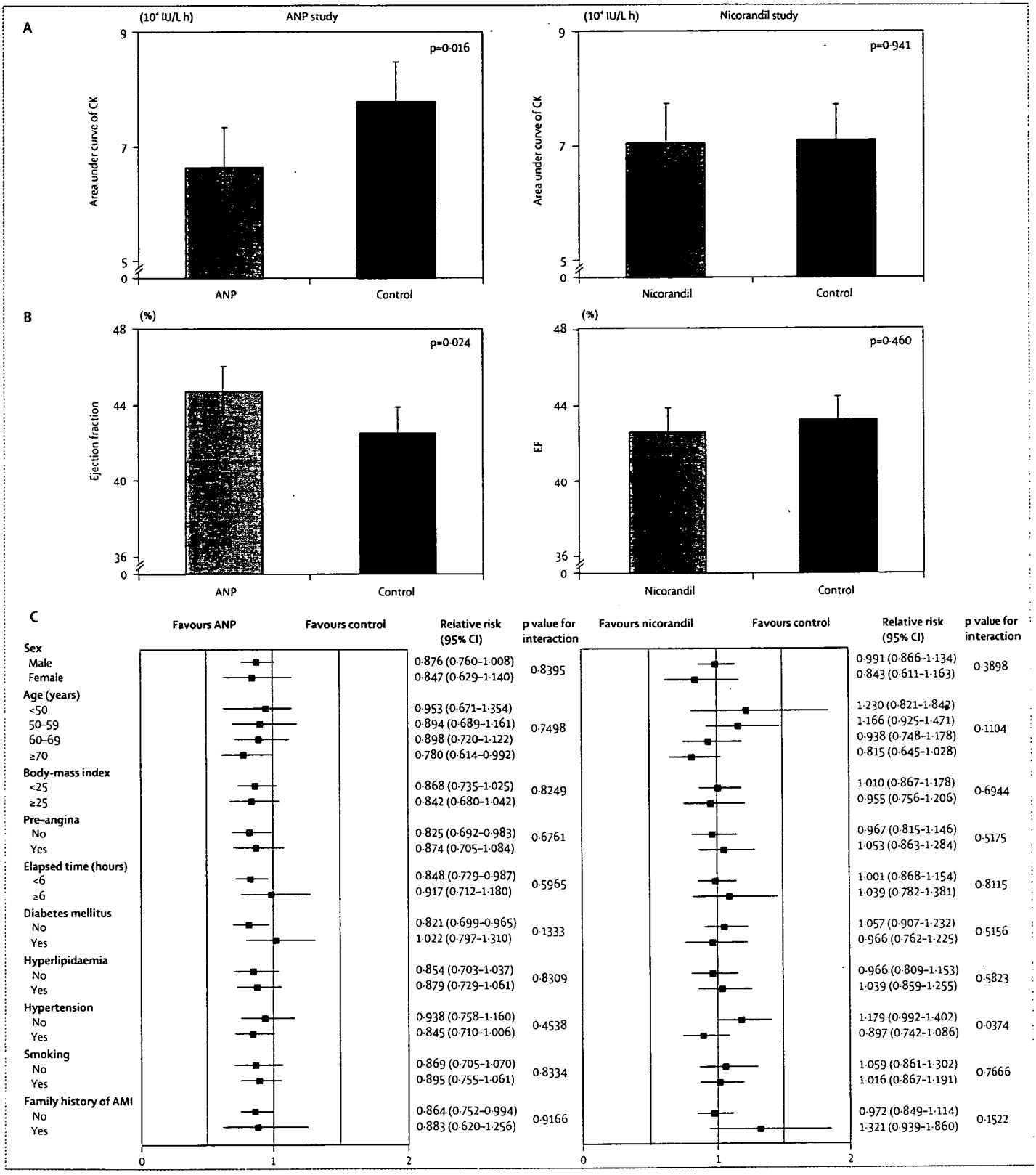

Figure 2: **Primary endpoints and subgroup analyses**

CK=creatine kinase. AMI=acute myocardial infarction. ANP=atrial natriuretic peptide. Panel A shows area under curve of creatine kinase concentration versus time. Panel B represents left ventricular ejection fraction measured at 6–12 months.

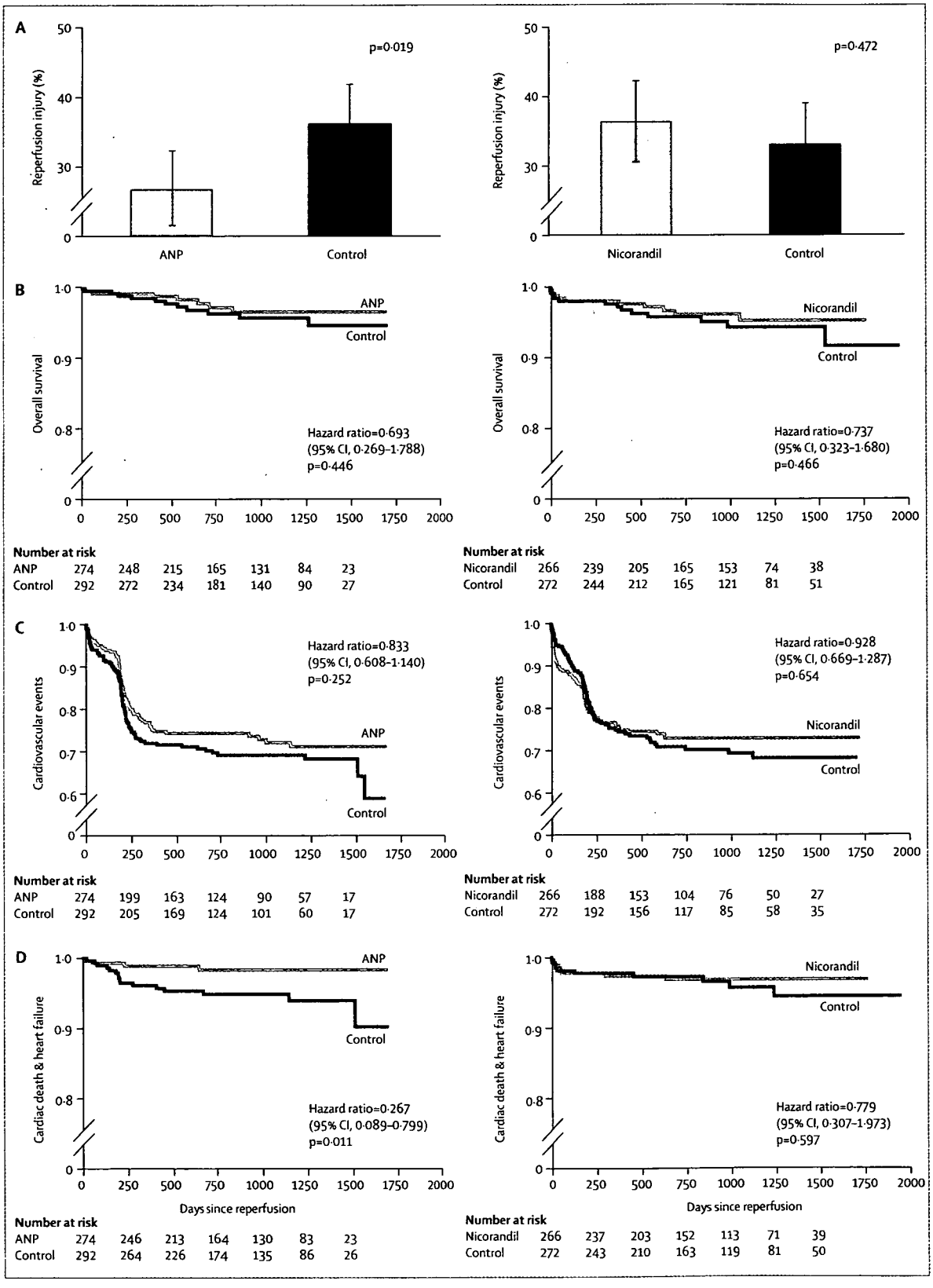

Figure 3: **Secondary endpoints and other subanalyses**
ANP=atrial natriuretic peptide.

The reduction of infarct size and the improvement of left ventricular ejection fraction might decrease mechanical stress on the non-infarcted myocardium, which might decrease hypertrophy and dilatation of the non-infarcted myocardium. Since cardiac hypertrophy and dilatation cause diastolic and systolic heart failure, a reduction of infarct size and an increase of left ventricular ejection fraction could mediate beneficial clinical outcomes. However, we need to do another large-scale clinical trial to target clinical outcomes such as cardiovascular death, because our primary aim here was to test the reduction of infarct size. Moreover, Hayashi and colleagues[20] showed that plasma concentrations of angiotensin II, aldosterone, and endothelin-1 were lower in patients given atrial natriuretic peptide than in controls. Sudden exposure to high concentrations of angiotensin II, aldosterone, and endothelin-1 for several days caused vascular or ventricular remodelling, and attenuation of these harmful effects by infusion of atrial natriuretic peptide could reduce the incidence of cardiac death and readmission to hospital for chronic heart failure.[20]

One reason that nicorandil treatment did not limit infarct size in our study could be the size of the dose. Ishii and colleagues[25] have reported that one intravenous administration of a dose of nicorandil that was three times higher than that which we used decreased the infarct size and reduced the rate of cardiovascular death or readmission to hospital for chronic heart failure in 368 patients with acute myocardial infarction.

Patients in the nicorandil study who were given nicorandil orally in the chronic phase had greater increases in left ventricular ejection fraction, irrespective of whether nicorandil was given intravenously or orally. Since microvascular obstruction ten days after myocardial infarction was associated with left ventricular remodelling and poor prognosis, coronary perfusion might be improved by opening KATP channels in coronary blood vessels during the healing stage. The IONA study[35] showed that nicorandil could reduce the incidence of unstable angina in patients with stable angina.

Our finding that treatment with atrial natriuretic peptide in the acute phase reduced the incidence of readmission to hospital for chronic heart failure could help to reduce the physical, medical, and economic burdens on people around the world. Moreover, since intravenous nicorandil in the acute phase, followed by oral administration in the chronic phase, increased the left ventricular ejection fraction, chronic treatment with nicorandil could improve ventricular function for patients with myocardial infarction in the chronic phase.

Several limitations of our study should be discussed. First, physicians knew the random assignment of patients, and treatment for acute myocardial infarction in the chronic phase was not restricted accordingly; this could have affected the difference in nicorandil treatment at the chronic phase. Second, although we planned to do angiography of the left ventricle when patients were admitted to hospital, some hospitals could not take angiographs, because of the additional medical cost. Therefore, baseline angiographs were absent for some patients. Third, the patterns of missing angiography data on left ventriculography differed between the two studies (which were done at different hospitals) and also between the atrial natriuretic peptide group and corresponding placebo group. We cannot explain this difference, but since we did not intervene in this procedure, we believe that it must be due to chance.

Contributors

Department of Cardiology, National Hospital Organisation Ehime National Hospital, Toon, Ehime, Japan (T Otani); Division of Cardiology, National Hospital Organisation Shizuoka Medical Centre, Suntoh-Gun, Shizuoka, Japan (H Yokoyama); Department of Cardiology, Kameda Medical Centre, Kamogawa, Chiba, Japan (Y Hashimoto); Division of Cardiovascular disease of Medicine, Hokkaido Junkanki Hospital, Sapporo, Hokkaido, Japan (N Funayama); Department of Cardiology, Kyushu Kosei Nenkin Hospital, Kitakyushu, Fukuoka, Japan (H Yamamoto); Department of Cardiology, Surugadai Nihon University Hospital, Chiyoda-Ku, Tokyo, Japan (E Tachibana); Department of Cardiology, St Mary's Hospital, Kurume, Fukuoka, Japan (K Yamamoto); Department of Cardiology, Miki City Hospital, Miki, Hyogo, Japan (K Awano); Division of Cardiology, Cardiovascular Centre, Tsuchiya General Hospital, Hiroshima, Hiroshima, Japan (T Sakuma); Department of Cardiology, Himeji Brain and Heart Centre, Himeji, Hyogo, Japan (T Kajiya); Department of Cardiovascular Centre, National Hospital Organisation Kumamoto Medical Centre, Kumamoto, Kumamoto, Japan (K Fujimoto); Department of Cardiology, Fukuyama Cardiovascular Hospital, Fukuyama, Hiroshima, Japan (H Kohno); Division of Cardiology, Tokuyama Central Hospital, Shunan, Yamaguchi, Japan (T Iwami); Division of Cardiology, Mito Saiseikai General Hospital, Mito, Ibaraki, Japan (M Murata); Division of Cardiology, Osaka General Medical Centre, Osaka, Osaka, Japan (M Fukunami); Department of Cardiology, Kobe General Hospital , Kobe , Hyogo, Japan (A Yamamuro); Department of Cardiology, Ogaki Municipal Hospital, Ogaki, Gifu, Japan (T Sone); Heart Centre Division of Cardiology, Social Insurance Kinan Hospital, Tanabe, Wakayama, Japan (Y Okumoto); Department of Circulatory Devision, National Hospital Organisation Ureshino Medical Centre, Ureshino, Saga, Japan (S Hata); Department of Cardiovascular Medicine, Matsuyama Shimin Hospital, Matsuyama, Ehime, Japan (M Abe); Cardiovascular Centre, Anjo Kosei Hospital, Anjo, Aichi, Japan (Y Murata); Cardiovascular Division of Medicine, National Cardiovascular Centre, Suita, Osaka, Japan (S Yasuda); Department of Cardiovascular and Renal Medicine, Saga University Faculty of Medicine, Saga, Saga, Japan (K Node); Department of Cardiology, Kawachi General Hospital, Higashiosaka, Osaka, Japan (M Mishima); Department of Cardiology, Engaru Kousei Hospital, Monbetsu-Gun, Hokkaido, Japan (H Honda); Department of Cardiology, Ehime Prefectural Imabari Hospital, Imabari, Ehime, Japan (H Matsuoka); Department of Cardiology, Tokushima Red Cross Hospital, Komatushima, Tokushima, Japan (Y Hiasa); Department of Cardiology, Musashino Red Cross Hospital, Musashino, Tokyo, Japan (T Miyamoto); Department of Cardiology, Fukuoka University School of Medicine, Fukuoka, Fukuoka, Japan (K Saku); Department of Cardiology, Chiba Emergency Medical Centre, Chiba, Chiba, Japan (I Ishibashi); Department of Cardiology, Saiseikai Fukuoka General Hospital, Fukuoka, Fukuoka, Japan (Y Yamamoto); Department of Cardiology, National Hospital Organisation Ibaraki-Higashi Hospital, Naka-Gun, Ibaraki, Japan (Y Eki); Department of Cardiology, Kawasaki Medical School Hospital, Kurashiki, Okayama, Japan (K Yoshida); Department of Cardiology,

