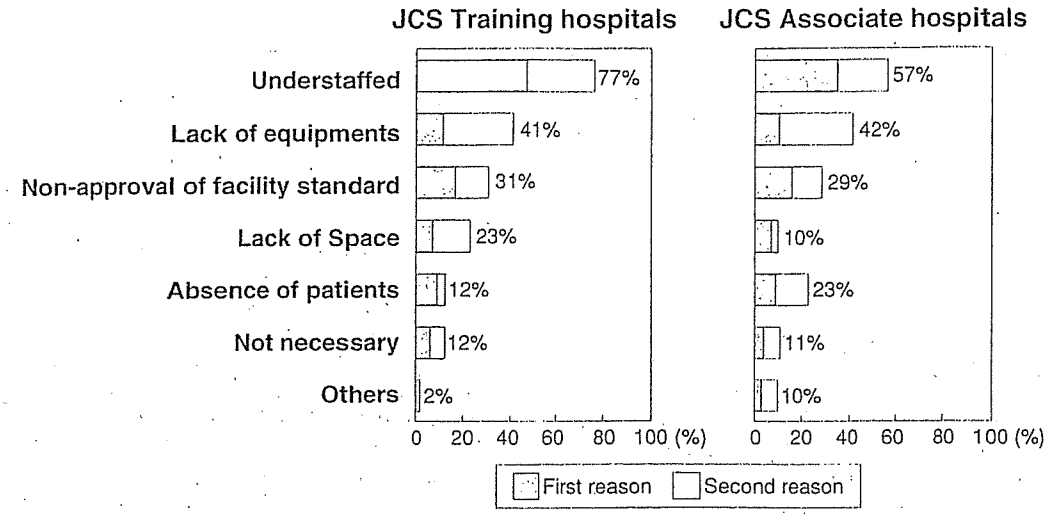

Fig 2. Reasons for not implementing cardiac rehabilitation (CR) in cardiology training hospitals authorized by the JCS. Data were collected from 222 of the 245 JCS training hospitals (Left) and 106 of the 128 JCS associate hospitals (Right) that were not performing any CR. The first and second reasons for non-implementation of rehabilitation were added. JCS, Japanese Circulation Society.

tient CR program of the same hospital when available (ie, a transfer rate of 100%), the participation rates of outpatient CR were only 10.6%, 0.4% and 0% among acute-phase survivors in THs, AHs, and NTHs, respectively. Furthermore, when we assumed the transfer rate from the in-hospital recovery-phase CR to outpatient CR to be 50%, the participation rates fell to 5.3%, 0.2%, and 0%, respectively.

From the data of this nationwide survey, the participation rates in CR after AMI in Japan were estimated. The numbers of patients who participated in any CR and recovery-phase CR after AMI in the whole of Japan in the year of 2003 were calculated from the average number of patients in each hospital in each category and the numbers of hospitals in the 3 categories (Table 3), yielding a total of 14,989 patients and 9,811 patients per year who participated in any CR and recovery-phase CR, respectively, in Japan. When we assumed that the transfer rates from the in-hospital recovery-phase CR to outpatient CR to be 100% and 50%, respectively, the number of participants in outpatient CR programs was estimated to be 4,896 and 2,443 patients/year, respectively, for the whole of Japan. When we assumed the acute-phase survival rate to be 90%[15,16] this yielded the acute-phase survivors (ie, denominator of CR participation rates) in Japan to be 64,809 patients/year. As a result, the estimated nationwide participation rates in any CR and recovery-phase CR were 23.1% and 15.1%, respectively, among the acute-phase survivors. Finally, the nationwide participation rate in outpatient CR was calculated to be only 3.8–7.6%, depending on a transfer rate from the in-hospital recovery-phase CR to outpatient CR after hospital discharge of 50–100% (Table 3).

Reasons for Non-Implementation of CR

In the present survey, 222 of the 245 THs and 106 of the 128 AHs that did not have any CR program for AMI gave reasons for not implementing rehabilitation (Fig 2). When the 1st and 2nd reasons were added, the 3 major reasons for non-implementation in both THs and AHs were lack of staff, lack of equipment and lack of achieving CR facility standards. The 4th reason was lack of CR space in THs, compared with lack of participating patients in AHs.

Discussion

Major Findings

This is the first nationwide survey of the implementation of CR for AMI patients in Japan. The major findings are: (1) in contrast to the broad dissemination of acute-phase invasive procedures for AMI, the implementation of recovery-phase and outpatient CR after AMI is extremely poor in Japan; the implementation rate of outpatient CR was only 9.3%, even in JCS THs, and the nationwide participation rate in outpatient CR was estimated to be only 3.8–7.6%; (2) the quality of CR programs reflected by implementation rates of patient education programs and exercise prescriptions based on exercise testing was also poor; and (3) the major reasons for not implementing CR were lack of staff, equipment and space, and not achieving the CR facility standards. These data clearly indicate that recovery-phase and outpatient CR for AMI is severely underutilized in Japan.

Hospital Implementation of CR in Japan

The present study has demonstrated that most THs and AHs in Japan are aggressively treating patients with AMI with invasive procedures such as emergency PCI, but that the implementation rates of all types of CR are disproportionately low relative to the high implementation rates of invasive procedures. In particular, THs have sufficient beds and staff cardiologists, an ICU and sufficient numbers of hospitalized AMI patients, so there seems to be no objective reason for not implementing CR for AMI.

A recent survey has reported that there are 2,621 CR programs in the USA[17] whereas according to the Japanese Association of Cardiac Rehabilitation, the number of hospitals approved for CR in Japan was only 186 in February 2005. This number accounted for only 15.9% of the total number (1,170 hospitals) of JCS-authorized THs (THs and AHs) that are treating 80% of all hospitalized AMI patients in Japan, and also accounted for only 15.0% of the 1,240 hospitals performing PCI according to the Japan Coronary Intervention Study[11] Clearly, this small number of CR-approved hospitals is a major obstacle for the nationwide

spread of CR in Japan[12].

