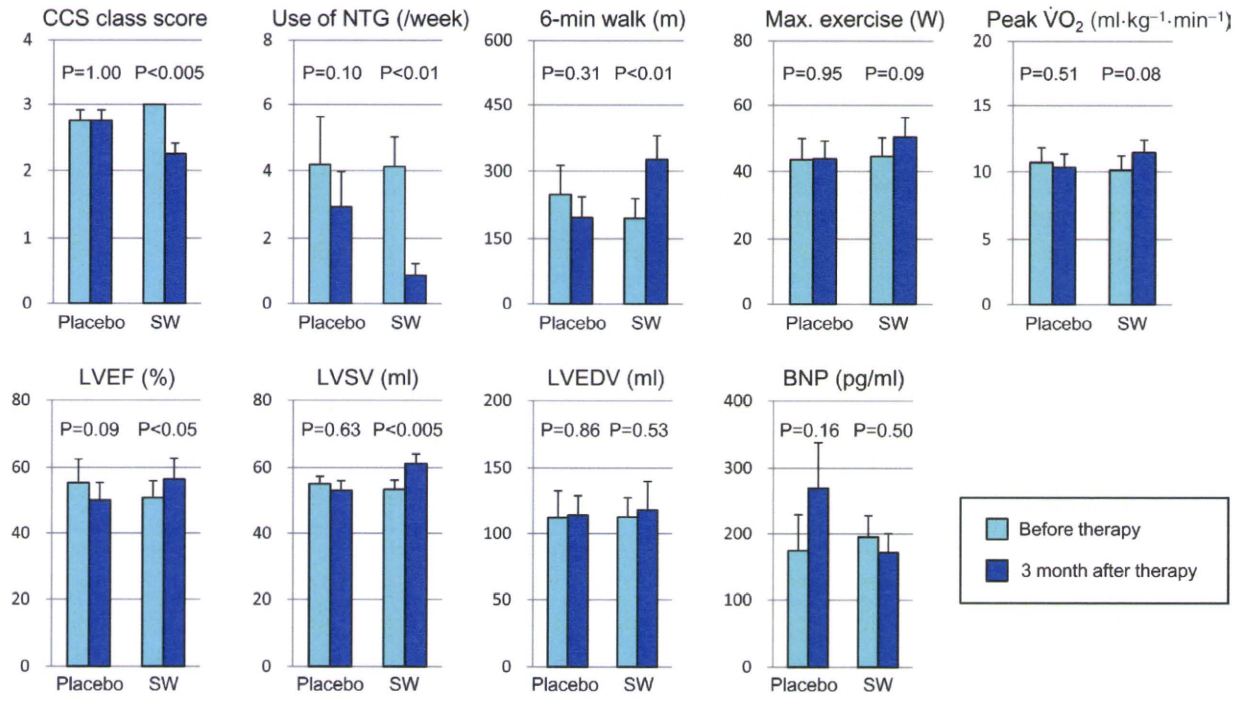

Figure. Effects of shock wave (SW) therapy. Results are mean ± SE (n=8 each). CCS, Canadian Cardiovascular Society; NTG, nitroglycerin; Max. exercise, maximum exercise capacity in watts (W); Peak $\dot{V}O_2$, peak oxygen uptake; LVEF, left ventricular (LV) ejection fraction; LVSV, LV stroke volume; LVEDV, LV end-diastolic volume; BNP, brain natriuretic peptide.

(technical problem in the remaining one patient).

The present study was approved by the Ethical Committees of Tohoku University in 2005, and informed consent was given by each patient.

Results are expressed as mean ± SEM. Comparisons during the time course after SW therapy were made by repeated measure ANOVA followed by Bonferroni/Dunn post hoc test. All statistical analyses were performed using StatView (SAS Institute, Cary, NC, USA), and $P<0.05$ was considered to be statistically significant.

Results

The SW therapy, but not placebo, significantly improved symptoms (CCS class score) and nitroglycerin use (Figure). The SW therapy also significantly improved the 6-min walking distance and tended to improve both maximum exercise capacity and peak oxygen uptake (peak $\dot{V}O_2$). LVEF and LV stroke volume evaluated by MRI were significantly improved only with the SW therapy, although LV end-diastolic volume and plasma brain natriuretic peptide level remained unchanged. The number of $CD34^+/KDR^+$ and $CD34^+/KDR^+/c\text{-}kit^+$ cells in peripheral blood also remained unchanged with both therapies (data not shown). No procedural complications or adverse effects were noted during or after either therapy as in the previous studies.[3–7]

Discussion

We have previously demonstrated that low-energy SW

therapy enhanced angiogenesis and improved myocardial ischemia in a pig model of chronic myocardial ischemia,[3,4] and that SW therapy improved the symptoms and myocardial perfusion in patients with severe angina pectoris in an open trial.[3,5] The present double-blind and placebo-controlled study further demonstrates that our extracorporeal cardiac SW therapy is an effective therapeutic option for severe angina pectoris, providing convincing evidence for its effectiveness and safety.

During the past 2 decades, regenerative therapies using genes, cytokines, and progenitor cells have been under investigation for ischemic cardiovascular diseases.[8] However, these therapies have not been consistently effective in humans, despite promising results in early preclinical studies.[9–12] A potential explanation for these inconsistent results is the complex crosstalk among multiple pathways, in which enhancement of only 1 factor among numerous angiogenic factors may not be enough to achieve clinical benefit. Furthermore, animal studies of cell therapy have revealed that the number of newly generated vascular cells is too low to induce any functional improvement, suggesting that the paracrine action of transplanted cells stimulates intrinsic angiogenic capacity.[13] In contrast, low-energy SW upregulates multiple angiogenic pathways (eg, VEGF, flt-1, SDF-1, and nitric oxide synthase).[4,7,14]

There are several limitations to the present study. First, the number of patients is small. Although more than 150 patients with severe angina pectoris were reviewed as potential candidates for this study, most of them were excluded due to insufficient midication, potential indications of CABG or PCI, and co-existence of malignant tumor. However, we were able to reconfirm the beneficial effects of SW therapy in the present double-blind and placebo-control study, as we had observed in a previous open study.[5] A future large-scale trial would validate the present results. Second, maximum exercise capacity and $p\dot{V}O_2$ were not significantly improved while the symptoms and 6-min walking distance were significantly improved by the SW therapy. In 6 of the 8 patients, exercise was stopped because of leg pain or fatigue before reaching the anaerobic threshold. Thus, exercise tolerance might have been underestimated because of arteriosclerosis obliterans and/or physical deconditioning. Another parameter, such as arteriovenous difference in lactate concentration under overdrive pacing, might have been a better index of ischemia. Third, the number of circulating progenitor cells in peripheral blood was not increased in the present study. Thus, it remains to be examined whether our SW therapy promotes recruitment of bone marrow-derived cells by the ischemic myocardium of humans.[14,15] Fourth, the detailed molecular mechanisms of the beneficial effects of SW in humans remain to be clarified in future studies.[3–7]

In conclusion, the present double-blind, placebo-controlled study further confirmed the effectiveness and safety of our extracorporeal cardiac SW therapy for the treatment of severe angina pectoris, although large-scale multi-center study is needed.

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EDITORIAL

Potential usefulness of fish oil in the primary prevention of acute coronary syndrome

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This editorial refers to 'Fish intake and acute coronary syndrome'†, by L.J. Bjerregaard *et al.* on page 29

Acute coronary syndrome (ACS) is a major health problem and leads to a large number of hospitalizations annually in Europe.[1] ACS refers to a group of clinical conditions caused by myocardial ischaemia, including unstable angina pectoris, non-ST-segment elevation myocardial infarction (NSTEMI), ST-segment elevation myocardial infarction (STEMI), and sudden cardiac death. ACS results from a common underlying pathophysiological mechanism, i.e. plaques vulnerable to rupture or erosion, with different degrees of superimposed thrombosis and distal embolization. Vulnerable plaques that are likely to rupture or erode have evidence of inflammation, together with thin fibrous caps and large lipid cores. The platelet-rich thrombus, developed in association with pro-coagulant-vulnerable blood, can release vasoconstrictor substances such as serotonin and thromboxane A_2 that may induce vasoconstriction at the site of plaque rupture or in the microcirculation.