Tokyo Metropolitan Bokutoh General Hospital, Sumida-Ku, Tokyo, Japan (I Kubo); Division of Cardiology, Omura Municipal Hospital, Omura, Nagasaki, Japan (Y Tanioka); Department of Cardiology, National Hospital Organisation Mito Medical Centre, Higashi Ibaraki-gun, Ibaraki, Japan (S Taguchi); Department of Cardiology, Tokyo Medical University, Shinjuku-ku, Tokyo, Japan (A Yamashina); Department of Cardiology, Fukuyama City Hospital, Fukuyama, Hiroshima, Japan (K Hashimoto); Department of Medicine,Nippon Medical School Chiba Hokuso Hospital, Inba-Gun, Chiba, Japan (K Mizuno); Department of Cardiology, Kanazawa Medical University, Kahoku-Gun, Ishikawa, Japan (S Okubo); Department of Internal Medicine,University of Yamanashi Faculty of Medicine, Chuo,Yamanashi, Japan (K Kugiyama); Department of Cardiology, Tsukazaki Memorial Hospital, Himeji, Hyogo, Japan (H Iida); Department of Cardiovascular Science and Medicine, Chiba University Graduate School of Medicine, Chiba, Chiba, Japan (I Komuro); Department of Cardiovascular Medicine, Graduate School of Medical Sciences Kumamoto University, Kumamoto, Kumamoto, Japan (H Ogawa); Department of Cardiology, Shizuoka Prefectural General Hospital, Shizuoka, Shizuoka, Japan (O Doi); Division of Cardiology, Tokyo Metropolitan Geriatric Hospital, Itabashi-Ku, Tokyo, Japan (K Harada); Department of Internal Medicine, Cardiovascular Division, Asahikawa Medical College, Asahikawa, Hokkaido, Japan (N Hasebe); Department of Cardiology, Sasebo City General Hospital, Sasebo, Nagasaki, Japan (T Yamasa); Department of Internal Medicine, Division of Coronary Heart Disease, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan (M Masutani); Department of Cardiovascular Medicine, Graduate School of Medical Sciences Kyushu University, Fukuoka, Fukuoka, Japan (K Egashira); Department of Cardiovascular Medicine, Tokyo Medical and Dental University, Bunkyo-Ku, Tokyo, Japan (M Isobe); Department of Internal Medicine and Cardiology, Graduate School of Medicine Osaka City University, Osaka, Osaka, Japan (M Yoshiyama); Department of Cardiology, Tokyo Women's Medical University, Shinjyuku-Ku, Tokyo, Japan (H Kasanuki); Department of Cardiology, Nagasaki Citizens Hospital, Nagasaki, Nagasaki, Japan (S Suzuki); Department of Emergency and Critical Care Medicine, Chikushi Hospital Fukuoka University, Chikushino, Fukuoka, Japan (H Mihara).

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

These studies were supported by grants for Comprehensive Research on Ageing and Health (H13-21seiki (seikatsu) 23), in Health and Labour Sciences Research from Ministry of Health, Labour and Welfare, Japan, and by a grant from the Japanese Cardiovascular Research Foundation. We thank Satomi Ihara for her excellent assistance with data management; Hidetoshi Okazaki, Hiroyuki Yamamoto, Masakatsu Wakeno, Atsushi Nakano, Hiroyuki Takahama, Shin Ito, Hideyuki Sasaki, and Kyungduk Min for analysis of angiographs of left ventricles; Yoshie Yanagi, Hiromi Ohara, Chikayo Tsujimoto, Naoko Matsuo, Yoshihiro Asano, Masashi Fujita, Shuichiro Higo, and Mitsutoshi Asai for data monitoring; Ms Uchida at SACT international for data analysis; Dr Tsutomu Yamazaki for data management; and Dr Ed Schweitzer for review of the manuscript.

References

1 Thom T, Haase N, Rosamond W, et al. Heart disease and stroke statistics–2006 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* 2006; **113**: 85–151.

2 Jessup M, Brozena S. Heart failure. *N Engl J Med* 2003; **348**: 2007–18.

3 Levy D, Kenchaiah S, Larson MG, et al. Long-term trends in the incidence of and survival with heart failure. *N Engl J Med* 2002; **347**: 1397–402.

4 Shiba N, Watanabe J, Shinozaki T, et al. Poor prognosis of Japanese patients with chronic heart failure following myocardial infarction—comparison with nonischemic cardiomyopathy. *Circ J* 2005; **69**: 143–49.

5 Kloner RA, Rezkalla SH. Cardiac protection during acute myocardial infarction: where do we stand in 2004? *J Am Coll Cardiol* 2004; **44**: 276–86.

6 The MIAMI Trial Research Group. Metoprolol in acute myocardial infarction (MIAMI). A randomised placebo-controlled international trial. *Eur Heart J* 1985; **6**: 199–226.

7 Ross AM, Gibbons RJ, Stone GW, Kloner RA, Alexander RW. A randomised, double-blinded, placebo-controlled multicenter trial of adenosine as an adjunct to reperfusion in the treatment of acute myocardial infarction (AMISTAD-II). *J Am Coll Cardiol* 2005; **45**: 1775–80.

8 van der Horst IC, Zijlstra F, van't Hof AW, et al. Glucose-insulin-potassium infusion inpatients treated with primary angioplasty for acute myocardial infarction: the glucose-insulin-potassium study: a randomised trial. *J Am Coll Cardiol* 2003; **42**: 784–91.

9 Grines CL, Browne KF, Marco J, et al. A comparison of immediate angioplasty with thrombolytic therapy for acute myocardial infarction. The Primary Angioplasty in Myocardial Infarction Study Group. *N Engl J Med* 1993; **328**: 673–79.

10 Sakurai K, Watanabe J, Iwabuchi K, et al. Comparison of the efficacy of reperfusion therapies for early mortality from acute myocardial infarction in Japan: registry of Miyagi Study Group for AMI (MsAMI). *Circ J* 2003; **67**: 209–14.

11 Lamas GA, Flaker GC, Mitchell G, et al. Effect of infarct artery patency on prognosis after acute myocardial infarction. The Survival and Ventricular Enlargement Investigators. *Circulation* 1995; **92**: 1101–09.

12 Verma S, Fedak PW, Weisel RD, et al. Fundamentals of reperfusion injury for the clinical cardiologist. *Circulation* 2002; **105**: 2332–36.

13 Zeymer U, Suryapranata H, Monassier JP, et al. The Na(+)/H(+) exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction. Results of the evaluation of the safety and cardioprotective effects of eniporide in acute myocardial infarction (ESCAMI) trial. *J Am Coll Cardiol* 2001; **38**: 1644–50.

14 European Study of Prevention of Infarct with Molsidomine (ESPRIM) Group. The ESPRIM trial: short-term treatment of acute myocardial infarction with molsidomine. *Lancet* 1994; **344**: 91–97.

15 Wall TC, Califf RM, Blankenship J, et al. Intravenous fluosol in the treatment of acute myocardial infarction. Results of the Thrombolysis and Angioplasty in Myocardial Infarction 9 Trial. TAMI 9 Research Group. *Circulation* 1994; **90**: 114–20.

16 Cody RJ, Atlas SA, Laragh JH, et al. Atrial natriuretic factor in normal subjects and heart failure patients. Plasma levels and renal, hormonal, and hemodynamic responses to peptide infusion. *J Clin Invest* 1986; **78**: 1362–74.

17 Emori T, Hirata Y, Imai T, Eguchi S, Kanno K, Marumo F. Cellular mechanism of natriuretic peptides-induced inhibition of endothelin-1 biosynthesis in rat endothelial cells. *Endocrinology* 1993; **133**: 2474–80.

18 Kitakaze M, Minamino T, Node K, et al. Role of activation of ectosolic 5′-nucleotidase in the cardioprotection mediated by opening of K+ channels. *Am J Physiol* 1996; **270**: 1744–56.

19 Mizumura T, Nithipatikom K, Gross GJ. Infarct size-reducing effect of nicorandil is mediated by the KATP channel but not by its nitrate-like properties in dogs. *Cardiovasc Res* 1996; **32**: 274–85.

20 Hayashi M, Tsutamoto T, Wada A, et al. Intravenous atrial natriuretic peptide prevents left ventricular remodeling in patients with first anterior acute myocardial infarction. *J Am Coll Cardiol* 2001; **37**: 1820–26.

21 Kuga H, Ogawa K, Oida A, et al. Administration of atrial natriuretic peptide attenuates reperfusion phenomena and preserves left ventricular regional wall motion after direct coronary angioplasty for acute myocardial infarction. *Circ J* 2003; **67**: 443–48.

22 Sugimoto K, Ito H, Iwakura K, et al. Intravenous nicorandil in conjunction with coronary reperfusion therapy is associated with better clinical and functional outcomes in patients with acute myocardial infarction. *Circ J* 2003; **67**: 295–300.

23 Sakata Y, Kodama K, Komamura K, et al. Salutary effect of adjunctive intracoronary nicorandil administration on restoration of myocardial blood flow and functional improvement in patients with acute myocardial infarction. *Am Heart J* 1997; **133**: 616–21.

24 Fukuzawa S, Ozawa S, Inagaki M, et al. Nicorandil affords cardioprotection in patients with acute myocardial infarction treated with primary percutaneous transluminal coronary angioplasty: assessment with thallium-201/iodine-123 BMIPP dual SPECT. *J Nucl Cardiol* 2000; **7**: 447–53.

25 Ishii H, Ichimiya S, Kanashiro M, et al. Impact of a single intravenous administration of nicorandil before reperfusion in patients with ST-segment-elevation myocardial infarction. *Circulation* 2005; 112: 1284–88.

26 Asakura M, Jiyoong K, Minamino T, Shintani Y, Asanuma H, Kitakaze M. Rationale and design of a large-scale trial using atrial natriuretic peptide (ANP) as an adjunct to percutaneous coronary intervention for ST-segment elevation acute myocardial infarction: Japan-Working groups of acute myocardial infarction for the reduction of Necrotic Damage by ANP (J-WIND-ANP). *Circ J* 2004; 68: 95–100.

27 Minamino T, Jiyoong K, Asakura M, Shintani Y, Asanuma H, Kitakaze M. Rationale and design of a large-scale trial using nicorandil as an adjunct to percutaneous coronary intervention for ST-segment elevation acute myocardial infarction: Japan-Working groups of acute myocardial infarction for the reduction of Necrotic Damage by a K-ATP channel opener (J-WIND-KATP). *Circ J* 2004; 68: 101–06.

28 Seino Y, Tomita Y, Hoshino K, Setsuta K, Takano T, Hayakawa H. Pathophysiological analysis of serum troponin T release kinetics in evolving ischemic myocardial injury. *Jpn Circ J* 1996; 60: 265–76.

29 Steen H, Giannitsis E, Futterer S, Merten C, Juenger C, Katus HA. Cardiac troponin T at 96 hours after acute myocardial infarction correlates with infarct size and cardiac function. *J Am Coll Cardiol* 2006; 48: 2192–94.

30 Vollmer RT, Christenson RH, Reimer K, Ohman EM. Temporal creatine kinase curves in acute myocardial infarction. Implications of a good empiric fit with the log-normal function. *Am J Clin Pathol* 1993; 100: 293–98.

31 Therneau TM, Grambsch PM. Modeling survival data: extending the Cox model. New York, USA: Springer, 2000.

32 Hess KR. Graphical methods for assessing violations of the proportional hazards assumption in Cox regression. *Stat Med* 1995; 14: 1707–23.

33 Mahaffey KW, Puma JA, Barbagelata NA, et al. Adenosine as an adjunct to thrombolytic therapy for acute myocardial infarction: results of a multicenter, randomised, placebo-controlled trial: the Acute Myocardial Infarction STudy of ADenosine (AMISTAD) trial. *J Am Coll Cardiol* 1999; 34: 1711–20.

34 Grover GJ, Sleph PG, Dzwonczyk S. Role of myocardial ATP-sensitive potassium channels in mediating preconditioning in the dog heart and their possible interaction with adenosine A1-receptors. *Circulation* 1992; 86: 1310–16.

35 IONA Study Group. Effect of nicorandil on coronary events in patients with stable angina: the Impact Of Nicorandil in Angina (IONA) randomised trial. *Lancet* 2002; 359: 1269–75.