From the present result, the number of hospitals that have an outpatient CR program is estimated to be only 85 in Japan (859×9.3%+311×1.5%), which is even less than half of the CR-approved hospitals (85/186=45.7%). In contrast, almost all of the 2,621 CR programs in the USA are conducted as outpatient programs[17] The length of hospital stay of AMI patients is rapidly decreasing because of early ambulation after aggressive reperfusion therapy (ie, less physical deconditioning) and the socioeconomic pressure on hospitals. Because the shorter hospital stay prevents patients from receiving enough education and instruction on life-style modification for secondary prevention, there is an increasing need for outpatient CR after discharge[2,18] Even when the smaller population (≈1/2) and the lower disease prevalence (≈1/5) in Japan than in the USA are taken into account, the number of the facilities for outpatient CR in Japan (approximately 1/30) is disproportionately small.

Patient Participation Rate in CR in Japan

The patient participation rates in the present survey can be compared with those in a previous report[12] which estimated the participation rate of recovery-phase CR in 1996–1998 to be 12% in JCS THs and 5% in all hospitals in Japan. The present result of a participation rate of 17.2% in THs in 2003 is slightly higher, but largely in accordance with the previous report, indicating that the participation rate in CR is slowly increasing but remains low in JCS THs. On the other hand, the nationwide participation rate in recovery-phase CR of 15.1% is higher than the previous estimation of 5%[12] possibly because of an increase in the proportion of patients hospitalized in THs or an increase in the implementation of recovery-phase CR in NTHs.

The low patient participation rate of 3.8–7.6% in outpatient CR in the whole of Japan (Table 3) is in accordance with the low implementation rate of outpatient CR programs. This is the first assessment of the patient participation rate in outpatient CR in Japan. Because the proportion of patients who subsequently participate in the outpatient CR program among the initial participants in the in-hospital CR program (ie, the transfer rate) is usually less than 50%, the estimated lower rate of 3.8% could even be overestimated. In the United States, the participation rate of AMI patients in phase II (usually outpatient-type) CR has been reported to be 11–47%[19–23] and a recent community survey reported an even higher participation rate of 55% in Omland County, Minnesota[24] Therefore, it is clear that the patient participation rate in outpatient CR is markedly lower in Japan than in the USA. Because the role of post-AMI outpatient CR is rapidly emerging in the era of short hospital stay, it is critically important to urgently increase both the number of CR-approved facilities and the patient participation rates in outpatient CR in Japan.

Quality of Care in CR

The present survey has revealed that the standard procedures in CR, such as patient education programs, exercise prescription based on exercise tests, and cardiopulmonary exercise tests with expiratory gas analysis, are poorly implemented even in JCS THs in Japan (Fig 1). All these activities and procedures are important components of a comprehensive CR program[1,2,7,25] Therefore, not only an increase in the implementation rate but also an enhancement of the quality of care in CR should be aimed for in Japan. Thus, future surveys should assess not only the implementation of exercise training but also the implementation of these comprehensive activities in CR.

Reasons for Non-Implementation of CR

The reasons for not implementing CR in THs were lack of staff, lack of equipments, lack of achieving approval for a CR facility, and lack of CR space. Before this survey, the difficulty in fulfilling the CR facility standards had been thought to be the main reason for the low implementation rate of CR in Japan. However, THs are usually large, general hospitals that would be expected to have sufficient staff, equipment and space. In addition, the present result that 73% (175/240) of THs that had been approved for specific intensive care did not have approval for CR despite their ability to fulfill the CR facility standards indicates that there are reasons other than the CR facility standards for the non-implementation of CR in these hospitals.

Ades et al reported that according to multivariate analysis, the strength of the physician's recommendation for participation was the most powerful predictor of entry into CR by patients after AMI or coronary bypass surgery[20] Thus, physicians' reluctance or ignorance regarding CR after AMI might be a reason for the low implementation rate of CR in Japan. Because the CR facility standards in Japan have been loosened in 2004 and 2006, the motivation of both physicians and the hospitals would be a critically important factor for the implementation of CR.

Because the beneficial effects of CR on exercise capacity, coronary risk factor reduction, quality of life, and prognosis (cardiovascular mortality and total mortality) in patients after AMI have been established[1–7,26] the low implementation rate of CR implies that patients are not participating in CR for reasons unrelated to their physical conditions. Thus, efforts should be made urgently to increase the implementation rate of CR in Japan. To achieve this goal, it appears necessary to increase the number of hospitals approved for CR and to enhance physicians' understanding of the benefits of CR after AMI.

Study Limitations

This was a hospital-based survey using a questionnaire, so the reliability of data depends on the accuracy of diagnosis and the patient statistics in the surveyed hospitals. However, the close agreement of the estimated total number of hospitalized AMI patients in the present survey in 2003 (71,201 patients) and that of a the previous nationwide survey in 2000 (66,459 patients)[14] suggests that the data collected in the present survey are reliable.

The relatively low response rate (59%) in the present survey compared with the previous survey[14] might have yielded a potential statistical bias. However, similar or even lower response rates have been reported in other nationwide surveys[27,28] In addition, when the hospitals that replied and those that did not reply were compared, there were no significant differences in the numbers of total hospital beds (THs: Reply 467±258 beds vs No-reply 446±241 beds, NS; AHs : Reply 262±133 beds vs No-reply 275±141 beds, NS; NTHs: Reply 138±114 beds vs No-reply 143±111 beds, NS) or in the regional distribution (ie, northeast or southwest Japan, urban or rural areas) between the 2 hospital groups, suggesting that a statistical bias caused by the low reply rate should be negligible.

Because the present survey did not investigate the actual numbers of acute-phase survivors and participants in outpatient CR in each hospital, the participation rate in outpatient

CR had to be estimated on the basis of some assumptions. However, because we used assumptions that would lead to higher participation rates, the results should be biased, if anything, toward overestimation, rather than underestimation of participation rates. Even with the possible overestimation, the participation rates in all CR in Japan were extremely low.