Bjerregaard *et al.* followed-up 25 573 men and 28 653 women in the large cohort of the Danish health system for 7.6 years.[2] During that period, ACS developed in a total of 1122 cases, among which the number of 'non-fatal' events was much higher than that of 'fatal' events ($n = 175$). A significant association was found between the intake of fatty fish and the incidence of ACS in men, whereas in women only a trend was seen in the highest quintile. A high consumption of fish or long-chain omega (*n*)-3 polyunsaturated fatty acids (*n*-3 PUFAs) rich in fish oil is thought to be associated with a reduction in deaths from coronary artery diseases, i.e. fatal myocardial infarction and sudden cardiac death.[3] Although there has been debate as to whether *n*-3 PUFAs may reduce 'non-fatal' cardiovascular events, the study by Bjerregaard *et al.* may provide a clue to this important issue.

n-3 PUFAs possess several beneficial effects on the pathological processes of ACS, including reduction in blood pressure and plasma levels of triglyceride, inhibition of thrombus formation and inflammation, and stimulation of endothelial production of nitric oxide.[4,5] These pleiotropic effects of *n*-3 PUFAs beyond their lipid-lowering effect (*Figure 1*) may not only prevent the development of atherosclerotic plaques but also stabilize them, as reported in an experimental study using Apo-E deficient mice[6] and in a clinical study using samples obtained at carotid endarterectomy.[7] A recent study using multidetector row computed tomography demonstrated a significant correlation between serum *n*-3 PUFA levels and the extent of coronary soft plaques and calcification in Japanese patients with ACS.[8] Even after partial or complete occlusion of the coronary artery, *n*-3 PUFAs could attenuate myocardial ischaemia/reperfusion injury, through mechanisms mediated in part by the opening of K(Ca) channels and nitric oxide in rabbits.[9] Importantly, fish oil or *n*-3 PUFAs have unique feature of suppression of arrhythmias

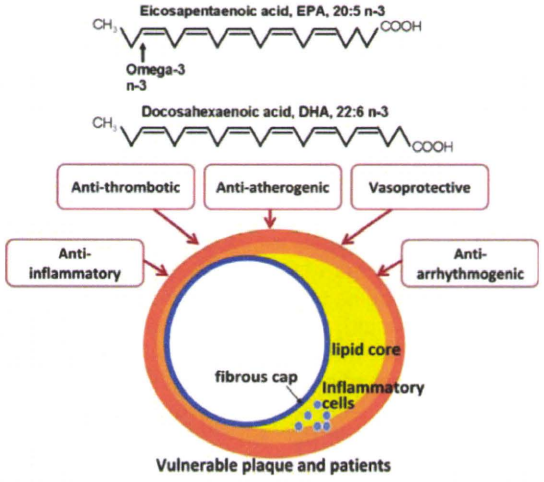

Figure 1 Pleiotropic effects of (*n*)-3 polyunsaturated fatty acids (*n*-3 PUFAs), which are beneficial for vulnerable plaque and patients who develop acute coronary syndrome (ACS).

The opinions expressed in this article are not necessarily those of the Editors of the *European Heart Journal* or of the European Society of Cardiology.

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through several actions on the ionic channels that regulate transmembrane action potentials.[10,11]

The study of Bjerregaard *et al.* indicates the potential role of fish oil in the primary prevention of 'non-fatal' cardiovascular events such as ACS.[2] However, several issues remain to be elucidated. First, baseline intake of *n*-3 PUFAs from the sources (fish), which would be associated with the frequency of consumption and the serving sizes of the specific fish, was not precisely assessed. Secondly, no evidence has been found for the effective dosage and combination of fish oil components such as eicosapentaenoic acid and docosahexaenoic acid. Thirdly, males and females appear to differ in their ability to synthesize *n*-3 PUFA with a resultant difference in their plasma concentrations. These issues remain to be clarified in future studies.

Conflict of interest: none declared.

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Clinical Characteristics and Long-Term Prognosis of Vasospastic Angina Patients Who Survived Out-of-Hospital Cardiac Arrest

Multicenter Registry Study of the Japanese Coronary Spasm Association

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Background—Coronary artery spasm plays an important role in the pathogenesis of ischemic heart disease; however, its role in sudden cardiac death remains to be fully elucidated. We examined the clinical characteristics and outcomes of patients with vasospastic angina in our nationwide multicenter registry by the Japanese Coronary Spasm Association.

Methods and Results—Between September 2007 and December 2008, 1429 patients with VSA (male/female, 1090/339; median, 66 years) were identified. They were characterized by a high prevalence of smoking and included 35 patients who survived out-of-hospital cardiac arrest (OHCA). The OHCA survivors, as compared with the remaining 1394 non-OHCA patients, were characterized by younger age (median, 58 versus 66 years; $P<0.001$) and higher incidence of left anterior descending coronary artery spasm (72% versus 53%, $P<0.05$). In the OHCA survivors, 14 patients underwent implantable cardioverter-defibrillator implantation while intensively treated with calcium channel blockers. Survival rate free from major adverse cardiac events was significantly lower in the OHCA survivors compared with the non-OHCA patients (72% versus 92% at 5 years, $P<0.001$), including appropriate ICD shocks for ventricular fibrillation in 2 patients. Multivariable analysis revealed that OHCA events were significantly correlated with major adverse cardiac events (hazard ratio, 3.25; 95% confidence interval, 1.39 to 7.61; $P<0.01$).

Conclusions—These results from the largest vasospastic angina cohort indicate that vasospasm patients who survived OHCA are high-risk population. Further studies are needed to determine whether implantable cardioverter-defibrillator therapy improves patient prognosis. **(*Circ Arrhythm Electrophysiol.* 2011;4:00-00.)**

Key Words: acetylcholine ■ angina ■ arrhythmia ■ prognosis ■ vasospasm

Out-of-hospital cardiac arrest (OHCA) is a major public health problem. Its estimated number is 300 000 to 400 000 per year in the United States.[1] A prospective study showed an incidence of 53 in 100 000 per year, with 25% of victims being younger than 65 years.[2] Causes of OHCA are strongly associated with coronary artery disease as evidenced at autopsy, and the survival rate from OHCA still remains to be substantially improved.[1] Importantly, a significant number of OHCA cases remained unexplained if victims have no structural abnormalities (eg, organic coronary stenosis) in the postmortem analysis.[3] This finding strongly suggests that functional abnormalities of the coronary artery are also involved in the pathogenesis of OHCA.[4]

Clinical Perspective on p ●●●

Recently, the prevalence of early access to emergency medical service, early bystander cardiopulmonary resuscitation, and early defibrillation has been increasing, with a resultant improvement of the survival rate from OHCA.[5–7] The progress of the chain of survival now opens the window to elucidate the underlying mechanisms of patients who survived OHCA. Coronary artery spasm plays an important

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The online-only Data Supplement is available at http://circep.ahajournals.org/cgi/content/full/CIRCEP.110.959809/DC1.

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role in the pathogenesis of a wide variety of ischemic heart disease, including sudden cardiac death, and thus could be one of the most important functional abnormalities of the coronary artery.[8–10] However, little is known about the clinical characteristics including sex difference and long-term prognosis of patients with vasospastic angina (VSA) who survived OHCA, except for the previous single-center studies with a small number of patients.[11,12]

In the present study, we thus conducted the nationwide multicenter registry study with the large patient number by the Japanese Coronary Spasm Association to elucidate the clinical characteristics and long-term prognosis of VSA patients, especially those who survived OHCA.

Methods

The Japanese Coronary Spasm Association was founded in 2006, and currently 68 institutes participate. The present study was approved by the institutional review boards or ethics committees of all participating institutions.

Study Patients

All VSA patients were referred or admitted to the participating institutes and were originally diagnosed between April 1, 2003, and December 31, 2008. The registration was made between September 1, 2007, and December 31, 2008. In the present study, data collection was conducted in a retrospective fashion for patients seen before September 2007 and in a prospective manner for those seen after that date. The diagnosis of VSA was made based on the Guidelines for Diagnosis and Treatment of Patients with Vasospastic Angina of the Japanese Circulation Society.[13] The definition of VSA included an angina attack at rest and/or on effort, accompanied by a transient ECG ST-segment elevation or depression of >0.1 mV or a newly appearance of negative U wave in at least 2 related leads, and/or a total or subtotal coronary artery narrowing during the provocation test of coronary spasm, accompanied by chest pain and/or ischemic ECG changes mentioned above.

Data Collection

The demographic and clinical data were submitted to a central data base, including age, sex, coronary risk factors, family history, type of angina episodes, circadian distribution of angina attacks, leads of ST-segment elevation or depression and arrhythmias during spontaneous or provoked angina attack, location of coronary spasm, device therapy such as implantable cardioverter-defibrillator (ICD), medical therapy, and its adherence. We defined reduction and discontinuation of medication as having a gap in use of any medication and no use of medication, respectively. Hypertension, dyslipidemia, and diabetes mellitus were diagnosed on the basis of guidelines of the Japanese Society of Hypertension, Japan Atherosclerosis Society and the Japan Diabetes Society, respectively.[14–16] Significant coronary stenosis was defined as >50% of luminal narrowing of major coronary arteries evaluated by coronary angiography. OHCA was defined as the cessation of cardiac mechanical activity as confirmed by the absence of signs of circulation.[17]

End Points

The primary end point was major adverse cardiac events (MACE), including cardiac death, nonfatal myocardial infarction, hospitalization for unstable angina pectoris and heart failure, and appropriate ICD shocks during the follow-up period, which began at the date of original VSA diagnosis. The secondary end point was all-cause mortality. Cardiac death was defined as sudden death (ie, death occurring unexpectedly without any apparent symptoms or within 1 hour of symptom onset or nonwitnessed death in the absence of any other possible cause) or death associated with acute myocardial infarction. Acute myocardial infarction was defined in patients with prolonged (>30 minutes) chest pain, associated with ST-segment changes and elevated levels of cardiac enzymes. Unstable angina pectoris was diagnosed if chest discomfort or pain became recurrent or worsening along with ischemic ECG changes. Heart failure was diagnosed if a patient showed signs of exertional dyspnea, orthopnea, rales in more than one-third of the lung fields, elevated jugular venous pressure, or pulmonary congestion on chest radiography related to cardiac dysfunction.