Journal of the American College of Cardiology
© 2006 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 48, No. 7, 2006
ISSN 0735-1097/06/$32.00
doi:10.1016/j.jacc.2006.05.069

Impact of Blockade of Histamine H_2 Receptors on Chronic Heart Failure Revealed by Retrospective and Prospective Randomized Studies

Jiyoong Kim, MD, Akiko Ogai, MS, Satoshi Nakatani, MD, PhD, Kazuhiko Hashimura, MD, Hideaki Kanzaki, MD, Kazuo Komamura, MD, PhD, Masanori Asakura, MD, PhD, Hiroshi Asanuma, MD, PhD, Soichiro Kitamura, MD, PhD, Hitonobu Tomoike, MD, PhD, Masafumi Kitakaze, MD, PhD

Suita, Japan

OBJECTIVES The goal of this work was to determine whether the blockade of histamine H_2 receptors is beneficial for the pathophysiology of chronic heart failure (CHF).

BACKGROUND Because CHF is one of the major life-threatening diseases, we need to find a novel effective therapy. Intriguingly, our previous study, which predicts the involvement of histamine in CHF, suggests that we should test this hypothesis in patients with CHF.

METHODS We selected 159 patients who received famotidine among symptomatic CHF patients for the retrospective study. We blindly selected age- and gender-matched CHF patients receiving drugs for gastritis other than histamine H_2 receptor blockers as a control group. For the prospective study, 50 symptomatic CHF patients were randomly divided into 2 groups. One group received famotidine of 30 mg/day for 6 months, and the other group received teprenone.

RESULTS In the retrospective study, famotidine of 20 to 40 mg decreased both left ventricular end-diastolic and end-systolic lengths (LVDd and LVDs, respectively) and the plasma B-type natriuretic peptide (BNP) levels (182 ± 21 vs. 259 ± 25 pg/ml, $p < 0.05$) with unaltered fractional shortening (FS). In a randomized, open-label study, compared with teprenone, famotidine of 30 mg prospectively decreased both New York Heart Association functional class ($p < 0.05$) and plasma BNP levels (183 ± 26 pg/ml vs. 285 ± 41 pg/ml, $p < 0.05$); this corresponded to decreasing both LVDd (57 ± 2 mm vs. 64 ± 2 mm, $p < 0.05$) and LVDs (47 ± 2 mm vs. 55 ± 2 mm, $p < 0.05$) with unaltered FS (15 ± 1% vs. 17 ± 1%). The frequency of readmission because of worsening of CHF was lower in the famotidine group (4% and 24%, $p < 0.05$). On the other hand, teprenone had no effects on CHF.

CONCLUSIONS Famotidine improved both cardiac symptoms and ventricular remodeling associated with CHF. Histamine H_2 receptor blockers may have therapeutic benefits for CHF. (J Am Coll Cardiol 2006;48:1378–84) © 2006 by the American College of Cardiology Foundation

Despite current medical therapy for patients with chronic heart failure (CHF) such as angiotensin-converting enzyme (ACE) inhibitors or beta-adrenergic receptor blockers (1), CHF remains one of the major causes of high morbidity and mortality worldwide. Chronic heart failure is characterized by cardiac symptoms, impaired cardiac performance, cardiac

See page 1385

mechanical stress, and neurohormonal imbalance (2). Indeed, increased levels of catecholamines, cytokines, and angiotensin II are thought to play important roles in the pathophysiology and development of CHF (3,4). Histamine is one of the neurohormonal factors that provoke various cellular functions via stimulation of histamine H_1-H_3 receptors (5,6). Specifically, because histamine H_2 receptors are known to be located in gastric cells and enhance the production of acids that cause gastric ulcers, the blocker of histamine H_2 receptors is developed as the drug for the treatment of gastric ulcers (7). Interestingly, we have previously predicted that histamine H_2 receptor blockers may be cardioprotective in patients with CHF using the data mining technique (8). The histamine H_2 receptor is also located in the cardiomyocytes, and this receptor is coupled to Gs protein as well as is the beta receptor (9–13). Indeed, it is reported that: 1) histamine provokes positive inotropic effects (11,14); and 2) the blocker of histamine H_2 receptors decreases cardiac output (14). The important roles of mast cells and released histamine are also accepted in the cardiovascular system (15).

We tested the hypothesis that the blockade of histamine H_2 receptors by famotidine is beneficial for the pathophys-

From the Cardiovascular Division, National Cardiovascular Center, Suita, Japan. This study was supported by Grants-in-Aid for Human Genome, Tissue Engineering and Food Biotechnology (H13-Genome-011), Health and Labour Sciences Research Grants, Comprehensive Research on Aging and Health (H13-21seiki(seikatsu)-23), Health and Labour Sciences Research Grants from the Ministry of Health, Labour and Welfare, Japan, and a grant from the Japan Cardiovascular Research Foundation. The funding sources had no involvement in the current study.

Manuscript received April 11, 2006; revised manuscript received May 18, 2006, accepted May 23, 2006.

Abbreviations and Acronyms

ACE = angiotensin-converting enzyme
AMP = adenosine monophosphate
BNP = brain natriuretic peptide
CHF = chronic heart failure
FS = fractional shortening
GERD = gastroesophageal reflux disease
LVDd = left ventricular end-diastolic volume
LVDs = left ventricular end-systolic volume
NYHA = New York Heart Association

iology of CHF in retrospective and prospective randomized studies.

METHODS

This study was approved by the ethical committee of National Cardiovascular Center. Informed consent was obtained from all patients before participation in this study in accordance with institutional approved protocols.

Study population and protocols. THE RETROSPECTIVE STUDY. A total of 1,104 consecutive subjects who were admitted to our hospital for treatment of CHF between January 2002 and April 2004 were candidates for this study. The criteria for enrollment in this study were: 1) clinical evidence of heart failure despite the conventional therapy; and 2) left ventricular fractional shortening (FS) below 30%, as assessed by 2-dimensional echocardiography. All the patients had New York Heart Association (NYHA) functional classifications of II to III, but were stable for 2 months after their discharge. Among these patients, we selected the patients who received famotidine of 20 to 40 mg (n = 159, the famotidine group). In the control group, the patients were selected so as to be matched for age, gender, and the cause of CHF (n = 159). We randomly selected age-, gender-, and cause-matched patients for the other drug of non-histamine H_2 blocker for gastritis (n = 159). Among the 159 patients in each group, the number of patients who suffered from dilated cardiomyopathy, hypertensive heart disease, ischemic cardiomyopathy, and valvular heart disease were 71, 11, 39, and 38, respectively. Clinical parameters of the plasma brain natriuretic peptide (BNP) levels and echocardiography were obtained. We estimated the NYHA functional classification of each patient by 3 independent cardiologists who were blinded to the medical treatment of CHF. If the estimations of all 3 doctors did not agree, we decided to take the median among 3 values of NYHA functional classification.

The prospective studies: the effects of famotidine. We studied 50 patients with symptomatic CHF and gastroesophageal reflux disease (GERD) in our institute. Gastroesophageal reflux disease was diagnosed by questionnaires reported previously (16). The criteria for enrollment in this study were clinical evidence of heart failure despite the conventional therapy and a left ventricular FS below 30%, as assessed by 2-dimensional echocardiography, and existence of GERD. All the patients had NYHA functional classifications of II to III. There were 32 men and 18 women with a mean age of 65 years. The number of patients diagnosed as CHF because of dilated cardiomyopathy, hypertensive heart disease, ischemic cardiomyopathy, and valvular heart disease were 17, 2, 4, and 2 in each group, respectively. Exclusion criteria included chronic obstructive pulmonary disease, pregnancy, and severe liver disease as defined by having hepatic enzymes >2 times the upper limit of normal values. All patients were treated by optimal and stable doses of beta-blockers and ACE inhibitors for at least 3 months before screening echocardiography and randomization. We did not change the doses of these drugs after the enrollment. Patients were randomly divided into 2 treatment groups: famotidine (n = 25, the famotidine group) and teprenone (n = 25, the control group). The doses of famotidine and teprenone were 30 and 150 mg per day, respectively, and there were no patients who discontinued the intake of either famotidine or teprenone, and drugs for CHF.

In the current study, we tested the hypothesis that famotidine, the histamine H_2 receptor blocker, may have therapeutic benefits for CHF in the clinical settings. The primary end point is to assess the changes in NYHA functional class and the plasma BNP levels from the baseline to 24 weeks. We estimated the NYHA functional classification of each patient by 3 independent cardiologists who were blinded to the treatment assignment of famotidine. If the estimations of all 3 doctors did not agree, we decided to take the median among 3 values of NYHA functional classification. Additional analyses were done using the echocardiogram to obtain the changes in left ventricular or atrial volume, and the pressure differences across the tricuspid valve from baseline to 24 weeks. Furthermore, the frequency of readmission because of worsening of CHF within 24 weeks was investigated.

Estimating from retrospective study results showing that the reduction of the plasma BNP levels was about 30%, 25 patients were required for each study group. A randomization was performed according to a computer generated randomization list by central telephone call or fax to Clinical Study Support Center Japan (Suita Osaka, Japan).

Effects of teprenone. There is a possibility that teprenone has deleterious effects on the pathophysiology of CHF, and if this were the case, famotidine would appear to be beneficial, when famotidine has no cardioprotective effects. To examine this possibility, we administered teprenone to 10 patients with CHF for 24 weeks, and compared 10 CHF patients without the teprenone treatment. The criteria for the enrollment, evaluated parameters, and the evaluation procedure were the same as in the study of famotidine described earlier in the text.

Analysis of parameters for CHF. Blood samples were collected in test tubes containing ethylenediaminetetraacetic acid at baseline and after 24 weeks of the treatment. The plasma was separated from blood cells by centrifugation and frozen at −80°C. Plasma concentrations of BNP were

JACC Vol. 48, No. 7, 2006
October 3, 2006:1378–84

measured using a specific immunoradiometric assay (17). The personnel performing these assays were blinded to the patients' treatment assignments.

M-mode echocardiography was performed with 2-dimensional monitoring using a Sono layer phased-array sector scanner (SONOS 5500, Hewlett Packard, Palo Alto, California) before and after 24 weeks of the treatment with famotidine or teprenone (18). All echocardiograms were read by the same physician, at baseline and after 24 weeks of the treatment, who was blinded to patients' treatment, assignment, and time point.

Statistical analysis. Data are presented as mean ± SEM. Statistical analysis was performed using paired or unpaired *t* test for numerical values, and either chi-square tests or Wilcoxon signed rank test for categorical values. B-type natriuretic peptide levels were logarithmically transformed to perform the statistical analysis. Furthermore, we used two-way repeated-measures analysis of variance when we compared the changes of each parameter in 2 groups. The chi-square tests were also performed to test the differences of the incidence of the readmission. All statistical analyses were performed using Stat View version 5.0 for Windows (SAS Institute, Cary, North Carolina) and SPSS 10.0.5J software (SPSS Inc., Chicago, Illinois).

Table 1. Clinical Parameters of CHF With or Without Famotidine

	Control Group (n = 159)	Famotidine Group (n = 159)
Age (yrs)	66 ± 1	66 ± 1
M/F gender (%)	97/62 (61/39)	97/62 (61/39)
Hypertension (%)	11 (7)	11 (7)
Duration of CHF (yrs)	8.7 ± 0.7	8.5 ± 0.8
Systolic blood pressure (mm Hg)	112 ± 9	105 ± 8*
Diastolic blood pressure (mm Hg)	67 ± 4	62 ± 5*
Heart rate (beats/min)	73 ± 5	66 ± 5*
Fractional shortening (%)	24 ± 1	23 ± 1
LV diastolic diameter (mm)	58 ± 2	54 ± 1*
LV systolic diameter (mm)	44 ± 1	41 ± 1*
LA diameter (mm)	40 ± 3	39 ± 3
Pressure across tricuspid valve (mm Hg)	30 ± 2	28 ± 2
Plasma BNP levels (pg/ml)	259 ± 25	182 ± 21*
NYHA functional class: II/III (%)	75/84 (47/53)	97/62 (61/39)*
Concomitant drugs, n (%)		
Digoxin	126 (80)	134 (84)
Diuretics except spironolactone	140 (88)	137 (86)
Nitrates	80 (25)	32 (20)
Beta-blockers	143 (90)	137 (86)
ACE inhibitors	127 (80)	121 (76)
ARB	118 (20)	38 (24)
Spironolactone	25 (20)	25 (20)

Values are either numbers of each group, range, or mean ± SEM. *$p < 0.05$ vs. the control group.

ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker; BNP = brain natriuretic peptide; CHF = chronic heart failure; LA = left atrium; LV = left ventricular; NYHA = New York Heart Association; Fractional shortening (%) = (left ventricle end-diastolic diameter − left ventricle end-systolic diameter)/left ventricle end-diastolic diameter.

Table 2. Baseline Characteristics of the Study Population

	Teprenone Group (n = 25)	Famotidine Group (n = 25)
Age (yrs)	65 ± 2	65 ± 2
M/F gender (%)	16/9 (64/36)	16/9 (64/36)
NYHA functional class: II/III (%)	8/17 (32/68)	10/15 (40/60)
Hypertension (%)	4 (16)	6 (24)
Duration of CHF (yrs)	11.2 ± 1.7	13.2 ± 2.3
Systolic blood pressure (mm Hg)	113 ± 3	112 ± 3
Diastolic blood pressure (mm Hg)	68 ± 3	67 ± 2
Heart rate (beats/min)	83 ± 3	83 ± 2
Fractional shortening (%)	15 ± 1	15 ± 1
LV diastolic diameter (mm)	65 ± 2	64 ± 2
LV systolic diameter (mm)	55 ± 2	55 ± 2
LA diameter (mm)	43 ± 2	42 ± 2
Pressure across tricuspid valve (mm Hg)	33 ± 3	36 ± 3
Plasma BNP levels (pg/ml)	268 ± 28	286 ± 41
Concomitant drugs, n (%)		
Digoxin	24 (96)	22 (84)
Diuretics except spironolactone	25 (100)	25 (100)
Nitrates	7 (28)	4 (16)
Beta-blockers	25 (100)	25 (100)
ACE inhibitors	23 (92)	21 (84)
ARB	2 (8)	4 (16)
Spironolactone	10 (40)	8 (32)

Values are either numbers of each group, range, or mean ± SEM. Abbreviations as in Table 1.