Conclusion

This first nationwide survey of CR demonstrated that, in contrast to the broad dissemination of acute-phase PCI for AMI, the implementation of recovery-phase CR, especially outpatient CR, is extremely poor in Japan. In addition, patient education programs and exercise prescriptions based on exercise testing are only poorly implemented. Considering the established benefits of CR in patients with AMI, urgent efforts should be made to improve this marked underutilization of recovery-phase and outpatient CR in Japan.

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Appendix 1

The Japanese Cardiac Rehabilitation Survey Investigators

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Enhanced cardiac production of matrix metalloproteinase-2 and -9 and its attenuation associated with pravastatin treatment in patients with acute myocardial infarction

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ABSTRACT

Previous experimental studies have demonstrated that MMPs (matrix metalloproteinases) contribute to LV (left ventricular) remodelling. We hypothesized that cardiac MMPs are activated in patients with AMI (acute myocardial infarction) and, if so, MMP production may be attenuated by statins (3-hydroxy-3-methylglutaryl-CoA reductase inhibitors) through their cardiovascular protective actions. We studied 30 patients, ten control patients with stable angina pectoris and 20 patients with AMI, in whom LV catheterization at the chronic stage was performed 22 ± 12 days (value is mean ± S.D.) after the onset of AMI. Blood samples were collected from the CS (coronary sinus) and a peripheral artery. In patients with AMI, the levels of MMP-2 and MMP-9 were significantly ($P < 0.05$) higher in the CS than the peripheral artery (MMP-2, 853 ± 199 compared with 716 ± 127 ng/ml; MMP-9, 165 ± 129 compared with 98 ± 82 ng/ml), whereas no significant differences were observed in the patients with angina pectoris. The CS–arterial concentration gradients of MMP-2 and MMP-9 correlated positively with BNP (brain natriuretic peptide) levels (MMP-2, $R = 0.68$, $P < 0.01$; MMP-9, $R = 0.59$, $P < 0.05$) and LV end-diastolic volume index (MMP-2, $R = 0.70$, $P < 0.01$; MMP-9, $R = 0.70$, $P < 0.01$). When patients with AMI treated with 10 mg of pravastatin or without ($n = 10$ in each group) were compared, this statin therapy significantly ($P < 0.05$) decreased the CS–arterial concentration gradients of MMP-2 (69 ± 43 compared with 213 ± 185 ng/ml) and MMP-9 (14 ± 27 compared with 119 ± 84 ng/ml). In conclusion, the enhanced production of cardiac MMP-2 and MMP-9 is associated with LV enlargement and elevated BNP levels in patients with AMI. A pleiotropic effect of statins appears to be associated with the modulation of cardiac MMP activation, which may be potentially beneficial in the attenuation of post-infarction LV remodelling.

Key words: acute myocardial infarction, angina pectoris, brain natriuretic peptide (BNP), metalloproteinase (MMP), remodelling, statin, tissue inhibitor of metalloproteinases (TIMP).

Abbreviations: ACE-I, angiotension-converting enzyme inhibitor; AMI, acute myocardial infarction; Ang II, angiotensin II; AP, angina pectoris; BNP, brain natriuretic peptide; CK, creatine kinase; CRP, C-reactive protein; CS, coronary sinus; LDL, low-density lipoprotein; LV, left ventricular; LVEDVI, LV end-diastolic volume index; LVEF, LV ejection fraction; MMP, matrix metalloproteinase; TGF-β, transforming growth factor-β; TIMP, tissue inhibitor of metalloproteinases; WBC, white blood cell.

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INTRODUCTION

The loss of myocytes as a consequence of AMI (acute myocardial infarction) results in progressive changes in ventricular architecture [1,2]. This process, defined as post-infarction ventricular remodelling, is associated with a higher mortality and a higher incidence of complications, such as the development of heart failure, aneurysm formation and ventricular rupture [3,4]. During the remodelling process, as well as intrinsic changes in cardiac myocytes, it has been recognized that important alterations also occur within the extracellular matrix of the myocardium [5,6].

MMPs (matrix metalloproteinases) belong to a family of zinc-containing endoproteinases responsible for extracellular protein degradation, and are inhibited by specific tissue inhibitors [TIMP (tissue inhibitor of metalloproteinases)] [5,6]. In experimental myocardial infarction, MMPs are up-regulated in myocardial tissues, and are the driving force in extracellular matrix remodelling and infarct expansion [7,8]. Among the MMPs, the importance of MMP-9 during the processes of infarct healing and LV (left ventricular) remodelling has been demonstrated in previous studies using genetically modified mice [9,10]. Infarcted mice with the targeted deletion of MMP-9 had a decreased incidence of early myocardial rupture [9] and progressive LV dilation [10]. However, in the clinical setting, there has been little evidence regarding the production of MMPs in the infarcted human heart.

Statins have various cardiovascular protective actions, including anti-inflammatory and anti-apoptotic actions, independent of their effects on cholesterol levels. A study using a mouse AMI model demonstrated that statin treatment attenuated LV remodelling [11], which was associated with decreased MMP activity [12].

In the present study, we hypothesized that cardiac MMP activation may be associated with the degree of LV enlargement and the level of BNP (brain natriuretic peptide), a biochemical marker of post-infarction remodelling [13,14]. If so, MMP production may be attenuated by statin treatment in patients with AMI.

MATERIALS AND METHODS

Patients

This study included 30 male patients. All of the patients gave their written informed consent prior to participation in the study. The Institutional Ethical Committee on Human Research approved the study protocol. Patients with the following disorders were excluded from the study: prior myocardial infarction, and liver (elevated activities of aminotransferases), kidney (elevated level of creatinine or urea) or lung dysfunction (restrictive or obstructive pattern in spirometry).