Statistics

Continuous variables are presented as medians and interquartile ranges and categorical variables as percentages. Group comparisons were performed with Mann-Whitney test for continuous variables, Fisher exact test for categorical variables, and log-rank test for survival curves. Survival free from death and MACE was analyzed by the Kaplan–Meier method. Multivariable analysis of correlated factors of MACE was performed with a Cox proportional hazard model. Variables depicted by univariable analysis to be correlated with MACE and well-known predictive variables were subjected to the forced entry method. The proportional hazards assumption was examined with the log minus log plot. Hazard ratio and 95% confidence intervals were also calculated. A value of $P<0.05$ was considered to be statistically significant.

Results

Clinical Characteristics of Patients With VSA

Among a total of 1528 VSA patients registered from 47 institutes, 99 patients were excluded because they did not meet the diagnostic criteria (n=7) or the inclusion criteria (n=92). Finally, 1429 patients were studied (online-only Data Supplement Figure 1). The clinical characteristics of those patients are summarized in Table 1. Among the coronary risk factors, smoking was observed most frequently (≈60%), especially in male patients. The prevalence of family history of ischemic heart diseases, previous myocardial infarction, and the existence of organic coronary stenosis was relatively low (≈10%). When compared with the female patients, the male patients were characterized by younger age, higher incidences of previous myocardial infarction, organic coronary stenosis, angina attack with ST-segment elevation, and lower incidence of family history of ischemic heart diseases. In contrast, no sex difference was noted in the prevalence of arrhythmia during spontaneous attacks, including OHCA (Table 1). Among the 1317 patients in whom ECG was recorded during spontaneous attack, significant ST-segment elevation and depression was documented in 272 and 121 patients, respectively. T1,AQ:4

Among the registered patients except for 169 patients (12%, not recorded), angina attacks occurred exclusively at rest in 634 patients (44%), whereas it occurred predominantly at rest but was also induced by effort in 513 patients (36%). In 113 patients (8%), angina attacks were induced only by effort. In 658 patients, typical circadian pattern was identified mostly from midnight to early morning as follows; from midnight to 4 AM (n=160), 4 AM to 8 AM (n=377), 8 AM to noon (n=122), noon to 4 pm (n=36), 4 pm to 8 pm (n=40) and 8 pm to midnight (n=66).

The provocation test was performed during coronary angiography in 1244 patients with either acetylcholine (n=713, 57.3%), ergonovine (n=497, 40.0%), both (n=23, 1.8%), or others (eg, hyperventilation) (n=11, 0.9%). The prevalence of arrhythmic events during provocation test (n=85, 6.8%)

Table 1. Demographic Characteristics of VSA Patients

	Overall	Men	Women	*P* Value
No. of patients, n (%)	1429 (100)	1090 (76)	339 (24)	<0.001
Age, median (IQR), y	66 (58–73)	66 (58–72)	69 (60–75)	<0.001
Coronary risk factor, n (%)				
Hypertension	666 (47)	511 (47)	155 (46)	0.38
Dyslipidemia	647 (45)	481 (44)	166 (49)	0.07
Diabetes mellitus	233 (16)	186 (17)	47 (14)	0.09
Smoking	848 (59)	781 (72)	67 (20)	<0.001
Family history of IHD, n (%)	168 (12)	118 (11)	50 (15)	0.033
Previous MI, n (%)	91 (6)	81 (7)	10 (3)	0.003
Organic stenosis >50%, n (%)	201 (14)	170 (16)	31 (9)	0.001
ST-segment change during spontaneous attack, n (%)				
ST-elevation	272 (19)	234 (21)	38 (11)	<0.001
ST-depression	121 (8)	83 (8)	38 (11)	0.027
Arrhythmic event during spontaneous attack, n (%)				
PVC	14 (1)	12 (1)	2 (1)	0.32
VT/VF	17 (1)	14 (2)	3 (1)	0.40
AV block	21 (1)	19 (2)	2 (1)	0.09
Bradycardia/sinus pause	28 (2)	20 (2)	8 (2)	0.34
Out-of-hospital cardiac arrest*	35 (2)	30 (3)	5 (1)	0.13

AV indicates atrioventricular; IHD, ischemic heart disease; IQR, interquartile range; MI, myocardial infarction; PVC, premature ventricular contraction; VF, ventricular fibrillation; VSA, vasospastic angina; and VT, ventricular tachycardia.

*Twenty-six patients (male/female, 23/3 patients) were also complicated by nonfatal VT/VF.

was similar with that during spontaneous attack (n=107, 7.5%) (online-only Data Supplement Table 1).

Medical Treatments

In the present study, 1331 patients (93%) were treated with calcium channel blockers (CCBs), either CCBs alone (48%), or combination of CCBs and long-acting nitrates including nicorandil (45%). Most of the patients (n=1162, 81%) were treated with one type of CCB: delayed- or modified-release formulations of first-generation of CCBs in 814 and the second- and third-generation of CCBs with longer plasma half-lives in 348.[18] Antiplatelet agents were used in 669 patients (47%). However, the use of β-blockers was limited to 61 patients (4%) in the present study.

Prognostic Factors of MACE by Multivariate Analysis

During the median follow-up period of 32 months (interquartile range, 17 to 46 months), 19 patients (1.3%) died, in which 6 patients had cardiac death. MACE occurred in 85 patients (5.9%), including myocardial infarction (n=9), hospitalization for unstable angina (n=68) and heart failure (n=4), and appropriate ICD shocks (n=2). Overall 5-year survival rate free from all cause death or MACE was 98% and 91%, respectively (Figure 1). Especially, 5-year survival rate free from nonfatal myocardial infarction was high (99%). F1

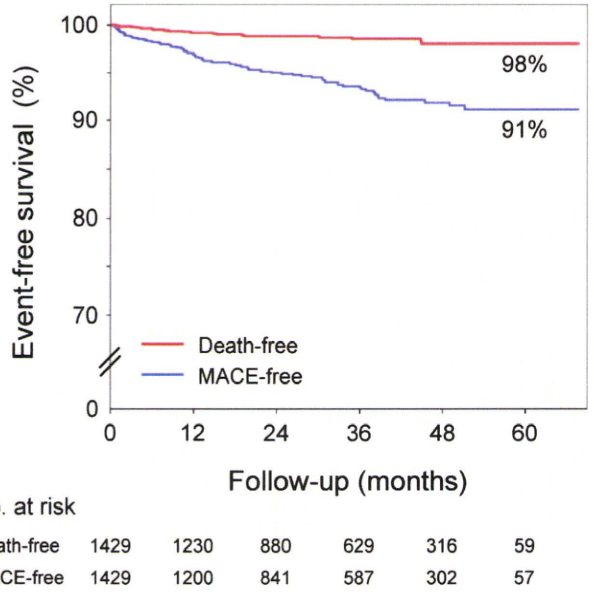

Figure 1. Kaplan–Meier curve for survival (red line) and MACE (blue line) in VSA patients. AQ: 7

Multivariable analysis demonstrated that in addition to the established prognostic factors (smoking, spontaneous attack with ST-segment elevation, multivessel spasm, and significant organic stenosis in major coronary arteries), history of OHCA was significantly correlated with MACE (Table 2). T2 Even when the analysis was limited to the patient without significant coronary stenosis, the survival curve (online-only Data Supplement Figure 2) and the correlated factors were unchanged (online-only Data Supplement Table 2).

Importantly, the rate of cardiac death and nonfatal myocardial infarction in patients in whom medications were reduced or discontinued (8%, 2 of 25 patients) was 10-fold higher than that in the patients with continued medications (0.7%, 10 of 1404 patients, $P=0.017$).

VSA Patients Who Survived Out-of-Hospital Cardiac Arrest Caused by Coronary Artery Spasm

The present study included 35 VSA patients who survived OHCA as their first manifestation of clinical events in 14 institutes, 7 of which had the emergency care department. In these 7 hospitals, coronary artery spasm was documented in 22 patients (6.0%) of 365 patients resuscitated from OHCA of cardiac origin between April 1, 2003, and December 31, 2008. The OHCA survivors with VSA were characterized by younger age and higher incidence of coronary spasm in the left anterior descending coronary artery as compared with the remaining non-OHCA patients (Table 3). However, the prevalence of significant coronary stenosis was comparable between the 2 groups. Appropriate ICD shocks for ventricular fibrillation (VF) were documented in 2 of the 14 patients with ICD implantation during intensive medical treatment. Sudden cardiac death occurred in 1 patient without an ICD who terminated medication himself before the fatal event. Hospitalization was needed because of nonfatal myocardial infarction (n=1) and unstable angina pectoris (n=3). Despite the comparable incidence of all-cause mortality (Figure 2A), T3 F2

Table 2. Correlated Factors for Major Adverse Cardiac Events in VSA Patients

	Univariable Analysis			Multivariable Analysis*		
	HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age	0.99	0.97–1.01	0.38			
Men	1.07	0.64–1.79	0.79			
Hypertension	0.90	0.58–1.38	0.62			
Dyslipidemia	1.17	0.76–1.79	0.48			
Diabetes mellitus	1.57	0.94–2.61	0.09	1.39	0.82–2.34	0.22
Smoking	1.96	1.21–3.19	0.006	1.67	1.02–2.73	0.041
Family history of IHD	1.10	0.58–2.06	0.78			
Previous MI	2.19	1.10–4.38	0.026	1.39	0.64–3.02	0.41
Significant organic stenosis	2.28	1.39–3.73	0.001	2.04	1.21–3.44	0.008
ST-elevation during spontaneous attack	1.62	1.01–2.60	0.045	1.77	1.09–2.87	0.022
VT/VF during spontaneous attack†	1.00	0.14–7.18	1.00			
History of OHCA	3.98	1.73–9.13	0.001	3.25	1.39–7.61	0.007
LAD spasm	1.15	0.75–1.77	0.51			
LCx spasm	0.92	0.55–1.53	0.74			
RCA spasm	1.17	0.76–1.78	0.48			
Multivessel spasm	1.45	0.93–2.26	0.10	1.62	1.03–2.56	0.037
Administration of β-blockers	2.34	1.08–5.06	0.032	1.56	0.64–3.79	0.33

CI indicates confidence interval; HR, hazard ratio; IHD, ischemic heart disease; LAD, left anterior descending artery; LCx, left circumflex artery; MI, myocardial infarction; OHCA, out-of-hospital cardiac arrest; RCA, right coronary artery; VF, ventricular fibrillation; and VT, ventricular tachycardia.