RESULTS

After age and gender matching, as shown in Table 1, the gender ratio and the average age of the 2 groups were similar. There were no significant differences of the variety of medical treatment drugs between the 2 groups. Blood pressure, heart rate, NYHA functional class, the plasma BNP levels, and left ventricular dimensions were smaller in the famotidine group compared with the control group. There were no differences between FS in the 2 groups. This result suggests that famotidine may be beneficial for pathophysiology of CHF.

As for the prospective randomized famotidine treatment protocol, medications were well tolerated over the 24-week period. The participants were recruited from September 2004 to October 2004. Participants attended clinic visits at the time of randomization (baseline) and at 4- to 8-week intervals for 24 weeks. All patients completed the protocol (50 of 50). No patients died during the 24-week study. In addition, the doses of beta-blockers, ACE inhibitors, and diuretics were not altered during the course of the entire study.

There were no differences in age, gender, or concurrent medications between the control and famotidine groups (Table 2). Blood pressure and heart rate were not different between the groups with and without famotidine before the treatment. Famotidine administration slightly decreased blood pressure (systolic and diastolic blood pressure 107 ± 3 mm Hg vs. 112 ± 3 mm Hg, $p < 0.01$ and 60 ± 3 mm Hg vs. 67 ± 2 mm Hg, $p < 0.05$) and heart rate (79 ± 2

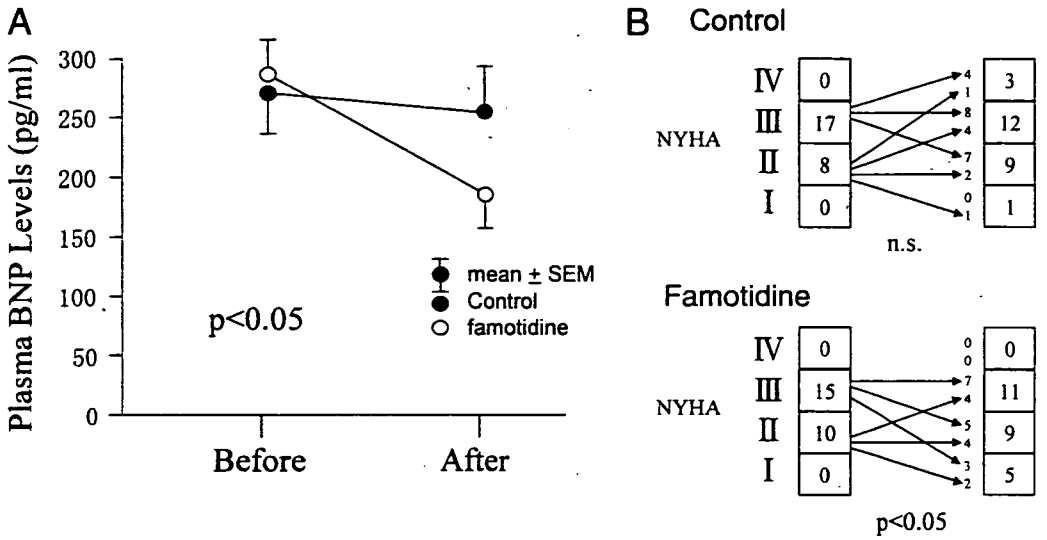

Figure 1. Changes in the plasma B-type natriuretic peptide (BNP) levels **(A)** and New York Heart Association (NYHA) functional classification **(B)** before and after the treatment with (the famotidine group) or without famotidine (the control group). The plasma BNP levels are statistically analyzed after the log transformation. The p values are obtained using 2-way repeated-measures analysis of variance **(A)** or Wilcoxon signed rank test **(B)**.

min^{-1} vs. 83 ± 2 min^{-1}, $p < 0.05$), whereas the control group did not exhibit changes in either blood pressure (systolic and diastolic blood pressure 113 ± 3 mm Hg vs. 113 ± 3 mm Hg and 68 ± 3 mm Hg vs. 68 ± 3 mm Hg) or heart rate (81 ± 3 min^{-1} vs. 83 ± 3 min^{-1}) before and 24 weeks after the treatment. The patients who received famotidine demonstrated improved functional capacity assessed by the plasma BNP levels and NYHA functional class (Fig. 1). Plasma BNP level and NYHA functional class were unchanged after 24 weeks in the group without famotidine. The functional improvement in the famotidine group was associated with improved cardiac performance. Compared with the group without famotidine, the patients treated with famotidine had lower left ventricular end-diastolic volume (LVDd) and left ventricular end-systolic volume (LVDs) while keeping FS unchanged (Fig. 2). The

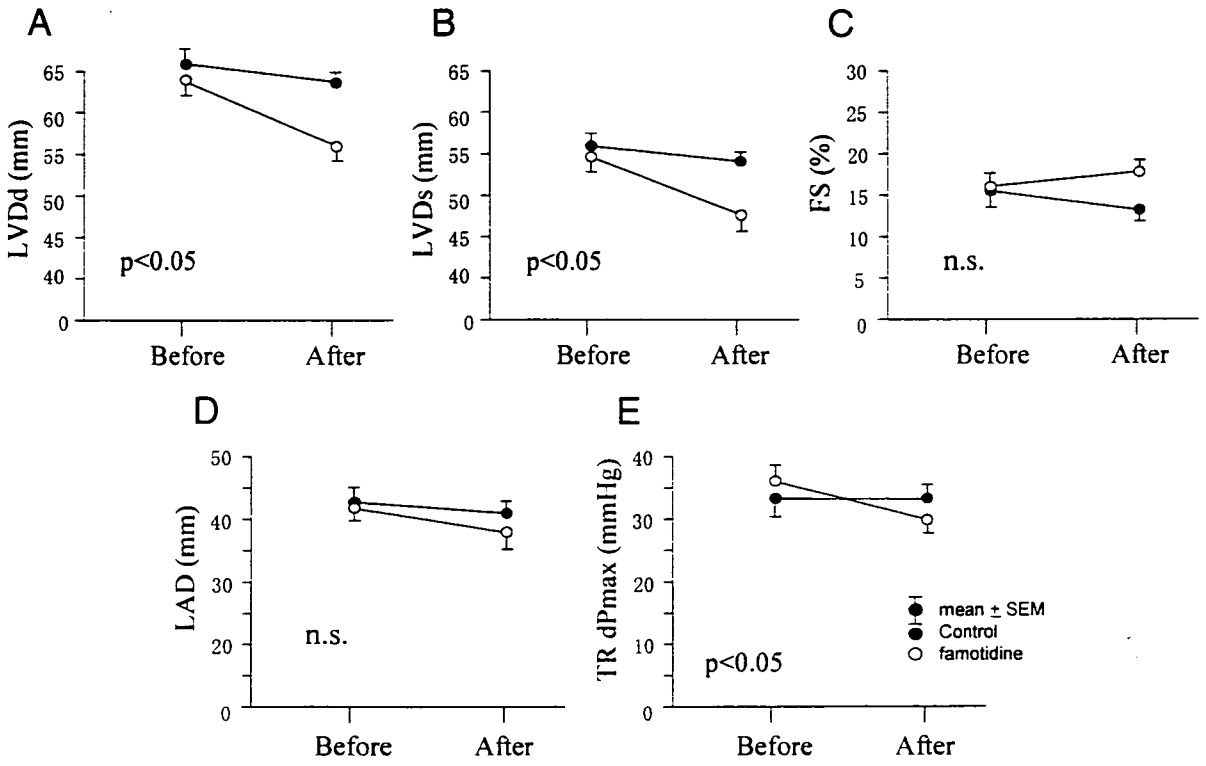

Figure 2. Changes in left ventricular (LV) end-diastolic volume (LVDd) **(A)** or end-systolic volume (LVDs) **(B)**, LV fractional shortening (FS) **(C)**, left atrial diameter (LAD) **(D)**, and the pressure differences across the tricuspid valve (TR dPmax) **(E)** before and after 24 weeks of treatment in the control and famotidine groups. The p values are tested using 2-way repeated-measures analysis of variance.

JACC Vol. 48, No. 7, 2006
October 3, 2006:1378–84

Table 3. Baseline Characteristics of the Study Population

	Teprenone Group (n = 10)	No Treatment Group (n = 10)
Age (yrs)	67 ± 4	68 ± 3
M/F gender (%)	7/3 (70/30)	7/3 (70/30)
NYHA functional class: II/III (%)	2/8 (20/80)	2/8 (20/80)
Hypertension (%)	2 (20)	2 (20)
Duration of CHF (yrs)	12.1 ± 2.8	12.0 ± 1.3
Systolic blood pressure (mm Hg)	109 ± 5	114 ± 4
Diastolic blood pressure (mm Hg)	64 ± 4	65 ± 32
Heart rate (beats/min)	75 ± 4	75 ± 2
Fractional shortening (%)	18 ± 3	18 ± 1
LV diastolic diameter (mm)	66 ± 4	65 ± 1
LV systolic diameter (mm)	53 ± 3	54 ± 1
LA diameter (mm)	45 ± 2	45 ± 2
Pressure across tricuspid valve (mm Hg)	36 ± 3	34 ± 2
Plasma BNP levels (pg/ml)	246 ± 48	248 ± 26
Concomitant drugs, n (%)		
Digoxin	10 (100)	10 (100)
Diuretics except spironolactone	10 (100)	10 (100)
Nitrates	2 (20)	1 (10)
Beta-blockers	10 (100)	10 (100)
ACE inhibitors	9 (90)	7 (70)
ARB	0 (0)	3 (30)
Spironolactone	5 (50)	5 (50)

Values are either numbers of each group, range, or mean ± SEM.
Abbreviations as in Table 1.

frequency of readmission because of worsening of CHF was lower in the famotidine group compared with the control group (1 [4%] and 6 [24%], difference [95% confidence interval] 20% [2 to 38], $p < 0.05$).

As for the prospective randomized teprenone treatment protocol, medications were well tolerated over the 24-week period. The participants were recruited from September 2004 to October 2004. Participants attended clinic visits at the time of randomization (baseline) and at 4- to 8-week intervals for 24 weeks. All patients completed the protocol (20 of 20). No patients died during the 24-week study. In addition, the doses of beta-blockers, ACE inhibitors, and diuretics were not altered during the course of entire study. There were no differences in age, gender, or concurrent medications between the control and famotidine groups (Table 3). We found that teprenone does not affect the severity of CHF (the plasma BNP levels: 246 ± 48 pg/ml vs. 234 ± 49 pg/ml, LVDd: 65.8 ± 3.6 mm vs. 65.6 ± 3.7 mm, LVDs: 53.2 ± 2.9 mm vs. 53.8 ± 3.6 mm, FS: 18.7 ± 2.8% vs. 18.3 ± 1.3% before and 24 weeks after an administration of teprenone) in comparison with the patients without the teprenone treatment (the plasma BNP levels: 248 ± 26 pg/ml vs. 238 ± 18 pg/ml, LVDd: 65.1 ± 1.3 mm vs. 66.7 ± 1.5 mm, LVDs: 53.6 ± 1.4 mm vs. 55.1 ± 1.2 mm, FS: 17.7 ± 1.3% vs. 17.2 ± 1.0% at observation time of 0 and 24 weeks). The frequency of readmission because of worsening of CHF was identical between the teprenone and control groups (2 [20%] and 2 [20%]).

DISCUSSION

In the present study, we demonstrate that the blockade of histamine H_2 receptors favors the improvements of the pathophysiology of CHF via retrospective and prospective clinical trials. These conclusions propose the novel findings that histamine that stimulates histamine H_2 receptors is one of the neurohumoral factors for the worsening of CHF, and that the blockade of histamine H_2 receptors becomes the novel strategy for the treatment of CHF.

Histamine in failing hearts. We have shown that histamine release is augmented in the ischemic myocardium compared with the non-ischemic myocardium in dogs (unpublished data). When the mast cells that store histamine are stimulated by ischemia or mechanical stress, mast cells actively release histamine. There are reports that mast cells are found in the human heart (19) and have been implicated in cardiovascular diseases (15,20,21). Indeed, the increase of mast cells have been observed in the hearts of patients with hypertrophy (22), dilated cardiomyopathy, ischemic cardiomyopathy (23), and ischemia/reperfusion (24), and the infarction-related coronary arteries (25). Furthermore, histamine is present in high concentrations in cardiac tissues in most animal species, including humans (10,26,27), and its release from cardiac stores and the subsequent actions on the heart may be of importance in the pathophysiology of heart disease. These lines of evidence agree with the present observation that the blockade of histamine H_2 receptors in failing hearts has an impact on the pathophysiology of CHF.