The control group consisted of ten patients with stable AP (angina pectoris), who complained of symptoms consistent with Canadian Cardiovascular Society Classification of angina level I, II or III, with evidence of myocardial ischaemia. All of the control patients had no evidence of a previous AMI, and had severe coronary artery stenosis and therefore underwent coronary angioplasty (with adjunctive stenting in five patients). The treated sites were the left anterior descending artery in four patients (40 %), the right or left circumflex artery in four patients (40 %), and both the left anterior descending and right coronary arteries in two patients (20 %).

We also studied 20 patients with AMI who fulfilled the following criteria: typical chest pain >30 min of duration, ST segment elevation >0.1 mV in two or more ECG leads with the subsequent evolution of a typical infarct pattern, and increased serum CK (creatine kinase) level. A total of 14 patients underwent PTCA (percutaneous transluminal coronary angioplasty) of the infarct-related artery (with adjunctive stenting in nine patients), and the remaining six patients received an intravenous administration of a tissue-type plasminogen activator and/or heparin in the acute phase. In all the patients, coronary angiography immediately after treatment showed a TIMI 3 grade flow in the infarct-related artery. The elapsed time to reperfusion was 4.6 h on average. The infarct sites were in the anterior wall in ten patients (50 %), the inferior wall in seven patients (35 %) and the postero-lateral wall in three patients (15 %). In this study, all of the patients with AMI were treated with the ACE-I (angiotension-converting enzyme inhibitor) enalapril (5 mg) after their hospital admission. Among them, ten patients with hyperlipidaemia (total cholesterol level > 220 mg/dl) were treated with 10 mg of pravastatin; the remaining ten patients did not have hyperlipidaemia and thus did not receive pravastatin. A recent Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA) trial [14a] has shown a similar decrease in coronary artery disease incidence following treatment with 10–20 mg of pravastatin used in Asia to that observed for 20–40 mg doses used in Europe and the United States.

Cardiac catheterization and analysis of LV function

In patients with AMI, chronic-stage cardiac catheterization was repeated approx. 3–4 weeks after the onset of AMI. A 5 French multipurpose catheter (Cathex) was introduced into the CS (coronary sinus) through the left subclavian vein under fluoroscopic guidance [14]. The position of the catheter tip was confirmed by the injection of contrast medium. Blood samples were collected from the CS before the intravenous administration of heparin. Following the collection of blood samples from the right brachial artery (as peripheral blood samples) through a 6 French sheath, heparin was administered and coronary

angiography and left ventriculography were performed, according to the conventional Judkins' technique. LV pressure was measured using a 2 French high-fidelity micromanometer catheter (Miller Instruments) advanced into the left ventricle via the lumen of a 6 French pig-tail catheter. The restenosis of a treated artery was defined as an arterial narrowing of >75%, as determined by coronary angiography.

LV volume was evaluated angiographically by a cardiologist who was blinded to the results of the biochemical assays. Ventricular silhouettes in a 30° right anterior oblique projection were digitized using an ANCHOR ventriculography analysis system (Siemens-Elema). Using the area-length method, LV end-systolic volume index, LVEDVI (LV end-diastolic volume index) and LVEF (LV ejection fraction) were calculated.

Biochemical assessment

Blood samples were centrifuged and serum was stored at −80 °C until assay. A sandwich enzyme immunoassay was performed to determine MMP-2 level (Fuji Chemical Industries) [15]. In addition, the level of MMP-9, another gelatinase-like MMP-2, and that of MMP-13, an interstitial collagenase, were analysed using MMP Biotrak enzyme-linked immunoadsorbent assay kits (Amersham Biosciences). The levels were back-calculated from the standard curve determined with the enzyme-linked immunoadsorbent assay kits using a 96-well microplate reader (Emax; Molecular Devices). These kits detect the pro-enzyme and the pro-enzyme complexed with TIMP. The detection limits were 0.5 ng/ml for MMP-2, 0.6 ng/ml for MMP-9 and 0.03 ng/ml for MMP-13.

We also measured levels of TIMP-1 (Fuji Chemical Industries) and TIMP-2 (Amersham Biosciences) using sandwich enzyme immunoassays [15]. The detection limits for TIMP-1 and TIMP-2 were 1.2 and 8.0 ng/ml respectively.

BNP was measured using specific immunoradiometric assay kits (Shionogi). The sensitivity of these kits was 2 pg/ml. Ang II (angiotensin II) and TGF-β (transforming growth factor-β) levels were also measured, as reported previously [16].

The serum CRP (C-reactive protein) level was measured by N Latex CRP II monoassay using a nephelometric analyser (BN II; Dade Behring). The lower detection limit of this test was 0.06 mg/dl. Total cholesterol, triacylglycerol (triglyceride) and HDL (high-density lipoprotein) cholesterol concentrations were determined by enzymatic methods using a Toshiba TBA 80M analyser. LDL (low-density lipoprotein) was calculated using Fredewald's formula. We also measured WBC (white blood cell) number.

Statistical analysis

The two groups were compared by Student's t test. Measurements from the CS and the peripheral artery were compared within a group by ANOVA. When a significant difference among groups was indicated by the initial analysis, individual paired comparisons were determined using the Student–Newman–Keuls method. A linear regression line was calculated by the least-square method to assess the correlation between two parameters. To investigate independent predictors, we used multivariate logistic regression analysis. In all cases, differences were considered significant at $P < 0.05$. Results are presented as means ± S.D., or medians.

Table 1 Clinical characteristics

*$P = 0.05$ and **$P < 0.01$ compared with control (patients with stable AP).