*Analysis was performed on 8 variables including diabetes mellitus, smoking, previous MI, significant organic stenosis, ST-elevation during spontaneous attack, history of OHCA, multivessel spasm, and administration of β-blockers.

†Patients complicated by OHCA were not included.

event-free survival was significantly lower in the OHCA survivors as compared with the non-OHCA patients (72 versus 92% at 5 years, $P<0.001$) (Figure 2B). In subgroup analysis between OHCA survivors who did (n=5) and did not (n=30) have later adverse events, left ventricular ejection fraction and the prevalence of significant coronary stenosis was comparable (online-only Data Supplement Table 3).

Discussion

To the best of our knowledge, the present multicenter study with 1429 patients is the largest cohort of VSA, in which the patients were registered on the basis of standardized criteria by the Japanese Circulation Society.[13] The present study also is characterized by the fact that ≈400 VSA cases with documented spontaneous attacks were included, which enhances the scientific level of the study. In the present study, we were able to demonstrate that VSA patients who survived OHCA are particularly high-risk population, even in the current era with long-acting CCBs.

VSA Patients Who Survived OHCA as a High-Risk Population

In the 2000s, early initiation of cardiopulmonary resuscitation and the widespread use of defibrillation programs have saved many patients with OHCA, making subsequent care of these patients more important than ever.[19] Accumulating evidence indicates that cardiac arrest in the absence of organic heart disease is more common than previously expected.[20] In the autopsy studies in patients with sudden cardiac death, the prevalence of no significant coronary stenosis was higher in Japanese (26%) than in European populations (4%),[21,22] indicating the potential importance of functional coronary abnormalities in the pathogenesis of sudden cardiac death in Japanese. In the present study, coronary spasm was documented in 6% of the patients resuscitated from OHCA of cardiac origin. The prevalence of vasospasm in OHCA patients appeared to be doubled in comparison with that (3%) reported in the previous study participating 4 French emergency units.[20] Because the racial differences may affect the diagnostic and therapeutic strategies (eg, use of the provocation test), the study with Japanese patients should provide important information for better understanding of the pathogenesis of VSA.

In the present study, the incidence of OHCA in VSA patients was 2.4%, which is 50-fold higher than that (0.05%) in the general Japanese population,[23] indicating that VSA patients, especially those who survived OHCA, are high-risk population. As shown in Figure 2B, event-free survival rate in the OHCA survivors was much lower than in the non-OHCA patients. The event of OHCA and worse clinical outcome may not be coincidental but could be explained in part by severe myocardial ischemia caused by left anterior descending artery spasm (Table 3).[24]

The multivariable analysis also demonstrated that prior history of OHCA events was a novel and significant correlated factor of MACE in VSA patients (Table 2). Although life-threatening arrhythmias may be related to increased disease activity of coronary spasm,[25,26] a potential involvement of an arrhythmic substrate in association with ventricular repolarization abnormalities has been suggested in the previous study.[27,28] In patients with variant angina compli-

Table 3. Demographic Characteristics and Angiographic Findings of VSA Patients With and Those Without Out-of-Hospital Cardiac Arrest

	OHCA (n=35)	Non-OHCA (n=1394)	*P* Value
Age, median (IQR), y	58 (44–65)	66 (58–73)	<0.001
Men, n (%)	30 (86)	1060 (76)	0.13
Coronary risk factor, n (%)			
Hypertension	13 (37)	653 (47)	0.17
Dyslipidemia	7 (20)	640 (46)	0.002
Diabetes mellitus	4 (11)	229 (16)	0.30
Smoking	24 (69)	824 (59)	0.17
Family history of IHD, n (%)	3 (9)	165 (12)	0.40
Family history of sudden death, n (%)	0 (0)	19 (1)	0.62
Previous MI, n (%)	5 (14)	86 (6)	0.07
Organic stenosis >50%, n (%)	5 (14)	196 (14)	0.56
LAD	4 (11)	112 (8)	0.32
LCx	1 (3)	62 (4)	0.54
RCA	2 (6)	69 (5)	0.53
ST-segment changes during spontaneous attack, n (%)			
ST-elevation	6 (17)	266 (19)	0.49
ST-depression	5 (14)	116 (8)	0.17
Spasm-positive arteries, n (%)*			
LAD	23 (72)	643 (53)	0.025
LCx	11 (34)	306 (25)	0.17
RCA	17 (53)	676 (56)	0.45
Multivessel	13 (41)	361 (30)	0.13

IQR indicates interquartile range; IHD, ischemic heart disease; MI, myocardial infarction; LAD, left anterior descending artery; LCx, left circumflex artery; OHCA, out-of-hospital cardiac arrest; and RCA, right coronary artery.

*Data analyzing 1244 patients (32 OHCA survivors and 1212 non-OHCA patients) who underwent vasospasm provocation test.

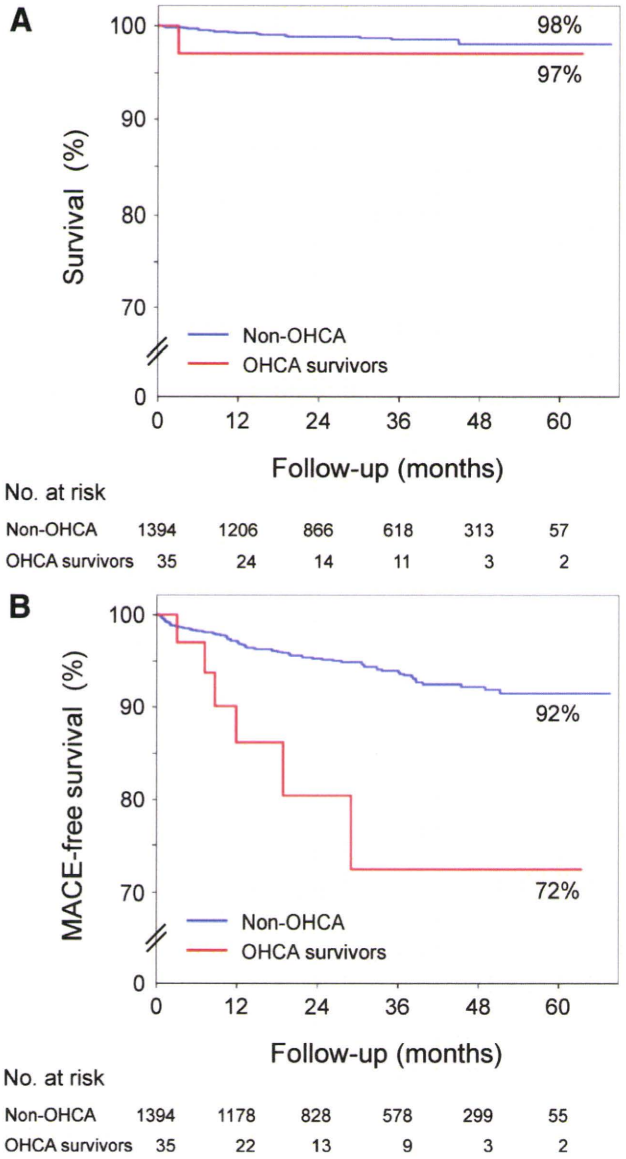

Figure 2. Kaplan–Meier curve for all-cause of death and MACE in VSA patients. **A,** The survival rate was comparable between the VSA patients who survived OHCA (red line, n=35) and those without OHCA (blue line, n=1394) (*P*=0.30). **B,** MACE-free survival was significantly worse in the VSA patients who survived OHCA (red line, n=35) compared with those without OHCA (blue line, n=1394) (*P*<0.001). MACE include cardiac death, nonfatal myocardial infarction, hospitalization for heart failure and unstable angina pectoris, and appropriate ICD shocks.

cated by cardiac arrest, the prevalence of QT dispersion was significantly higher compared with uncomplicated patients.[27] We also have recently reported that OHCA survivors with coronary spasm demonstrated concomitant idiopathic VF, indicating heterogeneity of the underlying mechanisms.[28]

The association of potentially lethal ischemia-induced ventricular arrhythmias may justify the use of an ICD.[29] Meisel et al[12] reported both the efficacy and limitation of ICD therapy in patients with refractory variant angina. In their 7 patients with variant angina complicated by VF, appropriate ICD shocks were documented in 4 patients, but 1 patient died of electromechanical dissociation even under intensive medical treatment with CCBs. However, in the previous studies with a small number of patients (n=6≈7), the prognosis was favorable in survivors of cardiac arrest caused by coronary spasm who did not receive ICD.[10,11] It remains to be examined in a future multicenter study whether ICD therapy can improve the prognosis of OHCA survivors with coronary spasm.