The role of histamine receptors in failing hearts. The histamine receptors (H_1, H_2, H_3, and H_4) are all G protein-coupled molecules, and they transduce extracellular signals via Gq, Gs, and Gi/o, respectively (5,6,28). Specifically, histamine H_2 receptors are linked to Gs proteins that facilitate the production of cyclic adenosine monophosphate (AMP) as beta-adrenoreceptors are (29). Histamine H_2-receptor-stimulated cAMP accumulation or adenylyl cyclase activator has been demonstrated in a variety of tissues including gastric cells (10,30), vascular smooth muscle cells (31), brain (10,32), and cardiac tissue (10,33). Beta-adrenoreceptor blockers are known to be cardioprotective in failing hearts because the accumulation of cyclic AMP after the activation of beta-adrenoreceptors enhances both myocardial contractility and oxygen consumption, which deteriorates heart function in patients with CHF (34,35). In addition, it has been reported that histamine is a powerful vasoconstrictor in atherosclerotic coronary arteries (36), which may locally provoke coronary spasm and thus contribute to the onset of myocardial infarction (23). The importance of beta-adrenoreceptor blockers depends on the presence of both catecholamine and beta-adrenoreceptors in the heart. Therefore, because histamine H_2 receptors and histamine are located in failing human hearts, it is likely that blockers of histamine H_2 receptors are as cardioprotective against failing hearts as beta-adrenoreceptor blockers are.

Because famotidine decreases both blood pressure and heart rate, this may improve the pathophysiology of CHF. Indeed, the reduction of afterload or preload and heart rate seems to be an important factor in the treatment of CHF. This is also the case in either beta-adrenoreceptor blockers or ACE inhibitors in patients with heart failure. Either beta-adrenoreceptor blockers or ACE inhibitors are still effective independent of the reduction of loading condition to the heart, because they inhibit the signal transduction for deterioration of cardiac function. Because histamine increases cyclic AMP levels in the cardiomyocytes via histamine H_2 receptors, famotidine may be beneficial through both load-reduction-dependent and -independent mechanisms.

Clinical importance. Beta-adrenoreceptor blockers have been shown to be effective for treating ischemic heart diseases and heart failure (37), and histamine receptor blockers are similar to beta-adrenoreceptor blockers. Histamine plays an important role in the regulation and malregulation of cardiac and coronary function. Furthermore, the histamine receptor blockers such as famotidine that are used for peptic ulcers or GERD all over the world could be used for ischemic heart diseases. Furthermore, beta-adrenoreceptor blockers ameliorate the severity of heart failure, and histamine receptor blockers may be beneficial for patients with CHF. However, we should note that the 3 H_2 receptor blockers administered for 7 days at clinical dosages had no significant effect on left ventricular systolic function, aerobic metabolic performance, or exercise capacity in men with class II or III stable CHF (38). This suggests that a relatively long-term administration of histamine receptor blockers is necessary to mediate the cardioprotective effects of histamine receptor blockers in patients with CHF as a relatively long-term administration of beta-adrenoreceptor blockers is necessary for the treatment of CHF (37).

Moreover, because famotidine was administered in addition to the aggressive treatments with beta-adrenoreceptors blockers, ACE inhibitors, and diuretics, and we proved that famotidine further improves the pathophysiology of CHF, it is possible to develop famotidine for the drug of CHF, although we need to plan and perform a large-scale clinical trial for the investigation of the effects of famotidine on CHF. We also need to clarify the best dose of famotidine for the treatment of CHF.

Study limitations. The present study has several limitations that we need to pay attention to. First of all, the first part of the data was obtained from the retrospective analysis, and seemed to be influenced by many factors, although Table 1 showed low BNP levels and low ventricular volumes in the famotidine group suggested the preventive effects of famotidine on cardiac remodeling. To strengthen the hypothesis obtained by the retrospective study, we performed the prospective analysis using either famotidine or teprenone.

The second limitation is that the second part of the study was an open-labeled, randomized trial using small sampling size. However, to decrease these weaknesses, we used the objective end points such as the plasma BNP levels and left ventricular dimensions, and we also tried to exclude the subjective scope of the assessment of NYHA functional classification.

Third, the severities of pathophysiology of CHF in the retrospective and prospective studies were different. The severity of CHF in enrolled patients in prospective study is higher than that in the retrospective study. This is because we enrolled the patients from the different protocols. Nevertheless, because both studies suggest that famotidine is effective for patients with CHF, we may be able to suggest the beneficial effects of famotidine to treat patients with CHF.

Fourth, if teprenone could be deleterious to the pathophysiology of CHF, famotidine seemed to be beneficial compared with teprenone even if famotidine has no beneficial effects on CHF. Before planning the present study, we tested the effects of pathophysiology of CHF, and we found that teprenone has no beneficial or deleterious effects on CHF in the present study.

Fifth, either famotidine or teprenone may directly affect the plasma half-life or excretion of BNP. If this is the case, the plasma BNP levels may be altered independent of the improvements of CHF. We cannot deny this possibility, however, because left ventricular dimension becomes smaller in the famotidine group, suggesting that famotidine is beneficial for the heart of CHF patients.

Sixth, we should notice that an interaction of gastritis with heart failure could confound their conclusion regarding the effect of histamine blockade. Indeed, it may be still possible to consider that famotidine improves cardiac function via an improvement of GERD if GERD worsens CHF, because we have no positive or negative data to link GERD and CHF. We should investigate this possibility to explain the effects of H_2 receptor blockers on CHF in further study.

In summary, despite these limitations, we proposed the hypothesis that H_2 receptor blockers are effective for the treatment of CHF, and we need to verify the beneficial effects of H_2 receptor blockers such as ranitidine or cimetidine as well as famotidine in CHF patients with a large-scale trial.

Reprint requests and correspondence: Dr. Masafumi Kitakaze, Cardiovascular Division, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita City, Osaka Pref. 565-8565, Japan. E-mail: kitakaze@zf6.so-net.ne.jp.

REFERENCES

1. Braunwald E, Bristow MR. Congestive heart failure: fifty years of progress. Circulation 2000;102:14–23.

2. Torre-Amione G. The syndrome of heart failure: emerging concepts in the understanding of its pathogenesis and treatment. Curr Opin Cardiol 1999;14:193–5.
3. Packer M. Neurohormonal interactions and adaptations in congestive heart failure. Circulation 1988;77:721–30.
4. Drexler H, Hornig B. Importance of endothelial function in chronic heart failure. J Cardiovasc Pharmacol 1996;27:S9–12.
5. Hough LB. Genomics meets histamine receptors: new subtypes, new receptors. Mol Pharmacol 2001;59:415–9.
6. Leurs R, Bakker RA, Timmerman H, et al. The histamine H3 receptor: from gene cloning to H3 receptor drugs. Nat Rev Drug Discov 2005;4:107–20.
7. Gantz I, Schaffer M, DelValle J, et al. Molecular cloning of a gene encoding the histamine H2 receptor. Proc Natl Acad Sci U S A 1991;88:429–33.
8. Kim J, Washio T, Yamagishi M, et al. A novel data mining approach to the identification of effective drugs or combinations for targeted endpoints—application to chronic heart failure as a new form of evidence-based medicine. Cardiovasc Drugs Ther 2004;18:483–9.
9. Matsuda N, Jesmin S, Takahashi Y, et al. Histamine H1 and H2 receptor gene and protein levels are differentially expressed in the hearts of rodents and humans. J Pharmacol Exp Ther 2004;309: 786–95.
10. Hill SJ, Ganellin CR, Timmerman H, et al. International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev 1997;49:253–78.
11. Eckel L, Gristwood RW, Nawrath H, et al. Inotropic and electrophysiological effects of histamine on human ventricular heart muscle. J Physiol 1982;330:111–23.
12. Du XY, Schoemaker RG, Bos E, et al. Effects of histamine on porcine isolated myocardium: differentiation from effects on human tissue. J Cardiovasc Pharmacol 1993;22:468–73.
13. Hattori Y. Cardiac histamine receptors: their pharmacological consequences and signal transduction pathways. Methods Find Exp Clin Pharmacol 1999;21:123–31.
14. Kirch W, Halabi A, Hinrichsen H. Hemodynamic effects of quinidine and famotidine in patients with congestive heart failure. Clin Pharmacol Ther 1992;51:325–33.
15. Maronle G. The role of mast cells in cardiovascular disease. In: Matsumori A, editor. Cardiomyopathies in Heart Failure: Biomolecular, Infectious and Immune Mechanisms. Norwell, MA: Kluwer Academic Publishers, 2003:185–228.
16. Carlsson R, Dent J, Bolling-Sternevald E, et al. The usefulness of a structured questionnaire in the assessment of symptomatic gastroesophageal reflux disease. Scand J Gastroenterol 1998;33:1023–9.
17. Pollock ML, Lowenthal DT, Phillips MI, Staples ED. Abnormal neuroendocrine responses during exercise in heart transplant recipients. Circulation 1992;86:1453–63.
18. Goldstein M, Vincent JL, Kahn RJ. Evaluation of cardiac function by echo-Doppler studies in critically ill patients. Intensive Care Med 1988;14:406–10.
19. Dvorak AM. Mast-cell degranulation in human hearts. N Engl J Med 1986;315:969–70.
20. Marone G, de Crescenzo G, Adt M, Patella V, Arbustini E, Genovese A. Immunological characterization and functional importance of human heart mast cells. Immunopharmacology 1995;31:1–18.
21. Hara M, Ono K, Hwang MW, et al. Evidence for a role of mast cells in the evolution to congestive heart failure. J Exp Med 2002;195: 375–81.
22. Panizo A, Mindan FJ, Galindo MF, Cenarruzabeitia E, Hernandez M, Diez J. Are mast cells involved in hypertensive heart disease? J Hypertens 1995;13:1201–8.
23. Patella V, Marino I, Arbustini E, et al. Stem cell factor in mast cells and increased mast cell density in idiopathic and ischemic cardiomyopathy. Circulation 1998;97:971–8.
24. Frangogiannis NG, Perrard JL, Mendoza LH, et al. Stem cell factor induction is associated with mast cell accumulation after canine myocardial ischemia and reperfusion. Circulation 1998;98:687–98.
25. Laine P, Kaartinen M, Penttila A, et al. Association between myocardial infarction and the mast cells in the adventitia of the infarct-related coronary artery. Circulation 1999;99:361–9.
26. Wolff AA, Levi R. Histamine and cardiac arrhythmias. Circ Res 1986;58:1–16.
27. Bristow MR, Ginsburg R, Harrison DC. Histamine and the human heart: the other receptor system. Am J Cardiol 1982;49:249–51.
28. Hofstra CL, Desai PJ, Thurmond RL, et al. Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. J Pharmacol Exp Ther 2003;305:1212–21.
29. Lohse MJ, Engelhardt S, Eschenhagen T. What is the role of beta-adrenergic signaling in heart failure? Circ Res 2003;93:896–906.
30. Gespach C, Bouhours D, Bouhours JF, et al. Histamine interaction on surface recognition sites of H2-type in parietal and non-parietal cells isolated from the guinea pig stomach. FEBS Lett 1982;149:85–90.
31. Ottosson A, Jansen I, Edvinsson L. Pharmacological characterization of histamine receptors in the human temporal artery. Br J Clin Pharmacol 1989;27:139–45.
32. Al-Gadi M, Hill SJ. The role of calcium in the cyclic AMP response to histamine in rabbit cerebral cortical slices. Br J Pharmacol 1987;91: 213–22.
33. Johnson CL, Weinstein H, Green JP. Studies on histamine H2 receptors coupled to cardiac adenylate cyclase. Blockade by H2 and H1 receptor antagonists. Mol Pharmacol 1979;16:417–28.
34. Packer M, Coats AJ, Fowler MB, et al. Effect of carvedilol on survival in severe chronic heart failure. N Engl J Med 2001;344:1651–8.
35. Dargie HJ. Effect of carvedilol on outcome after myocardial infarction in patients with left-ventricular dysfunction: the CAPRICORN randomised trial. Lancet 2001;357:1385–90.
36. Ginsburg R, Bristow MR, Davis K, et al. Quantitative pharmacologic responses of normal and atherosclerotic isolated human epicardial coronary arteries. Circulation 1984;69:430–40.
37. Metra M, Nardi M, Giubbini R, et al. Effects of short- and long-term carvedilol administration on rest and exercise hemodynamic variables, exercise capacity and clinical conditions in patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol 1994;24:1678–87.
38. Lucas BD Jr., Williams MA, Mohiuddin SM, LaMadrid LJ, Schroeder LJ, Hilleman DE. Effect of oral H2-receptor antagonists on left ventricular systolic function and exercise capacity in patients with chronic stable heart failure. Pharmacotherapy 1998;18:824–30.

ELSEVIER

Cardiovascular Research 67 (2005) 705 – 713

Cardiovascular Research

www.elsevier.com/locate/cardiores

Exacerbation of heart failure in adiponectin-deficient mice due to impaired regulation of AMPK and glucose metabolism

Yulin Liao[a], Seiji Takashima[a], Norikazu Maeda[b], Noriyuki Ouchi[b], Kazuo Komamura[c], Iichiro Shimomura[b], Masatsugu Hori[a], Yuji Matsuzawa[b], Tohru Funahashi[b], Masafumi Kitakaze[c,*]

[a]*Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[b]*Department of Internal Medicine and Molecular Science, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan*
[c]*Cardiovascular Division of Medicine, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan*

Received 13 January 2005; received in revised form 5 April 2005; accepted 19 April 2005
Available online 23 May 2005
Time for primary review 24 days

Abstract

Objective: Insulin resistance (IR) was reported to be associated with chronic heart failure (CHF). Adiponectin, an insulin-sensitizing hormone with anti-inflammatory activity, improves energy metabolism via AMP-activated protein kinase (AMPK). AMPK deficiency is associated with depressed cardiac function under stress conditions. However, it is not clear whether adiponectin plays an important role in CHF. We hypothesize that deficiency of adiponectin might result in deterioration of heart failure.
Methods: Using adiponectin null mice and their littermates, we examined the effects of adiponectin on LV pressure overload-induced cardiac hypertrophy and failure, and investigated the mechanisms involved.
Results: Three weeks after transverse aortic constriction (TAC), cardiac hypertrophy (evaluated from the heart-to-body weight ratio: 7.62±0.27 in wild-type (WT) mice, 9.97±1.13 in knockout (KO) mice, $P<0.05$) and pulmonary congestion (lung-to-body weight ratio: 9.05±1.49 in WT mice, 14.95±2.36 in KO mice, $P<0.05$) were significantly greater in adiponectin KO mice than WT mice. LV dimensions were also increased in KO mice. Compared with WT TAC mice, expression of AMPKα protein was lower, while IR was higher in KO TAC mice.
Conclusion: These findings indicate that adiponectin deficiency leads to progressive cardiac remodeling in pressure overloaded condition mediated via lowing AMPK signaling and impaired glucose metabolism.