Characteristic	Patients with AMI ($n = 20$)	Patients with stable AP ($n = 10$)
Age (years)	66 ± 9	67 ± 6
Peak CK (units/l)	1986 (801–8574)	–
Cardiac function		
LVEF (%)	48 ± 7**	58 ± 7
LVEDVI (ml/m^2)	95 ± 18**	55 ± 21
Vessels >75% stenosed (n)	1.5 ± 0.7	1.6 ± 0.7
Risk factors (n)		
Hypertension	11 (55%)	7 (70%)
Diabetes mellitus	15 (75%)	6 (60%)
Hyperlipidaemia	10 (50%)	6 (60%)
Smoking	12 (60%)	6 (60%)
Biochemical parameters†		
Total cholesterol (mg/dl)	193 ± 27	198 ± 20
LDL (mg/dl)	120 ± 30	122 ± 31
WBC count (cells/μl)	6615 ± 1571	5600 ± 1063
CRP (mg/dl)	0.34 ± 0.33*	0.13 ± 0.06
Medication used (n)		
ACE-I	20 (100%)	4 (40%)
β-Blockers	11 (55%)	6 (60%)
Statins	10 (50%)	6 (60%)
Calcium antagonists	7 (35%)	5 (50%)
Nitrates	4 (20%)	2 (20%)
Aspirin	20 (100%)	10 (100%)

† Data obtained on the day when cardiac catheterization was performed.

RESULTS

The baseline clinical characteristics of the patients with AMI and the control patients with AP (without evidence of AMI) are summarized in Table 1. In the patients with AMI, cardiac function data were obtained at chronic-stage cardiac catheterization performed 22 ± 12 days after the onset of AMI. Coronary angiography revealed 90% stenosis of the infarct-related artery in two patients and 100% stenosis in three patients. These five patients with restenosis had received intravenous thrombolysis alone in the acute stage. In the remaining 15 patients, the treated

Table 2 Comparisons of BNP, MMP and TIMP levels in the CS and peripheral artery

*$P < 0.05$ compared with levels in artery; †$P < 0.05$ compared with control (patients with stable AP).

	Patients with AMI ($n = 20$)		Patients with stable AP ($n = 10$)	
Peptide	CS	Artery	CS	Artery
BNP (pg/ml)	400 ± 376*†	126 ± 176	54 ± 25	52 ± 25
MMP-2 (ng/ml)	853 ± 199*†	716 ± 127	631 ± 44	630 ± 46
MMP-9 (ng/ml)	165 ± 129*†	98 ± 82	68 ± 25	71 ± 24
MMP-13 (ng/ml)	0.05 ± 0.04	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
TIMP-1 (ng/ml)	155 ± 59	150 ± 53	130 ± 33	134 ± 32
TIMP-2 (ng/ml)	112 ± 18	108 ± 14	94 ± 11	97 ± 16

sites remained patent. With the exception of cardiac function (LVEF and LVEDVI) and the prevalence of ACE-I use, clinical characteristics were similar between patients with AMI and AP.

Enhancement of cardiac MMP production in patients with AMI

Table 2 shows the comparison of BNP, MMP and TIMP levels between blood samples from the CS and peripheral artery. In patients with AMI, levels of BNP, MMP-2 and MMP-9 were significantly ($P < 0.05$) higher in the CS than in the peripheral artery, whereas the levels of MMP-13, TIMP-1 and TIMP-2 were similar. In control patients with AP, no significant differences in the levels of BNP, MMPs and TIMPs were observed between the CS and peripheral artery. These findings indicate that the production of MMP-2 and MMP-9, as well as that of BNP, is enhanced in an infarcted heart.

Correlation of cardiac MMP production with post-infarction LV remodelling

In patients with AMI, the CS–arterial concentration gradients of MMP-2 and MMP-9 correlated positively with those of BNP and LVEDVI respectively (Figure 1), but not with LVEF, peak CK level and circulating WBC counts. These myocardial gradients were not different between patients with and without progression to restenosis (MMP-2, 87 ± 32 compared with 152 ± 173 ng/ml; MMP-9, 83 ± 86 compared with 61 ± 82 ng/ml).

Comparisons between pravastatin-treated patients with AMI and non-pravastatin-treated patients with AMI

We then compared levels of MMPs between ten patients treated with 10 mg of pravastatin and ten patients not treated with pravastatin (Table 3). Although the total cholesterol level before treatment was higher ($P < 0.05$) in the pravastatin-treated patients with AMI (223 ± 7 mg/dl in treated patients compared with 195 ± 17 mg/dl in

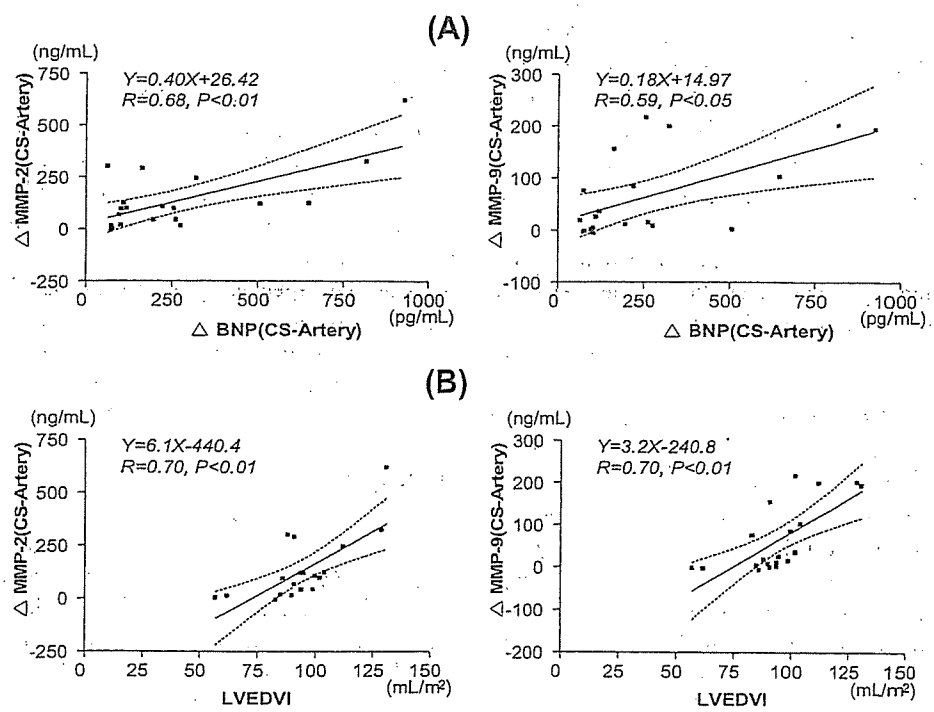

Figure 1 Correlations between CS–arterial concentration gradients of MMP-2 and -9 and BNP (A) and LVEDVI (B) in 20 patients with AMI

Table 3 Comparisons of MMPs between pravastatin-treated and non-pravastatin-treated patients

* $P < 0.05$ compared with levels in artery; † $P < 0.05$ compared with levels in non-pravastatin-treated patients. CS–artery, CS–arterial concentration gradient.