Importance of Continued Medical Treatment for VSA

After withdrawal of CCB, silent myocardial ischemia with fatal arrhythmias[30] and a rebound phenomenon of the spasm[31,32] could occur. In the present study, the Fisher exact test also demonstrated that the incidence of cardiac death and nonfatal myocardial infarction was significantly increased in patients in whom medications were reduced or discontinued. These findings indicate that medications should not be withdrawn carelessly, even if symptomatic attacks appear to be controlled. Although CCBs remain the mainstay of the current clinical practice, it has been reported that 6-month CCB therapy did not completely normalize coronary vasoconstricting responses to acetylcholine despite the absence of symptomatic angina.[33] Even after 1-year CCB therapy, myocardial fatty acid metabolic images assessed using ^{123}I-15-(p-iodophenyl)-3-R,S-methyl pentadecanoic acid have been re-

ported to appear abnormal in VSA patients.[34] These findings suggest that there are also limitations of these classes of drug. Recently, the accumulating evidence demonstrated that small GTPase RhoA and its downstream effector Rho-kinase play a central role by increased Ca^{2+} sensitivity of vascular smooth muscle cells in the molecular mechanism of coronary vasospasm in animal models and VSA patients.[35] The inhibition of Rho-kinase with fasudil has been reported to result in the disappearance of coronary vasospastic activity[36] and is a novel therapeutic option that could target specific abnormalities with a resultant remission of VSA.

Changing Characteristics of VSA Patients

In association with the epidemics of obesity and metabolic syndrome, the general population has been rapidly growing older and the Westernization of lifestyle has been progressing, especially in Japan.[37] Thus, the present nationwide multicenter registry study also focuses on the clinical characteristics and outcomes of VSA patients in the current era of the 2000s.

Coronary spasm was most frequently noted in middle-aged men, who otherwise did not exhibit coronary risk factors except for higher prevalence of smoking. In male VSA patients (Table 1), the prevalence of smoking still remains high (≈70%). The lower incidence of previous myocardial infarction and of organic coronary disease were comparable with the previous report on the clinical characteristics of Japanese patients as compared with Caucasian patients.[38]

Several prognostic studies with a few hundreds of patients were performed in the 1980s. Yasue et al[39] reported that 5-year survival rate free from death or myocardial infarction was 97% and 83% in 245 patients. In general, as reported in the previous comparative study,[38] the prognosis was much worse in a Western population than in a Japanese population.[26,40] In the current era, the clinical outcome of VSA patients appears to be further improved in the 2000s as compared with the 1980s.[26,38–40]

Limitations of the Study

Several limitations should be mentioned for the present study. First, the present study is a retrospective observational study and thus the association found in the present study is not necessarily causal. To address this important issue, we have recently started prospective studies by our Japanese Coronary Spasm Association. Second, the follow-up period was variable, and it is highly possible that many arrhythmic events were missed during the periods of time that the patients were not being monitored. Third, a complex composite primary end point, including ICD shocks, was used in the present study. Appropriate ICD shocks are not certainly a surrogate for sudden cardiac death. Fourth, management decisions were left to the discretion of each attending physician. Fifth, there is no sufficient information available about the date of reduction or discontinuation of medications and thus this variable was not included in the present Cox proportional hazard model. However, despite these limitations, the present findings should merit emphasis for better understanding of the pathogenesis and the long-term prognosis of VSA in the current era.

Conclusions

The present multicenter study by the Japanese Coronary Spasm Association describes the largest cohort of patients with vasospastic angina and a cohort who survived cardiac arrest. Especially, VSA patients who survived OHCA are a high-risk population, and the importance of continued medications should be emphasized.

Acknowledgments

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Disclosures

None.

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CLINICAL PERSPECTIVE

Myocardial ischemia is an important cause of out-of-hospital cardiac arrest (OHCA). Coronary artery spasm is a known cause, but there is limited information about the clinical characteristics and long-term prognosis of patients with vasospastic angina (VSA) who survive OHCA. The present multicenter study by the Japanese Coronary Spasm Association describes a large cohort of 1429 patients with VSA and compares 35 who survived OHCA with those without OHCA. Survival rate free from major adverse cardiac events was significantly lower in the OHCA survivors as compared with the non-OHCA patients, including appropriate implantable cardioverter-defibrillator shocks for ventricular fibrillation in 2 patients. Subgroup analysis of all OHCA cases presenting to 7 hospitals suggests a 6% incidence of VSA in survivors of OHCA from cardiac cause. These results indicate that VSA patients who survived OHCA are a high-risk population. Further studies are needed to determine whether implantable cardioverter-defibrillator therapy improves their prognosis.

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Long-Term Treatment With Eicosapentaenoic Acid Ameliorates Myocardial Ischemia-Reperfusion Injury in Pigs In Vivo

– Involvement of Rho-Kinase Pathway Inhibition –

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Background: Eicosapentaenoic acid (EPA), the major n-3 fatty acid in fish oil, exerts cardioprotective effects against ischemic heart disease; however, the detailed mechanisms remain to be elucidated. Rho-kinase plays an important role in the pathogenesis of cardiovascular diseases including ischemia-reperfusion (I/R) injury. Thus, the hypothesis that long-term EPA treatment ameliorates myocardial I/R injury through Rho-kinase pathway inhibition in pigs in vivo was investigated.

Methods and Results: Male pigs were treated with either a control chow or EPA ($600 \cdot mg \cdot kg^{-1} \cdot day^{-1}$) for 3 weeks (n=8 each) and were subjected to myocardial ischemia by 90-min occlusion of the left circumflex coronary artery and subsequent 60-min reperfusion. The EPA group had an increased EPA level in red blood cells (4.4±0.3mol%). The EPA treatment significantly ameliorated myocardial I/R injury, including regional wall motion abnormality (EPA 5.3±3.6 vs. control 35.1±3.8 unit, P<0.0001), left ventricular ejection fraction (EPA 43±9% vs. control 32±7%, P<0.05), occurrence of ventricular arrhythmias (EPA 181±73 vs. control 389±51 events, P<0.0001) and histological accumulation of inflammatory cells (P<0.01). Importantly, the EPA treatment significantly inhibited myocardial Rho-kinase activity (assessed by the extent of the myosin-binding subunit phosphorylation) (EPA 0.47±0.11 vs. control 0.77±0.14, P<0.05) and preserved myocardial eNOS activity (EPA 0.56±0.13 vs. control 0.23±0.07, P<0.01) with a significant correlation noted between them.

Conclusions: Long-term treatment with EPA ameliorates I/R injury partly through Rho-kinase pathway inhibition in vivo. (*Circ J* 20■■; ■■: ■■■■–■■■■)

Key Words: Eicosapentaenoic acid (EPA); Inflammation; Nitric oxide; Reperfusion

Reperfusion therapy by percutaneous coronary intervention (PCI) reduces infarct size and improves left ventricular (LV) function, with improved clinical outcomes in patients with acute myocardial infarction (AMI).[1] However, reperfusion therapy could also elicit adverse reactions that might limit its beneficial action, leading to irreversible cardiac damage.[2] In order to reduce and/or prevent those adverse reactions, cardioprotective agents are emerging in patients with AMI.

The previous studies demonstrated that high intake of fish oil and n-3 polyunsaturated fatty acids could reduce myocardial infarction and death including sudden cardiac death.[3–7] Eicosapentaenoic acid (EPA), the major component of fish oil, exerts several beneficial effects in the pathological processes of AMI, including inhibition of thrombus formation[8] and inflammation[9] and stimulation of endothelial production of nitric oxide (NO).[10] EPA also inhibits sphingosylphosphorylcholine-induced Rho-kinase activation, leading to inhibition of vasospasm.[11,12]

Rho-kinase has been identified as one of the effectors of the small GTP-binding protein, Rho.[13,14] Rho-kinase is involved in various cellular functions, including not only contraction of

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Table 1. Fatty Acids Composition in the Plasma, Red Blood Cells and the Heart (n=8)

	AA	EPA	AA/EPA	DHA
Plasma				
Control	6.88±1.93	0.38±0.16	17.36±2.39	1.18±0.51
EPA	2.18±0.77*	11.38±3.18*	0.21±0.11*	0.32±0.19*
RBC				
Control	4.74±0.25	0.23±0.06	21.55±6.27	1.62±0.36
EPA	2.68±0.36*	4.47±0.81*	0.79±0.56*	1.05±0.21*
Heart				
Control	14.32±1.13	1.30±0.51	12.24±3.54	1.34±0.38
EPA	6.51±1.69*	14.01±1.79*	0.49±0.20*	0.67±0.18*

Results (mol% to total fatty acids) are expressed as mean±SD. *P<0.01 vs. Control group.
AA, arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; RBC, red blood cells.