Keywords: Adiponectin; Heart failure; Myocardial hypertrophy; Metabolic syndrome

1. Introduction

The metabolic syndrome (MetS) has been identified as a constellation of important risk factors for cardiovascular disease (CVD) [1,2]. The Adult Treatment Panel III report (ATP III)[3] identified insulin resistance (IR)±glucose intolerance as an important component of MetS that is related to CVD. Clinical evidence suggests that LV hypertrophy is associated with either impaired glucose tolerance (IGT) or an increase in IR [4]. An increase in IR is also common in CHF patients with either ischemic heart disease or idiopathic dilated cardiomyopathy [5–7]. These findings lead to the concept that a strategy targeting improvement of IGT or IR should be beneficial for cardiac remodeling.

To date, there is compelling evidence that an impaired myocardial energy metabolism strongly influences cardiac remodeling [8–11]. The important role of the AMP-activated protein kinase (AMPK) in cardiac hypertrophy and failure seems to be deserving of more attention. AMPK

* Corresponding author. Tel.: +81 6 6833 5012x2225; fax: +81 6 6836 1120.
E-mail address: kitakaze@zf6.so-net.ne.jp (M. Kitakaze).

0008-6363/$ - see front matter © 2005 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.cardiores.2005.04.018

activity and protein expression were both reported to be increased by pressure overload hypertrophy [8], which should be considered a compensatory mechanism for cardiac remodeling, because the overexpression of mutations of this enzyme leads to deterioration of post-ischemic cardiac dysfunction [10] or experimental glycogen storage cardiomyopathy [11]. Accordingly, we considered that AMPK might play an important role in limiting cardiac remodeling and that an increase of AMPK in the heart might inhibit remodeling by regulation of cellular metabolism to maintain energy homeostasis under stress conditions. Intriguingly, adiponectin, an endogenous adipocyte-derived insulin-sensitizing hormone, has been shown to attenuate inflammation, regulate glucose and lipid metabolism. In addition, adiponectin is able to stimulate glucose utilization and fatty acid oxidation through the activation of AMPK [12]. Furthermore, administration of adiponectin reverses IR in mice with lipoatrophy and diabetes [13,14]. The importance of adiponectin has also been demonstrated by other evidence that it may directly influence the development of cardiovascular disease [15–17]. A recent clinical investigation demonstrated that a high plasma adiponectin concentration was associated with a lower risk of myocardial infarction in men [17]. These lines of evidence strongly suggest that adiponectin might play an important role in the inhibition of cardiac remodeling via its beneficial effects on MetS. Interestingly, a recent experimental study shows that 1 week pressure overload in adiponectin-deficient mice resulted in enhanced concentric cardiac hypertrophy with an increased mortality [18]. However, to our knowledge, no previous study has evaluated the role of AMPK or adiponectin on chronic heart failure (CHF). Therefore, we aimed to test the hypothesis that adiponectin might act as an endogenous protective modulator of chronic cardiac remodeling via regulation of AMPK.

In this study, we evaluated the role of adiponectin in the progression of cardiac hypertrophy and heart failure in a model of LV pressure overload using adiponectin knockout mice, and explored the potential mechanisms involved.

2. Methods

2.1. Adiponectin knockout (KO) mice

Adiponectin KO mice were generated as described previously [19]. Wild-type (WT) littermates served as the control.

2.2. TAC model

All procedures were performed in accordance with our institutional guidelines for animal research and comply with the Declaration of Helsinki and the NIH Guide. Mice (male, 9–10 weeks old, wt 25–29 g) were anesthetized with a mixture of xylazine (5 mg/kg) and ketamine (100 mg/kg, i.p.), and transverse aortic constriction (TAC) was created as we described previously. In order to confirm that pressure overload was similar between the wild-type and the KO mice, three mice in each group were selected for measurement of the ascending aortic pressure using a 1.4 F Millar pressure catheter on the second day after TAC. The other mice were killed after 3 weeks for morphological analysis. Mice were divided into four groups: WT sham ($n=5$), WT TAC ($n=24$), KO sham ($n=5$), and KO TAC ($n=24$).

2.3. Histology

Hearts were fixed with 10% formalin. The cardiac myocyte cross-sectional surface area was measured using three hearts in each group after images were captured from HE-stained sections as described elsewhere [20]. One hundred myocytes per heart were counted, and the average area was determined. Myocardial and perivascular fibrosis were stained with Azan [21].

2.4. Echocardiography

Transthoracic echocardiography was performed with a Sonos 4500 and a 15–6 L MHz transducer (Philips, the Netherlands). Mice were fixed while conscious and good two-dimensional short-axis LV views were obtained for guided M-mode measurements of the LV posterior wall thickness (LVPW), LV end-diastolic diameter (LVEDd), LV end-systolic diameter (LVESd), LV fractional shortening (LVFS), and LV ejection fraction (EF). $LVFS=(LVEDd-LVESd)/LVEDd*100$, $LVEF=[(LVEDd)^3-(LVESd)^3]/(LVEDd)^3*100$.

2.5. Measurement of glucose and insulin

Fasting plasma glucose was measured using a blood glucose test meter (Glutestace GT-1640, Arkray Company, Japan). After 14 h withdrawal of food from the cages, whole blood sample (3 μl) was taken from mouse tails with a glucose sensor inserted in Glutestace, and the result of plasma glucose concentration was read-out 30 s later. Serum insulin levels were measured according to the protocols of the manufacturers (EIA-3440 ELISA kit, DRG, German). IR was assessed with the homeostasis model: HOMA-IR = fasting glucose level (mg/dl) × fasting insulin level (ng/ml) ÷ 22.5.

2.6. Western blot analysis

SDS-PAGE was performed with 50 μg of protein extracted from mouse hearts. Blots were incubated with a mouse monoclonal antibody directed against anti-AMPKα_1, anti-AMPKα_2 antibodies (upstate). Signals obtained by Western blotting were quantified using Scion Image software.

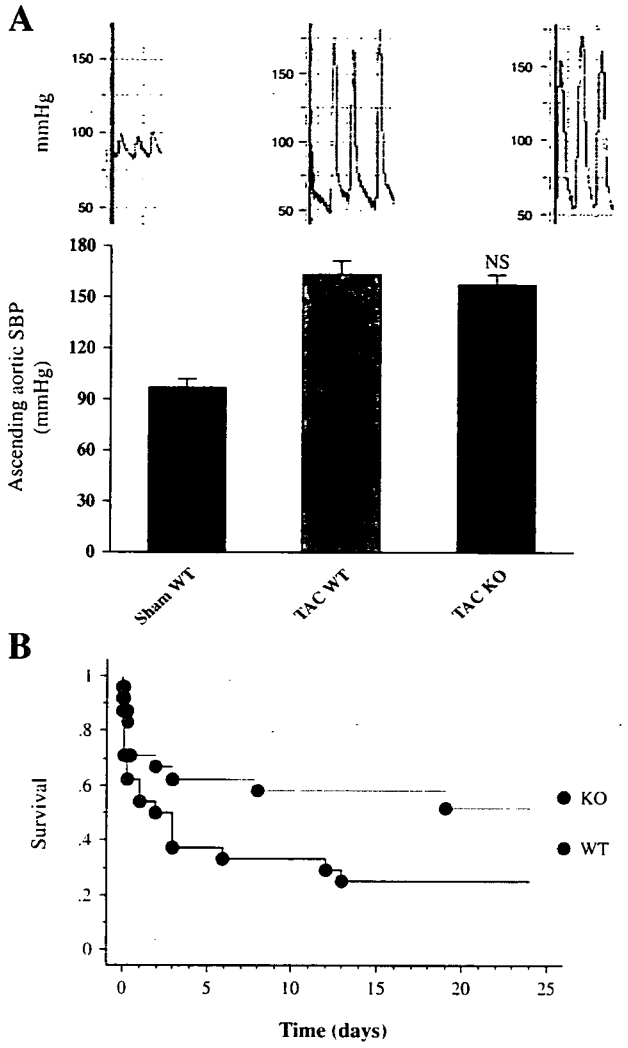

Fig. 1. Left ventricular pressure overload and survival. A) The ascending aortic systolic pressure measured with a 1.4 F catheter was similar in adiponectin KO and WT mice. NS: not significant vs. TAC WT. B) Kaplan–Meier survival analysis showed a significant higher mortality in adiponectin KO mice after TAC (Mantel–Cox test: $P=0.031$, $n=24$ in both WT and KO groups).

2.7. Statistical analysis

For all statistical tests, multiple comparisons were performed by one-way ANOVA with the Tukey–Kramer exact probability test. Survival analysis was performed using the Kaplan–Meier method. Variables with skewed distribution were transformed to logarithmic data. Results are reported as the mean ± SEM and $P<0.05$ was considered statistically significant.

3. Results

3.1. LV pressure overload and survival

To evaluate the role of adiponectin in cardiac remodeling, we used mice lacking the adiponectin/*CRP30* gene. During development up to 16 weeks of age, there were no differences in growth rate and food intake between WT mice and KO (homozygous) mice [19]. The results showed that LV pressure overload was similar in WT and KO mice (Fig. 1A). The mortality after TAC was significantly higher in KO mice than WT mice (Fig. 1B). We found that acute or subacute heart failure was the main cause of death confirmed by postmortem examination (pulmonary edema or hemorrhage was noted in most of the dead mice. Lung-to-body weight ration was 13.1 ± 2.3 mg/g for dead mice in adiponectin KO mice, 11.4 ± 1.9 mg/g for dead mice in WT group). Body weight (BW) and blood pressure (determined by tail cuff measurement) were similar before TAC (BW: 27.1 ± 0.4 g in KO, 27.7 ± 0.4 g in WT) and 3 weeks after TAC (BW: 24.5 ± 1.4 g in KO, 25.5 ± 0.7 g in WT).

3.2. Earlier transition from hypertrophy to heart failure in KO mice

Serial echocardiographic examinations showed that the heart function evaluated by LVEF and LVFS progressively

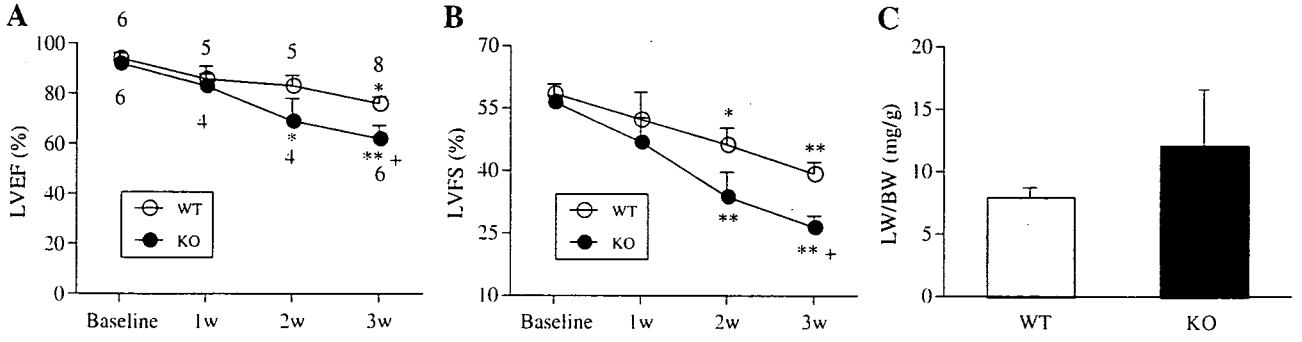

Fig. 2. The transition from hypertrophy to heart failure. A) Left ventricular ejection fraction (LVEF) and B) left ventricular fractional shortening (LVFS) were progressively depressed in adiponectin KO mice after 1 week of TAC, and the transition to heart failure occurred at 2 weeks after TAC in KO mice, which was confirmed by sacrifice to show an significant increase of lung-to-body weight ratio (C, $n=4$ for both WT and TAC mice). The number of mice in each time point for echocardiographic examination is indicated above or under the data points. $^{*}P<0.05$, $^{**}P<0.01$ vs. baseline, $^{\dagger}P<0.05$ vs. WT mice.

depressed in both adiponectin KO and WT mice over the course of 3 weeks (Fig. 2A, B). Two weeks after TAC, a significant reduction of LVEF and LVFS was noted in KO mice, indicating a proceeded transition to heart failure. To confirm the occurrence of heart failure, we sacrificed four mice in both KO and WT groups at 2 weeks after TAC and found a marked pulmonary congestion in KO mice (Fig. 2C).