	Patients with AMI						Patients with stable AP					
	Pravastatin-treated ($n = 10$)			Non-pravastatin-treated ($n = 10$)			Pravastatin-treated ($n = 6$)			Non-pravastatin-treated ($n = 4$)		
MMP (ng/ml)	CS	Artery	CS–artery	CS	Artery	CS–artery	CS	Artery	CS–artery	CS	Artery	CS–artery
MMP-2	808 ± 182	739 ± 158	69 ± 43†	897 ± 216*	684 ± 84	213 ± 185	631 ± 53	624 ± 51	7 ± 23	629 ± 32	639 ± 43	−9 ± 53
MMP-9	94 ± 61†	80 ± 59	14 ± 27†	236 ± 142*	117 ± 100	119 ± 84	68 ± 20	72 ± 16	−4 ± 4	68 ± 20	69 ± 29	0 ± 5
MMP-13	0.06 ± 0.06	0.03 ± 0.03	0.03 ± 0.06	0.03 ± 0.02	0.05 ± 0.03	−0.01 ± 0.03	0.03 ± 0.04	0.04 ± 0.02	−0.01 ± 0.01	0.04 ± 0.02	0.03 ± 0.02	0.01 ± 0.03

non-treated patients), no significant differences were observed after treatment between the two groups (183 ± 31 mg/dl in treated patients compared with 201 ± 20 mg/dl in non-treated patients). Levels of CRP (0.18 ± 0.13 mg/dl in treated patients compared with 0.50 ± 0.40 mg/dl in non-treated patients; $P = 0.03$) and the CS–arterial concentration gradients of MMP-2 and MMP-9 (Table 3) were significantly different between the two groups. However, the concentration gradients of TGF-β and Ang-II were similar between patients treated with pravastatin and those not treated (Ang-II, 19.5 ± 20.2 compared with 36.9 ± 32.4 pg/ml respectively; TGF-β, 1.2 ± 3.3 compared with 2.1 ± 4.7 pg/ml respectively).

We then performed multivariate analysis for the predictors of CS–arterial concentration gradients of MMP levels, including age, sex, coronary risk factors, peak CK, infarct site (anterior wall), CRP, TIMP, pravastatin treatment, LVEF and LVEDVI. The association between pravastatin treatment and cardiac MMP-2 production was modest, with an odds ratio of 0.074 (95 % confidence interval, 0.005–1.109; $P = 0.06$), and did not reach statistical significance.

DISCUSSION

The major findings of the present clinical study are that after AMI, the cardiac production of MMP-2 and MMP-9 is enhanced and associated with LV enlargement and BNP secretion, and that the pleiotropic effect of statins appears to be associated with the modulation of cardiac MMP activation.

Among the MMP species, MMP-2 and MMP-9 play an important role in LV remodelling, as these MMPs are activated in the myocardium and it has been reported that the targeted deletion of these MMPs prevents post-infarction cardiac dysfunction and rupture [9,10]. In the clinical setting, circulating MMP-2 and MMP-9 levels have been measured in previous studies of patients with AMI [17–19]; however, these results were conflicting. Squire et al. [17] reported that circulating MMP levels were inversely correlated with LV dilatation, whereas Matsunage et al. [18] and Nakaya et al. [19] found that serum MMP levels and activity were positively correlated with LV dilatation. In addition, circulating MMP levels could be affected at the acute stage following reperfusion therapy and by the clinically vulnerable state [20–23]. In the present study, we focused on cardiac production of MMP [14], and the measurement was performed at the clinically stable stage following AMI. As shown in Table 2, despite similar levels of TIMPs, significant differences in levels of BNP, MMP-2 and MMP-9 were observed between the CS and the peripheral artery in patients with AMI. To our knowledge, this is the first study demonstrating the enhanced production of MMP-2 and MMP-9 in a human infarcted heart. Moreover, as shown

in Figure 1, the CS–arterial concentration gradients of MMP-2 and MMP-9 correlated positively with those of BNP and LVEDVI. Taking into account the delicate balance between MMPs and TIMPs in tissue remodelling, the present findings indicate that excessive cardiac production of MMPs may play an important pathological role in the progression of post-infarction LV dysfunction.

A previous experimental study of an AMI model using BNP-transgenic mice demonstrated a potential interaction of BNP with inflammation [24]. The overexpression of BNP leads to neutrophil infiltration and MMP-9 expression in the infarct region and increases the incidence of cardiac rupture. These findings suggest the significance of inflammatory reaction in the heart accompanied by changes in LV function. 3-Hydroxy-3-methylglutaryl-CoA reductase inhibitors, such as statins, exert various cardiovascular protective effects beyond their lipid-level lowering actions [12,25]. These pleiotropic effects include the inhibition of inflammatory responses. In the present study, we have shown that the CS–arterial concentration gradients of MMP-2 and MMP-9 were smaller in the pravastatin-treated group than in the non-pravastatin-treated group, which was accompanied by a decrease in CRP level. These findings indicate that pravastatin may modulate cardiac MMP production in patients with AMI, probably via its anti-inflammatory effects. Similar observations of decreased circulating MMP-2 levels in patients with AMI treated with 10 mg of paravastain have been reported previously [19].