Table 2. Hemodynamic Changes During Ischemia and Following Reperfusion

Condition (min)	Pre	Ischemia			Reperfusion	
		10	20	30	0–5	60
HR (/min)						
Control	120±8	137±9*	139±12*	140±11*	132±15*	126±4
EPA	119±20	135±23*	138±23	133±26	127±21	125±22
SBP (mmHg)						
Control	105±6	83±7*	85±8*	80±10*	92±5*	91±6*
EPA	106±11	81±3*	83±2*	86±3*	90±5*	93±10*
DBP (mmHg)						
Control	77±9	68±6*	65±6*	68±11*	64±9*	78±9*
EPA	78±9	67±4*	62±3*,†	65±4*	67±7*	65±8*
MBP (mmHg)						
Control	87±6	73±6*	72±6*	69±6*	76±8*	73±6*
EPA	87±9	72±3*	69±2*,†	72±4*	75±6*	74±9*

Results are expressed as mean±SD. *P<0.05 vs. pre. †P<0.05 vs. Control group.
HR, heart rate; EPA, eicosapentaenoic acid; SBP, systolic blood pressure (BP); DBP, diastolic BP; MBP, mean BP.

vascular smooth muscle cells but also actin cytoskeleton organization, cell adhesion and motility, cytokinesis, and gene expression.[15–17] Rho-kinase also upregulates pro-inflammatory molecules[15] and downregulates endothelial NO synthase (eNOS),[18,19] which might play an important role in the pathogenesis of ischemia-reperfusion (I/R) injury.[20–22]

In the present study, we thus tested our hypothesis that long-term treatment with EPA ameliorates myocardial I/R injury partly through inhibition of the Rho-kinase pathway in pigs in vivo.

Methods

The present study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH 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 of Tohoku University (20MdA-46).

Animals and EPA Treatment

A total of 16 domestic male pigs (2–3 months old and weighing 20–30 kg) were randomly divided into the following 2 groups: 8 pigs were orally given EPA (600 mg·kg^{-1}·day^{-1}; EPA ethyl ester of purity >99%; Mochida Pharmaceutical, Tokyo, Japan) for 21 days (EPA group), and the remaining 8 pigs were fed with a standard chow alone (control group). The present dose and duration of the EPA treatment were determined based on a previous study with rabbits.[23] The fatty acids composition of the plasma, red blood cells (RBC) and homogenized heart tissue extracted from the interventricular septum (100 mg of tissue/ml of saline) was determined by capillary gas chromatography.[24] Total lipids were extracted by Folch's procedure and then fatty acids were methylated with boron trifluoride and methanol, and then methylated fatty acids were analyzed using a gas chromatograph (Shimadzu GC-17A, Shimadzu Corporation, Kyoto, Japan) and a BPX70 capillary column (0.25 mm in internal diameter×30 m in length, SGE International Ltd, Melbourne, Australia).[24] Tricosanoic acid, C23:0 was used as an internal standard.[24]

Porcine Model of Myocardial I/R

After the 3-week treatment, the animals were anesthetized with ketamine hydrochloride (20 mg/kg, IM) and sodium pentobarbital (20 mg/kg, IV). Surface ECG, heart rate and arterial blood pressure were continuously monitored by a polygraph recording system (LEG1000, Nihon-Kohden, Tokyo, Japan). We inserted a 7 Fr sheath into the left carotid artery for cardiac catheterization. A bolus of heparin (5,000 IU) was administered intravenously and 2,000 IU was injected every hour. We performed left ventriculography (LVG) and coronary angiography (CAG) in a left oblique view with the use of a cineangiography system (Toshiba Medical, Tochigi, Japan).[25] LV volume and LV ejection fraction were calculated using

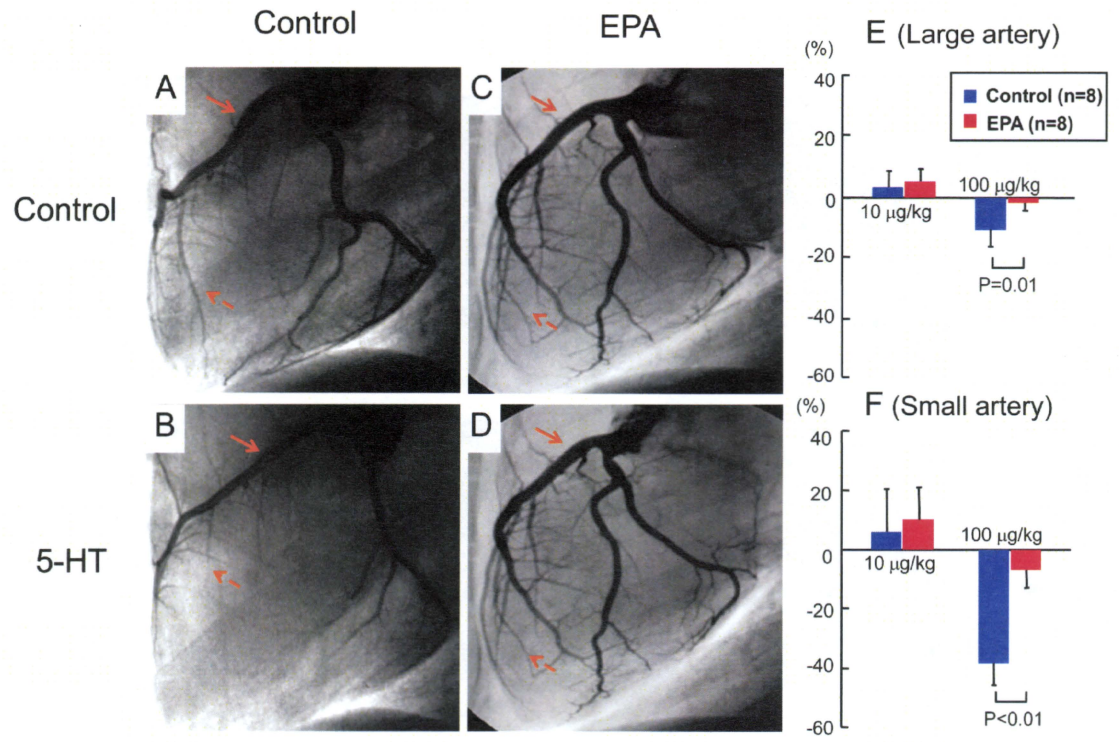

Figure 1. Eicosapentaenoic acid (EPA) treatment suppresses serotonin-induced coronary vasoconstriction. As compared with the baseline coronary angiography (**A**,**C**), a high dose of serotonin (5-HT, 100 μg/kg, IC) caused coronary vasoconstriction in a control pig (**B**), whereas these responses were significantly attenuated in an EPA-treated pig (**D**). The quantitative data shows that these inhibitory effects of EPA were noted in both the large (arrow) (**E**) and small (dashed arrow) segments of the left descending coronary arteries (**F**). Results are expressed as mean±SD (n=8 each).

Simpson's method. The centerline method was used to assess regional LV contractility.[26] As reported previously,[27] coronary vasomotor responses to serotonin (5-HT, 10 and 100 μg/kg, IC) and then endothelium-independent vasodilating responses to nitroglycerin (10 μg/kg, IC) were examined. The measurements were made in a blinded manner for the left anterior descending coronary artery, at both the large (just proximal to the first diagonal branch) and small (distal portion of branch with a baseline diameter of ~500 μm) coronary arteries.[27] A coronary angioplasty balloon (2.5–3.5 mm in diameter depending on the vessel size) was then introduced into the left circumflex coronary artery (LCX) and inflated to induce myocardial ischemia at the lowest pressure that completely occluded distal coronary flow. After 90 min of myocardial ischemia, reperfusion was made by completely deflating the angioplasty balloon for 60 min. Both CAG and LVG were repeated to assess the patency of LCX and the LV wall motion following I/R, respectively. Finally, the animals were euthanized with a lethal dose of sodium pentobarbital (40 mg/kg, IV). The heart tissues were immediately extracted from the interventricular septem as a non-ischemic area sample and those from the LV posterior wall as an ischemic area sample, frozen in tissue-freezing medium and stored at –80°C for subsequent histological and molecular analyses. A preliminary experiment with Evans blue staining demonstrated that the extent of the risk area relative to the LV was comparable between the 2 groups (EPA 38±2% vs. control 34±2%, NS) (n=4 each).

Histological Analysis

Histological analysis was performed in a blinded manner on 5-μm cryosections of the tissue. The sections were examined with a fluorescence microscope, using an NIB filter, and subsequently stained with hematoxylin and eosin for light microscopy study. The number of infiltrating neutrophils was determined by counting the cells in 10 randomly selected high-power fields from various samples of each experiment. The stained sections were examined at a magnification of ×100. The number of neutrophils (/mm^2) in the interstium and in vessels in each section was determined in a blinded manner from 25 random fields (0.01 mm^2 each) and was averaged to give the number of the cells (/mm^2).