3.3. Greater cardiac hypertrophy in KO mice

Three weeks after TAC, mice were sacrificed after echocardiographic examination. The wet heart-to-body weight ratio (HW/BW) was increased by 53% in TAC WT mice compared with sham WT mice, whereas HW/BW was dramatically increased by 110% in adiponectin TAC KO mice vs. sham KO mice. There was a significant difference of HW/BW between WT and KO TAC mice (Fig. 3A–C, E). The cross-sectional surface area of cardiac myocytes was significantly larger in KO mice than WT mice (Fig. 3F). There were no significant differences of HW/BW and cardiac myocyte cross-sectional surface area between WT and KO sham mice. These findings indicate that cardiac hypertrophy was far more extensive in adiponectin KO mice. We also examined myocardial and perivascular fibrosis and did not find significant difference between WT and KO TAC mice (Fig. 3D).

3.4. Worse pulmonary congestion in KO mice

We confirmed in previous studies that pulmonary edema is a reliable index of cardiac function in this model [22–24]. Severe pulmonary congestion was found in adiponectin KO mice. Compared with sham mice, the lung-to-body weight ratio (LW/BW) was increased by 170% in KO TAC mice,

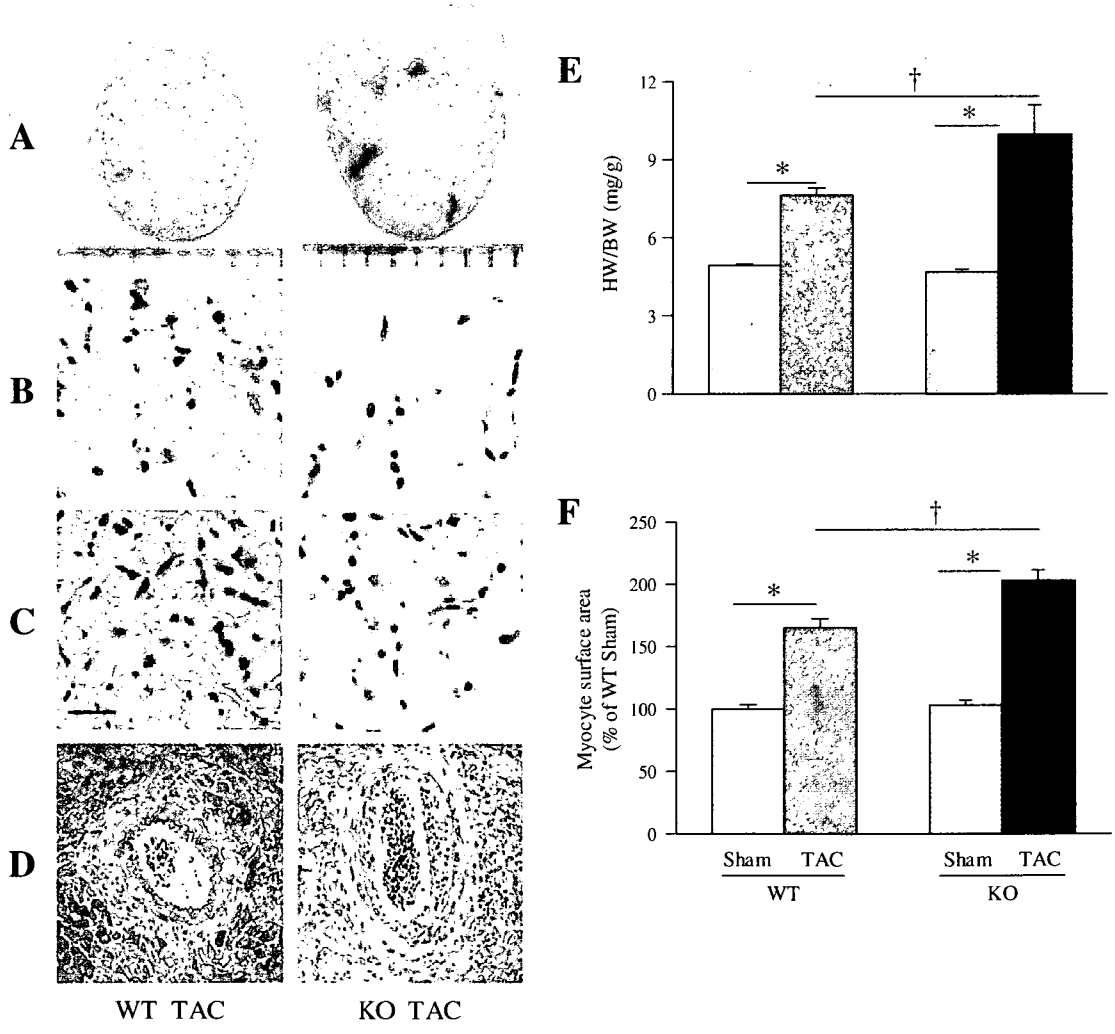

Fig. 3. Cardiac remodeling was more severe in KO mice. A) Representative pictures of cardiac hypertrophy in WT and KO mice at 3 weeks after TAC. B and C) Represent long-axis and cross-sectional views of cardiac myocytes with HE staining. D) Represents cardiac fibrosis with Azan staining (×100 magnification). HW/BW (E, $n=5$ in both sham groups, $n=8$ in WT TAC group, and $n=6$ in KO TAC group) and the cardiac myocyte cross-sectional surface area (F, $n=2$ in each sham group and $n=3$ in each TAC group) were increased significantly in KO mice compared with their wild-type (WT) littermates. *$P<0.01$, †$P<0.05$. Bar=20 μm for B and C.

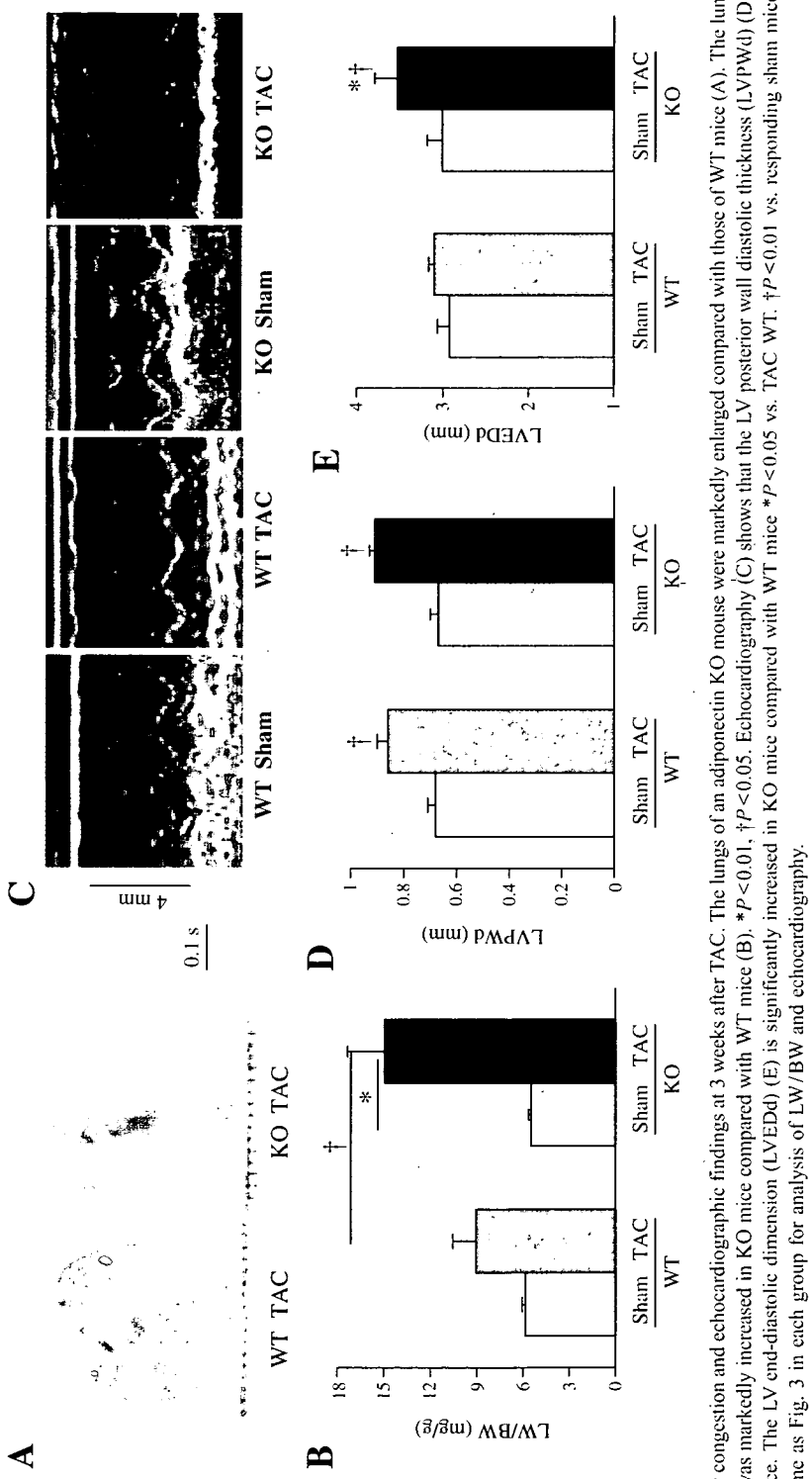

Fig. 4. Pulmonary congestion and echocardiographic findings at 3 weeks after TAC. The lungs of an adiponectin KO mouse were markedly enlarged compared with those of WT mice (A). The lung-to-body weight ratio (LW/BW) was markedly increased in KO mice compared with WT mice (B). *$P<0.01$, †$P<0.05$. Echocardiography (C) shows that the LV posterior wall diastolic thickness (LVPWd) (D) is similar in KO and WT TAC mice. The LV end-diastolic dimension (LVEDd) (E) is significantly increased in KO mice compared with WT mice *$P<0.05$ vs. TAC WT. †$P<0.01$ vs. responding sham mice. The number of animals is the same as Fig. 3 in each group for analysis of LW/BW and echocardiography.

whereas there was only a 55% increase in WT TAC littermates (Fig. 4A, B). There was no significant difference in LW/BW between KO and WT sham mice. We did not evaluate LV hemodynamics using a Millar pressure catheter because most of the KO mice appeared to be too weak to endure this procedure (including anesthesia) at 3 weeks after TAC.

3.5. Echocardiography findings

Because anesthesia has a significant influence on echocardiography data in mice [25] and most of the KO TAC mice were too weak for anesthesia at 3 weeks after TAC, we developed a method of performing echocardiographic examination in conscious mice. Compared with WT TAC mice, there was a significant decrease in both LV fractional shortening (LVFS) and the LV ejection fraction (LVEF) in KO TAC mice (Fig. 2A, B), and marked LV chamber dilation was observed in KO TAC mice (Fig. 4C, D). In contrast, there were no significant differences in these parameters between WT sham and KO sham mice. These findings indicate an increase in cardiac remodeling under pressure overload in adiponectin KO mice.

3.6. Myocardial AMPK expression

AMPK consists of one catalytic subunit (α) and two noncatalytic subunits (β and γ). Because AMPKα was reported to be activated by adiponectin [12], we examined the AMPKα_1 and α_2 protein expression in the hearts of WT and KO mice. As shown in Fig. 5, in the presence of LV pressure overload, AMPKα expression increased significantly, but the increment of AMPKα protein was less in KO than in WT hearts. These findings suggested that adiponectin deficiency means that the expression of AMPK cannot be increased sufficiently enough to provide adequate cardiac protection under stress conditions.

3.7. Increase of fasting glucose and IR

As IR is closely associated with cardiac remodeling [4–7] and adiponectin deficiency can lead to diet-induced IR [19], we determined the influence of adiponectin deficiency on glucose metabolism and IR in mice with LV pressure overload. As shown in Fig. 6A, fasting glucose levels increased by 40% in KO mice at 3 weeks after TAC, but rose by only about 20% in WT littermates, suggesting that the glucose metabolisms were more impaired in the adiponectin KO mice. Meanwhile, a similar increase in serum insulin was noted in both WT and KO TAC mice (Fig. 6B). As an index of IR, HOMA-IR was more increased in adiponectin KO mice than in WT mice at three weeks after TAC (Fig. 6C). Furthermore, we found a significant positive correlation between IR and the heart weight-to-body weight ratio in adiponectin KO mice rather than in WT

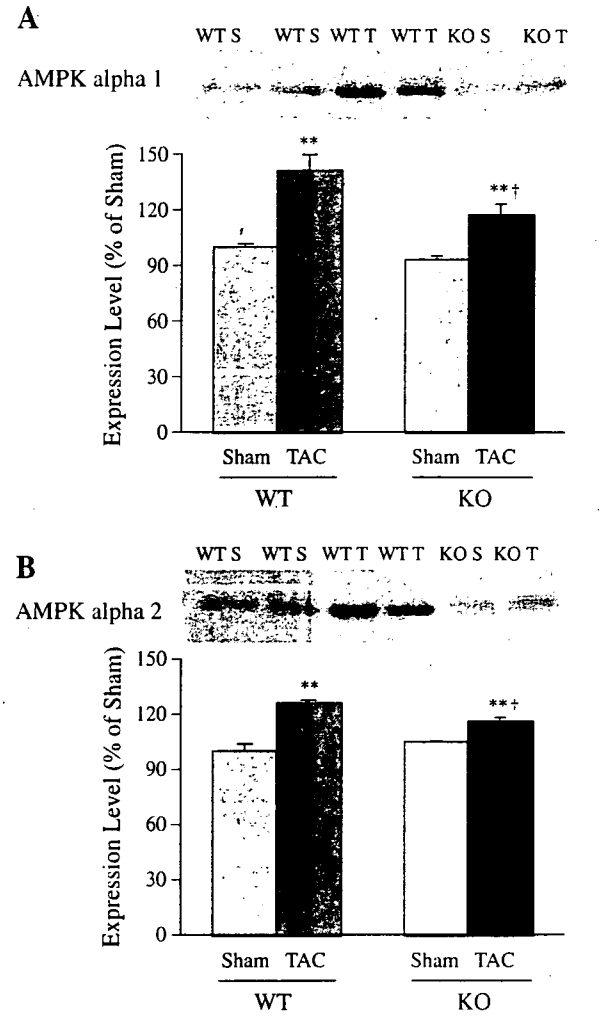

Fig. 5. Myocardial expression of AMPK. AMPKα_1 (A), α_2 (B) were increased in TAC mice, but the change was smaller in KO mice ($n=3$ in each group. $^{**}P<0.01$ vs. responding sham mice: $^{\dagger}P<0.05$ vs. WT TAC). S: sham, T: TAC.

mice (Fig. 6D), indicating that IR might also be involved in cardiac remodeling in adiponectin KO mice.