There are several potential limitations of the present study. First, this study was not randomized. Pravastatin was administered to a small number of patients with AMI with hyperlipidaemia. In such a pro-inflammatory state, tissue MMPs might have been activated before treatment [26], which could affect the results. Therefore prospective studies will be required to determine if pravastatin has a causal role in reducing cardiac MMP production in patients with AMI. Secondly, the present study was carried out over the short term, whereas ventricular remodelling is known to progress over months or years. Thirdly, previous studies have shown that the renin–angiotensin system is also involved in the induction of post-infarction ventricular remodelling [27] and can be inhibited by statins [28,29]. However, we have shown that the CS–arterial concentration gradients of Ang II were similar between pravastatin-treated patients and non-pravastatin-treated patients. This may be related, in part, to the fact that all our patients with AMI had been treated with 5 mg of enalapril.

In conclusion, the present study demonstrates the enhancement of MMP production in an infarcted heart. Pleiotropic effects of statins may be associated with the modulation of cardiac MMP activation, which is potentially beneficial in the attenuation of post-infarction LV remodelling.

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Crystallization and preliminary X-ray crystallographic analysis of two vascular apoptosis-inducing proteins (VAPs) from *Crotalus atrox* venom

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VAPs are haemorrhagic snake-venom toxins belonging to the reprolysin family of zinc metalloproteinases. *In vitro*, VAPs induce apoptosis specifically in cultured vascular endothelial cells. VAPs have a modular structure that bears structural homology to mammalian ADAMs (a disintegrin and metalloproteinases). VAP1 is a homodimer with a MW of 110 kDa in which the monomers are connected by a single disulfide bridge. VAP2 is homologous to VAP1 and exists as a monomer with a MW of 55 kDa. In the current study, several crystal forms of VAP1 and VAP2 were obtained using the vapour-diffusion method and diffraction data sets were collected using SPring-8 beamlines. The best crystals of VAP1 and VAP2 generated data sets to 2.5 and 2.15 Å resolution, respectively.

1. Introduction

Haemorrhagic snake venoms contain factors that induce apoptosis specifically in cultured vascular endothelial cells (Araki *et al.*, 1993). The vascular apoptosis-inducing proteins VAP1 and VAP2 were originally isolated from the venom of the western diamondback rattlesnake *Crotalus atrox* (Masuda *et al.*, 1997, 1998) and similar apoptotic toxins (VAPs) have been isolated from other snake venoms (Masuda *et al.*, 2001; You *et al.*, 2003; Trummal *et al.*, 2005). VAP1 is a disulfide-bonded homodimeric protein with a molecular weight of 110 kDa and an isoelectric point of 8.5. VAP2 is an acidic single-chain protein with a molecular weight of 55 kDa and an isoelectric point of 4.5 (Masuda *et al.*, 1997, 1998). VAP1 (Masuda *et al.*, 2000) and VAP2 (S. Masuda, H. Hayashi & S. Araki, in preparation) are modular metalloproteinases with nucleotide-sequence homology to genes encoding the mammalian membrane-anchored metalloproteinases known as ADAMs. ADAMs are an emerging class of metalloproteinases whose function has been implicated in cell–cell and cell–matrix adhesion and signalling. They also appear to be associated with numerous diseases including arthritis, Alzheimer's disease and cancer (White, 2003; Blobel, 2005; Seals & Courtneidge, 2003; Moss & Bartsch, 2004; Duffy *et al.*, 2003).

Viperidae snake venoms contain a number of metalloproteinases, the snake-venom metalloproteinases (SVMPs), that induce local and systemic haemorrhage by disrupting the wall of the blood vessels in envenomed patients (Gutierrez *et al.*, 2005). All known VAPs belong to the P-III class of SVMPs, which have been shown to be the most potent haemorrhagic toxins from snake venoms. The P-III SVMPs have a modular structure consisting of metalloproteinase (M), disintegrin (D) and cysteine-rich (C) domains (Fox & Serrano, 2005). SVMPs and ADAMs are members of the reprolysin group of zinc-dependent metalloproteinases, which together with astasins, serralysin and matrix metalloproteinases comprise the metzincin superfamily of metalloproteinases (Bode *et al.*, 1993). All these enzymes share a signature consensus zinc-binding motif, HE*XX*H*XX*G*XX*H, in their catalytic region that defines proteins of the class, as well as a methionine-containing turn that serves as a structural base for the three active histidine residues (Bode *et al.*, 1993).

The crystal structures of several SVMPs of the P-I class, which contain only an M domain, and of isolated domains of ADAMs have

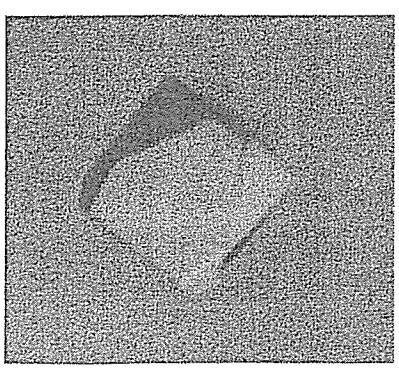

Table 1
Data-collection statistics for VAP1 crystals.

Values in parentheses are for the highest resolution shell. For each data set, a single crystal was used for measurement.

	Form 1-1	Form 1-2
Space group	$P4_12_12$	$P2_12_12_1$
Unit-cell parameters		
a (Å)	93.9	86.7
b (Å)	93.9	93.3
c (Å)	244.8	137.7
$\alpha = \beta = \gamma$ (°)	90	90
Beamline (detector)	BL45PX (Rigaku Jupiter)	BL45PX (Rigaku R-AXIS V)
Wavelength (Å)	0.98	1.0
Resolution (Å)	50–2.50 (2.59–2.50)	50–2.50 (2.59–2.50)
No. of unique reflections	38868 (3773)	38926 (3800)
R_{merge}†	0.084 (0.380)	0.072 (0.369)
$I/\sigma(I)$	18.7 (7.1)	14.4 (2.9)
Completeness (%)	99.7 (99.6)	99.4 (98.8)
Redundancy	12.7	3.91
No. of molecules in ASU	1	1
Matthews value (Å^3 Da^{-1})	2.5	2.5
Solvent content (%)	51	51

† $R_{merge} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl and $\langle I(hkl)\rangle$ is its average.

been determined. However, structures of SVMPs or ADAMs containing M, D and C domains have not been determined. To understand more about the structure of P-III SVMPs and ADAMs and how it relates to the molecular mechanism of VAP-induced apoptosis, we initiated the crystallographic analysis of VAP1 and VAP2. This is the first report of the crystallization and preliminary X-ray analysis of apoptotic SVMPs. Three-dimensional crystal structures of VAP1 derived from the two distinct crystal forms described in this report have recently been described (Takeda *et al.*, 2006); the structural analysis of VAP2 is ongoing.