Western Blot Analysis

The myocardial tissue was homogenized in a sample buffer that contained 50 mmol/L HCL (PH 7.4), 150 mmol/L NaCl, 10% glycerol, 1% Triton-X, 10 mmol/L sodium pyrophosphate, 10 mmol/L β-glycerophosphate, 1 mmol/L orthovanadate, 10 mmol/L NaF, 1 mmol/L DDT, 5 mmol/L ethylene diamine tetraacetic acid and 1% protease inhibitor cocktail. The tissue lysate was then centrifuged (15,000 rpm, 4°C, 20 min) and the supernatant was collected. The protein concentration was quantified by a bicinchoninate protein assay kit (Pierce Chemical Rockford, IL, USA). The extracted samples (20 μg of protein) were subjected to 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis/immunoblot analysis (100A for 1 h) and subsequently transferred to a polyvinylidine difluoride membrane electrophoretically (100V for

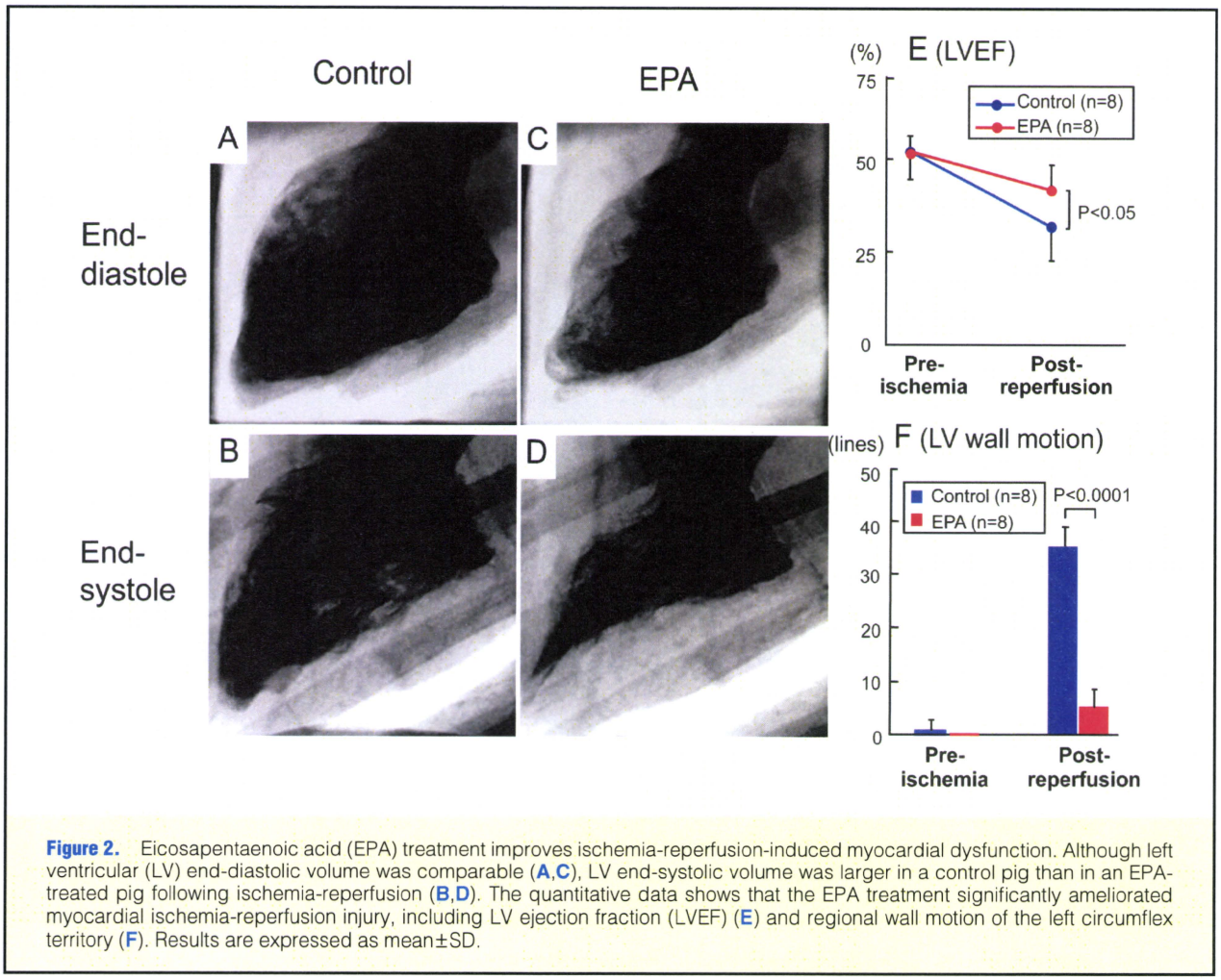

Figure 2. Eicosapentaenoic acid (EPA) treatment improves ischemia-reperfusion-induced myocardial dysfunction. Although left ventricular (LV) end-diastolic volume was comparable (**A**,**C**), LV end-systolic volume was larger in a control pig than in an EPA-treated pig following ischemia-reperfusion (**B**,**D**). The quantitative data shows that the EPA treatment significantly ameliorated myocardial ischemia-reperfusion injury, including LV ejection fraction (LVEF) (**E**) and regional wall motion of the left circumflex territory (**F**). Results are expressed as mean±SD.

1 h). The membranes were incubated by the specific antibody for Rho-kinase (ROCK) -I and -II (BD Transduction Laboratories), phosphorylated (p)-myosin binding subunit (MBS) (Thr696; Cosmo Bio Co, Ltd, Tokyo, Japan) and p-eNOS (Ser1177; Cell Signaling, Tokyo, Japan). Anti-mouse IgG was used as a secondary antibody (1:5,000). The ischemic and non-ischemic regions containing proteins were visualized by an electrochemiluminescence Western blotting luminal reagent (RPN2132; GE Healthcare UK Ltd, UK). Immnoreactivity was detected by enhanced chemiluminescence autoradiography (ECL Western blotting detection kit; Amersham Pharmacia Biotechnology, UK). Normalization for loading differences was accomplished using ratios of the densitometry signals for proteins of interest to GAPDH or β-actin. Scanning densitometry was used to quantify signal density from luminograms.

Statistical Analysis

Continuous variables are expressed as mean±SD and categorical variables as percentages. An unpaired Student's t-test was used to analyze differences in continuous variables. One way analysis of variance followed by Bonferroni's test was used to examine differences among multiple variables. Correlation among variables was determined using linear regression analysis. A linear regression line was calculated by the least-square method to assess the correlation between 2 parameters. Statistical analyses were performed with GraphPad Instat V3.06 for Windows (GraphPad Software Inc, La Jolla, CA, USA) and SigmaStat for Windows version 3.00.0 (SPSS Inc, Chicago, IL, USA). A value of $P<0.05$ was considered to be statistically significant.

Results

Effects of EPA Treatment on Fatty Acid Components

The long-term EPA treatment markedly increased the proportion of EPA (mol%) not only in the plasma but also in RBC and cardiac tissue (all $P<0.01$) (Table 1). In contrast, the EPA treatment significantly decreased the proportion of arachidonic acid (AA), AA/EPA ratio and docosahexaenoic acid (DHA) (mol%) in the plasma, RBC and heart tissue (all $P<0.01$) (Table 1).

Coronary Vascular Responses to Serotonin In Vivo

Between the EPA and the control group (n=8 each), there were no significant differences in hemodynamic variables (heart rate and blood pressure) at any measurement points except at 20 min during ischemia (Table 2). After I/R, the heart rate increased and blood pressure decreased in both groups, but to a similar extent (Table 2).

Figure 1 shows coronary vascular responses to serotonin (5-HT). There was no significant difference in baseline coronary diameter between the 2 groups (data not shown). A low dose of serotonin (10 μg/kg, IC) caused mild and insignificant

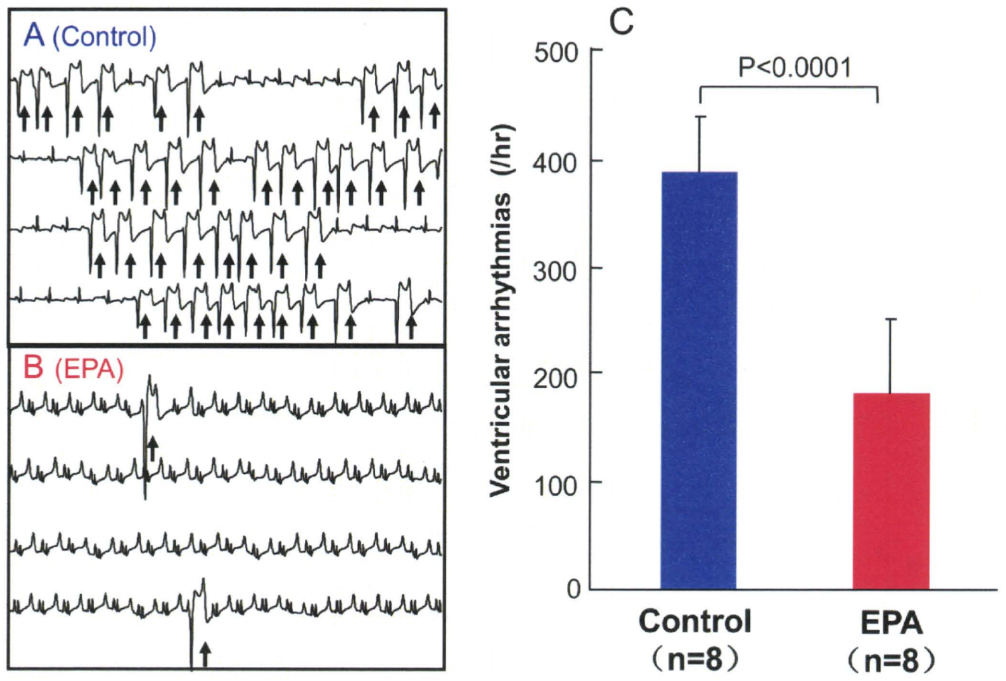

Figure 3. Eicosapentaenoic acid (EPA) treatment reduces ischemia-reperfusion-induced ventricular arrhythmias. Following ischemia-reperfusion, premature ventricular contractions (arrows) were induced frequently in a control pig (**A**), whereas they were suppressed in an EPA-treated pig (**B**). The quantitative data shows that the EPA treatment decreased the prevalence of ventricular arrhythmias including premature ventricular contraction, ventricular fibrillation and tachycardias as compared with the control group (**C**). Results are expressed as mean±SD.

coronary vasodilatation in both groups, whereas a high dose of serotonin (100 μg/kg, IC) caused coronary vasoconstriction in large and small arteries in the control group, but these responses were significantly attenuated in the EPA group (**Figure 1**).