4. Discussion

In this study, we found that adiponectin deficiency worsens cardiac remodeling induced by LV pressure overload, and this change was associated closely with a decrease in the expression of AMPK, and an increase in IR. These results are consistent with a recent study by Shibata et al. [18] showing that pressure overload for one week in adiponectin KO mice resulted in greater cardiac hypertrophy and higher mortality. Differently, this study further investigated the potential role of adiponectin-deficiency on the development of cardiac hypertrophy and chronic heart failure. We demonstrated that the transition from hypertrophy to heart failure proceeded in adiponectin KO mice. Additionally, we investigated the influence of adiponectin

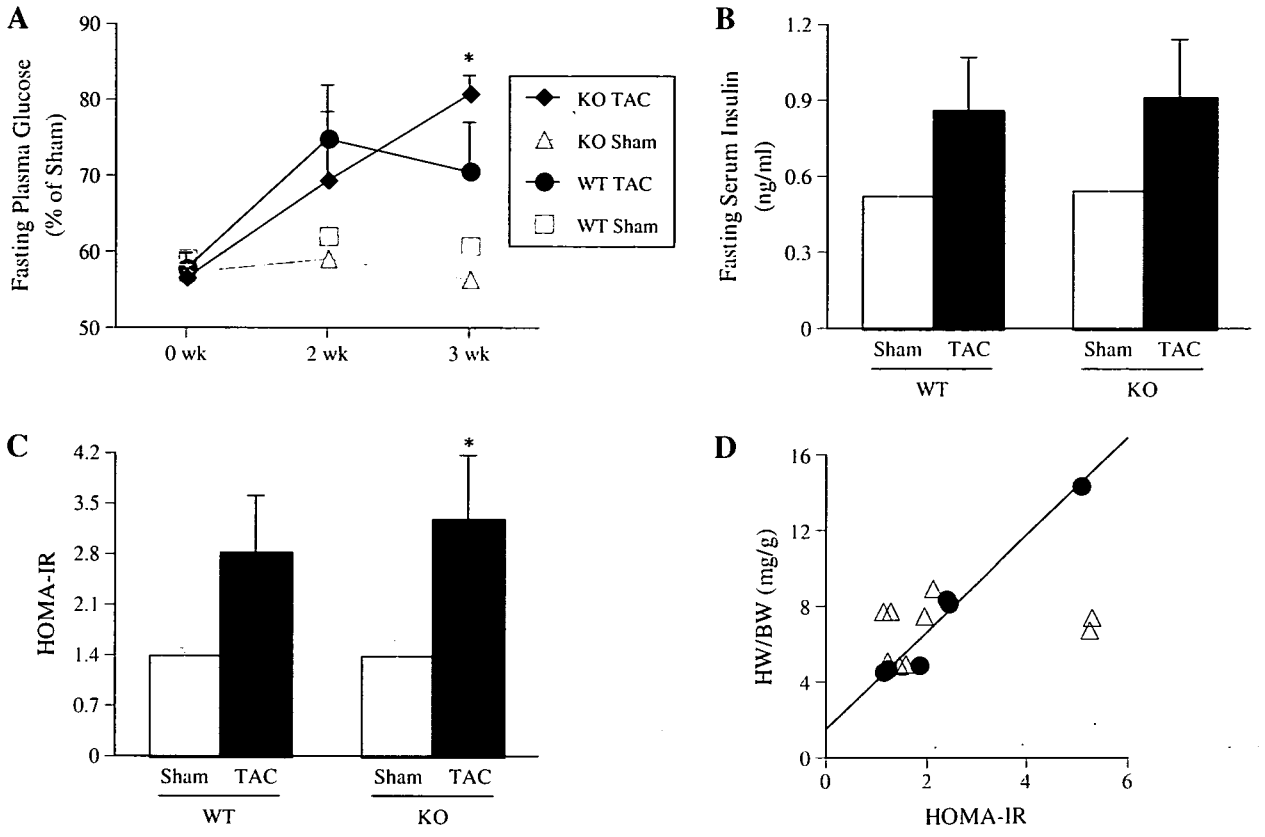

Fig. 6. Changes in glucose metabolism. Fasting glucose levels (A) were increased in adiponectin KO mice at 3 weeks after the onset of TAC, $*P<0.01$ vs. WT TAC ($n=5$ for all the groups at 0 week and for both sham groups at other two time points; $n=4$ for WT and KO TAC mice at 2 weeks, and $n=5$ and 3 for WT and KO TAC mice at 3 weeks, respectively). Serum insulin (B) was increased after TAC, but no significant difference was found between WT and KO mice, while the insulin resistance index HOMA-IR (C) was increased in KO mice. $\dagger P<0.05$ vs. KO sham ($n=3$ in both KO sham and TAC groups, $n=3$ in WT sham and $n=6$ in TAC groups). Linear correlation between HOMA-IR and HW/BW in both WT and KO mice groups (D) irrespective of TAC, $r=0.982$, $P<0.0001$, $n=6$ for KO mice (solid circle), while no significant correlation was found for WT mice ($n=9$, open triangle).

on glucose metabolism and addressed the important relation between metabolism and cardiac remodeling.

An increase in IR, glucose intolerance, and a proinflammatory state are among the six components of the MetS related to CVD, which is viewed as the primary outcome of this syndrome. In the present study, we noted that adiponectin deficiency induced an increase in IR and fasting glucose levels in the presence of pressure overload, suggesting that adiponectin has a strong influence on MetS and subsequently on cardiac remodeling. An increase in IR appears to downregulate adiponectin receptor expression via the phosphoinositide 3-kinase/Foxo1-dependent pathway [26]. In addition, Foxo1 is recognized as a negative regulator of insulin sensitivity [27], so it is theoretically acceptable that adiponectin knockout leads to MetS or that adiponectin KO mice are more susceptible to MetS under pathological stress. Although the exact relationship between MetS and CVD is not clear, both genetic and environmental factors may be involved. There is evidence that neuroendocrine factors [28] or the RAS (review [29]) may play an important role in MetS. We previously showed that plasma concentrations of catecholamines and renin were increased by LV pressure overload in mice [23]. In the present study, in addition to endogenous adiponectin deficiency, activation of the sympathoadrenal system and renin–angiotensin system (RAS) may have contributed to the onset of MetS.

The impact of MetS on CVD mortality has been investigated in several clinical studies [30–32]. It is generally agreed that CVD mortality is higher in subjects with MetS than in those without it. We found a positive correlation between IR and cardiac hypertrophy in adiponectin KO mice rather than in WT mice in this study, with both IR and HW/BW higher in adiponectin KO mice than in WT mice, suggesting that deficiency of adiponectin contributed to enhanced cardiac remodeling. Consistent with our results, a recent case-control study found that abnormal LV geometry and LV dysfunction were related to MetS [33]. Additionally, it is well known that type 2 diabetic patients are susceptible to diabetic cardiomyopathy, and the fasting plasma insulin level was reported to be the strongest independent predictor of LV mass in type 2 diabetes [34]. Taken together, these findings support the concept that MetS has an impact on cardiac remodeling. Although IR is known to be an important contributor to the

progression of heart failure, our data reported here are not enough to delineate the causal relationship between IR and cardiac remodeling. In spite of an increase tendency of IR showing in mice with cardiac hypertrophy, we did not find a significant correlation between IR and heart-to-body weight ratio in a relatively small sample of wild-type mice. In accordance with this study, previous clinical observations have shown IR to be related to the thickness of LV walls rather than LVH [35,36].

Adiponectin was reported to reduce the production of TNFα, and to improve both glucose metabolism and IR via the AMPK signaling pathway [12], suggesting that it may improve MetS. Evidence is emerging to demonstrate a critical role of AMPK in cardiac remodeling. Mutation of the gamma 2 subunit of AMPK has been shown to cause glycogen storage cardiomyopathy, and the influence of AMPKα on cardiac remodeling is another attractive research field. Both AMPKα_1 and AMPKα_2 expression were increased in hypertrophied hearts in the present study, which is only partially consistent with a previous investigation by Tian et al. [8]. They reported that α_1 was increased, α_2 expression was decreased, whereas activity of both AMPKα_1 and α_2 was increased in pressure overload rats. The reasons for this discrepancy are not clear. Generally, the activity of both AMPKα_1 and α_2 was reported to increase under stress conditions such as ischemia and pressure overload [8,10,18]. The protein expression of myocardial AMPK was seldom investigated and the reports are inconsistent. Acute ischemia [37] or short-term pressure overload [18] stimulates activity of myocardial AMPK without changing the AMPK protein expression, whereas both AMPKα_2 activity and expression were decreased at three weeks following volume-overload [38]. AMPK deficiency is reported to result in depressed LV function, increased myocardial necrosis, and apoptosis following ischemia/reperfusion injury [10]. The finding that AMPKα protein expression was increased in WT mice after TAC suggests that the augmentation of AMPKα signaling is a compensatory mechanism that attempts to maintain energy homeostasis in the heart under pressure overload. This mechanism may be partly controlled by adiponectin, because AMPK signaling was impaired in adiponectin KO mice and there was consequent progression of cardiac remodeling. Thus, this study provided a new link between adiponectin and AMPK in the process of cardiac remodeling. Apart from its influence on IR, AMPK, and TNFα, other mechanisms may also be involved in the beneficial effect of adiponectin on cardiac remodeling. Adiponectin has been reported to suppress superoxide generation and enhance eNOS activity [39], to have an antiproliferative effect [40], and to counteract beta adrenergic stimulation [41], all of which are closely related to cardiac remodeling [42]. Interestingly, AMPK and eNOS co-localize in hearts and AMPK was reported to activate eNOS [43,44]. Thus, it is reasonable for adiponectin deficiency to lead to progressive cardiac remodeling in response to pressure overload, as we showed in this study.

Acknowledgments

We thank Dr. Hidetoshi Okazaki, Hui Zhao and Dr Masakatsu Wakeno for their technical assistance. This work was supported by Grants (H13-Genome-011, H13-21seiki (seikatsu)-23) from the Ministry of Health, Labor and Welfare, Japan. Dr Liao is supported by a grant from the Japan Society for the Promotion of Science (P05228).

References

[1] Rutter MK, Meigs JB, Sullivan LM, D'Agostino RB, Wilson Sr. PW. C-reactive protein, the metabolic syndrome, and prediction of cardiovascular events in the Framingham Offspring Study. Circulation 2004;110:380–5.
[2] Isomaa B, Almgren P, Tuomi T, Forsen B, Lahti K, Nissen M, et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. Diabetes Care 2001;24:683–9.
[3] Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002;106:3143–421.
[4] Rutter MK, Parise H, Benjamin EJ, Levy D, Larson MG, Meigs JB, et al. Impact of glucose intolerance and insulin resistance on cardiac structure and function: sex-related differences in the Framingham Heart Study. Circulation 2003;107:448–54.
[5] Swan JW, Anker SD, Walton C, Godsland IF, Clark AL, Leyva F, et al. Insulin resistance in chronic heart failure: relation to severity and etiology of heart failure. J Am Coll Cardiol 1997;30:527–32.
[6] Paolisso G, De Riu S, Marrazzo G, Verza M, Varricchio M, D'Onofrio F. Insulin resistance and hyperinsulinemia in patients with chronic congestive heart failure. Metabolism 1991;40:972–7.
[7] Kemppainen J, Tsuchida H, Stolen K, Karlsson H, Bjornholm M, Heinonen OJ, et al. Insulin signalling and resistance in patients with chronic heart failure. J Physiol 2003;550:305–15.
[8] Tian R, Musi N, D'Agostino J, Hirshman MF, Goodyear LJ. Increased adenosine monophosphate-activated protein kinase activity in rat hearts with pressure-overload hypertrophy. Circulation 2001;104:1664–9.
[9] Asakawa M, Takano H, Nagai T, Uozumi H, Hasegawa H, Kubota N, et al. Peroxisome proliferator-activated receptor gamma plays a critical role in inhibition of cardiac hypertrophy in vitro and in vivo. Circulation 2002;105:1240–6.
[10] Russell III RR, Li J, Coven DL, Pypaert M, Zechner C, Palmeri M, et al. AMP-activated protein kinase mediates ischemic glucose uptake and prevents postischemic cardiac dysfunction, apoptosis, and injury. J Clin Invest 2004;114:495–503.
[11] Arad M, Moskowitz IP, Patel VV, Ahmad F, Perez-Atayde AR, Sawyer DB, et al. Transgenic mice overexpressing mutant PRKAG2 define the cause of Wolff–Parkinson–White syndrome in glycogen storage cardiomyopathy. Circulation 2003;107:2850–6.
[12] Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288–95.
[13] Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941–6.
[14] Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med 2001;7:947–53.