LV Function and Arrhythmias Following Reperfusion

The baseline LV volumetric data were comparable between the 2 groups (data not shown). Also, myocardial blood flow in the LCX region during ischemia, when measured by colored microspheres, was low and comparable between the 2 groups (EPA 0.10±0.04 vs. control 0.10±0.08 $ml \cdot min^{-1} \cdot kg^{-1}$, n=4 each). After I/R, both global and regional LV functions were significantly reduced in the control group, whereas the EPA treatment significantly ameliorated the I/R injury, including LV ejection fraction (EPA, 43±9% vs. control, 32±7%, P<0.05) and ischemic regional wall motion abnormality (EPA, 5.3±3.6 vs. control, 35.1±3.8 unit, P<0.0001) (**Figure 2**). The EPA treatment also suppressed the occurrence of ventricular arrhythmias, including ventricular fibrillation, ventricular tachycardia and premature ventricular contraction, after I/R (EPA, 181±73 vs. control, 389±51 events, P<0.0001) (**Figure 3**).

Inflammatory Cell Infiltration and Myocardial eNOS and Rho-Kinase Activities

The EPA treatment also significantly attenuated I/R-induced neutrophil infiltration in the ischemic region as compared with the control group (EPA, 69±34 vs. control, 170±107 cells/mm^2, P<0.001) (**Figure S1**).

In comparison with the non-ischemic myocardium, Rho-kinase activity (as assessed by the extent of MBS phosphorylation) was markedly increased by ~50–100 fold after I/R in both groups (**Figure 4**). However, the EPA treatment significantly inhibited the I/R-induced Rho-kinase activation (EPA, 0.47±0.11 vs. control, 0.77±0.14, P<0.05) (**Figure 4**). The EPA treatment also preserved eNOS activity (as assessed by the extent of eNOS phosphorylation) in the ischemic myocardium (EPA, 0.56±0.13 vs. control, 0.23±0.07, P<0.01) (**Figure 5**). In contrast, myocardial Rho-kinase and eNOS activities in the non-ischemic myocardium were comparable between the 2 groups (**Figures 4,5**). Importantly, there was a significant negative correlation between myocardial Rho-kinase activity and myocardial eNOS activity (R=–0.584, P=0.01) (**Figure 6**). The expression of ROCK-I was not changed by I/R or by the treatment (**Figure S2**), whereas the expression of ROCK-II was decreased by I/R, but was not affected by the treatment (**Figure S3**).

Discussion

The major findings of the present study were as follows: (1) the long-term EPA treatment significantly ameliorated myocardial I/R injury, including LVEF and regional wall motion abnormality, occurrence of ventricular arrhythmias and myocardial accumulation of neutrophils; and (2) these beneficial effects of EPA were associated with inhibition of myocardial Rho-kinase activity and preserved myocardial eNOS activity with a significant negative correlation between them.

Previous Clinical Studies of EPA Treatment and Cardiovascular Events

In 1976, it was first demonstrated that the high consumption

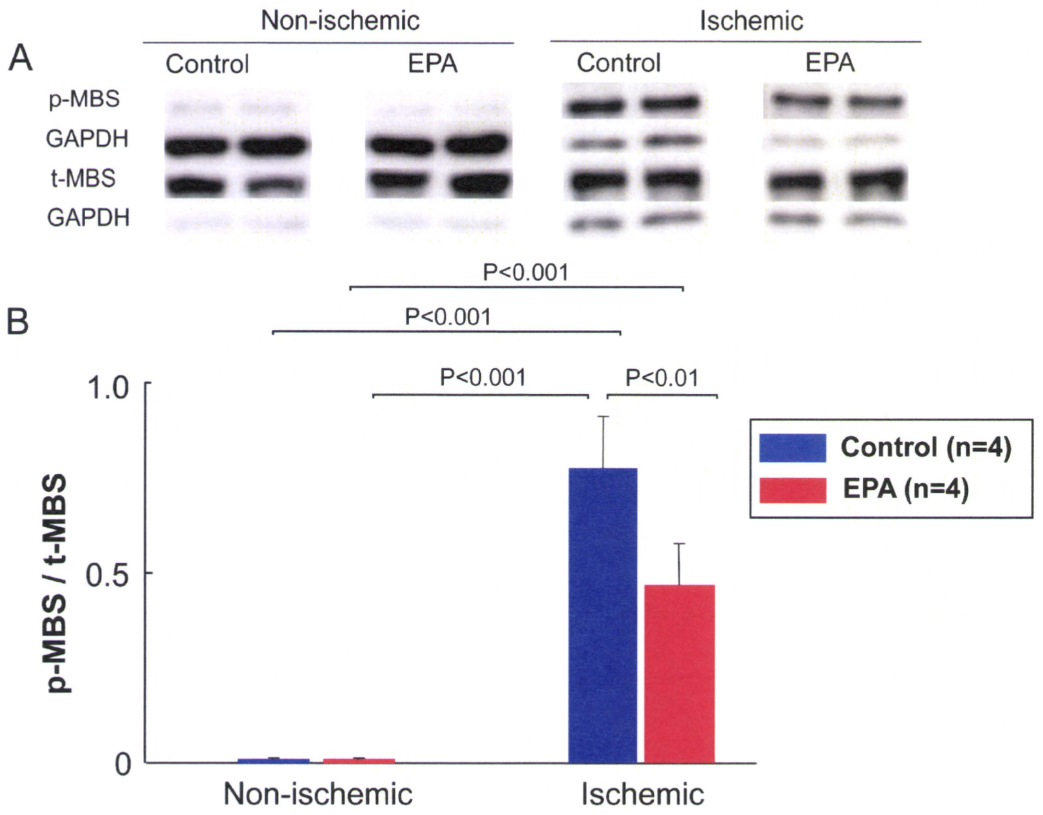

Figure 4. Eicosapentaenoic acid (EPA) treatment inhibits myocardial Rho-kinase activation in the ischemic region. (**A**) Representative Western blots of myocardial expression of phosphorylated (p)- and total (t)-MBS. (**B**) Quantitative results of the rario of p-MBS to t-MBS, indicating Rho-kinase activity. Results are expressed as mean±SD. MBS, myosin binding subunit.

of fish oil was associated with reduced cardiovascular risk in Inuit in Greenland.[3] Since then, accumulating evidence has demonstrated the beneficial effects of n-3 fatty acids, specifically those of EPA and DHA, especially for primary and secondary prevention of coronary artery diseases.[28,29] Dietary supplementation with n-3 fatty acids are now known to exert multiple beneficial effects, including a reduction in lipid levels, blood pressure and arrhythmias, improvement of endothelial and autonomic functions and inhibition of platelet aggregation.[30] In the present study, the EPA treatment markedly increased its concentration, not only in the plasma but also in RBC and heart tissue, while it conversely decreased the concentration of AA and DHA. The EPA levels in the RBC membrane in the present model (4.4±0.3 mol%) were comparable with those found in patients treated with 2,400 mg EPA for 2 years (average 5.8±1.2 mol%).[31,32] Also, in the present study, highly purified EPA was used, which might have been an advantage as compared with the previous studies where fish oil or a combination of EPA and DHA was used.[29]

Pleiotropic Effects of EPA on I/R Injury

In patients with AMI, the extent of infarct size is crucial because residual LV function determines their prognosis.[33,34] It has been well established that the most effective strategy for limiting infarct size is early restoration of coronary blood flow to the ischemic myocardium by thrombolysis, PCI or their combination.[35,36] Despite the success of contemporary reperfusion therapy and the effective restoration of epicardial coronary flow, many patients have a suboptimal flow at the coronary microcirculatory level. This could be due to I/R injury, which is a complex process involving various interrupting factors, such as microthrombus embolization, activated inflammatory cascade and enhanced production of reactive oxygen species.[37] Those patients with impaired coronary microcirculation have an impaired recovery of LV function and poor long-term prognosis.[38–40]

EPA could exert beneficial effects against myocardial I/R injury. In the present study, although the EPA effect on blood pressure (found only at 20 min during ischemia) seems to be minimized and platelet aggregation activity following EPA treatment was not assessed, the EPA treatment significantly attenuated coronary vasoconstricting responses to serotonin and ameliorated regional LV wall motion abnormality, LV dysfunction and occurrence of ventricular arrhythmias. It has been previously demonstrated that cardioprotective effects of EPA are mediated, in part, by its anti-thrombotic and anti-inflammatory effects and improved ion channel functions.[23,41–44] However, the detailed mechanisms of the beneficial effects of EPA remain to be fully elucidated.

Pathological Role of Rho-Kinase Pathway Activation and Its Inhibition by EPA

In the present study, we focused on the Rho-kinase pathway, as we and others have previously demonstrated an involvement of Rho-kinase activation in the pathogenesis of myocardial I/R injury.[19,45] Rho-kinase is a downstream effector of the