2. Methods

2.1. Purification

VAP1 and VAP2 were purified as described previously (Maruyama *et al.*, 2005; Masuda *et al.*, 1998) with some modifications. Briefly, crude *C. atrox* venom (Sigma–Aldrich, USA) was dissolved in buffer containing 10 m*M* Tris–HCl pH 7.0 and 10 m*M* NaCl and then applied onto a CM-Sepharose (Amersham Bioscience, USA) column equilibrated with the same buffer. VAP2 was eluted from the column with the above buffer, whereas VAP1 was eluted with buffer containing 10 m*M* Tris–HCl pH 7.0 and 50 m*M* NaCl.

The VAP1 was further purified on a hydroxylapatite column. The VAP1-containing CM-Sepharose fraction was first diluted with an equal amount of distilled water and then applied onto a hydroxylapatite column equilibrated with 25 m*M* sodium phosphate pH 7.0. VAP1 was eluted using buffer containing 50 m*M* sodium phosphate pH 7.0 and then concentrated using an Amicon Ultra membrane (Millipore) with a nominal molecular-weight limit (NMWL) of 50 000 Da. The final protein concentration was 6.5 mg ml^{-1}. During the concentration step, the buffer was replaced with 10 m*M* Tris–HCl pH 7.0.

The VAP2-containing CM-Sepharose fraction was loaded onto a Resource Q (GM Healthcare) column equilibrated with 10 m*M* Tris–HCl pH 8.0 and 50 m*M* NaCl and then eluted with a gradient of NaCl. 55 kDa molecular-weight fractions, which were eluted at about 130 m*M* NaCl, were pooled and concentrated by Amicon Ultra with a 30 000 NMWL membrane. The final protein concentration was 3.8 mg ml^{-1} in buffer containing 10 m*M* Tris–HCl pH 8.0.

2.2. Initial crystallization screen

Initial screening for appropriate crystallization conditions for VAP1 and VAP2 was carried out using the sitting-drop vapour-diffusion method and Crystal Screen (Hampton Research, USA), with or without 63 µg ml^{-1} (almost twice the molar protein concentration) of the hydroxyamate inhibitor 3-(*N*-hydroxycarboxamide)-2-isobutyl-propanoyl-Trp-methylamide (GM6001, Calbiochem) in the protein solution. A volume of 0.3–0.5 µl protein solution was mixed with an equal amount of reservoir solution and droplets were allowed to equilibrate against 0.1 ml reservoir solution at 293 K.

2.3. Diffraction data collection

Crystals were cryoprotected, mounted in a nylon loop (Hampton Research, USA) or in a Lytho Loop (Protein Wave Corp., Japan) and immediately exposed to a stream of nitrogen gas at 100 K to flash-freeze the samples. The preliminary X-ray data were collected using an in-house X-ray diffractometer (Rigaku Micromax-007 X-ray generator with R-AXIS VII imaging-plate detector) and crystals that diffracted well were selected for data acquisition using the beamlines at SPring-8. All diffraction data sets were collected using undulator beamlines (BL41XU, BL45XU) at 100 K and diffraction images were processed using the *HKL*2000 software (Otwinowski & Minor, 1997).

3. Results

3.1. VAP1 crystals

3.1.1. Crystallization. VAP1 was reproducibly crystallized in two distinct crystal forms. Crystals were initially obtained using Crystal

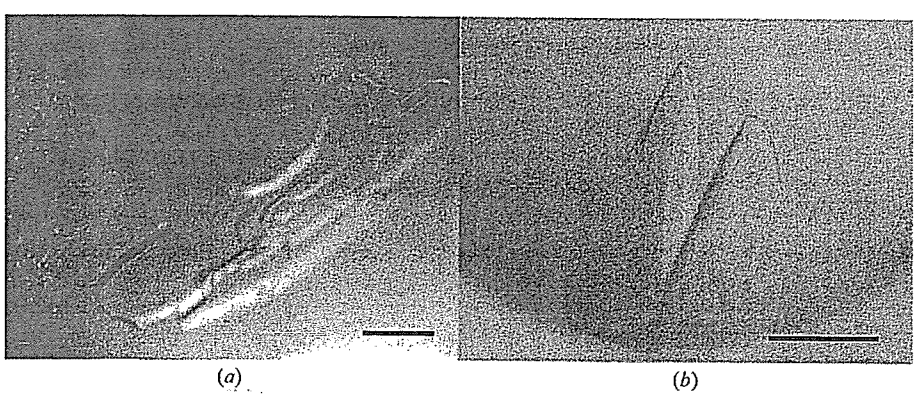

Figure 1
VAP1 crystals. (*a*) Form 1-1. (*b*) Form 1-2. The scale bars indicate 0.1 mm.

Table 2
Data-collection statistics for VAP2 crystals.

Values in parentheses are for the highest resolution shell. For each data set, a single crystal was used for measurement.

	Form 2-1	Form 2-2	Form 2-3	Form 2-4	Form 2-5
GM6001	+	+	+	+	−
Space group	$P2_1$	$P2_12_12_1$	$P4_1$	$P6_322$	$C2$
Unit-cell parameters					
a (Å)	56.9	57.7	60.7	156.8	220.7
b (Å)	138.0	118.2	60.7	156.8	79.5
c (Å)	59.2	138.5	257.9	95.6	58.7
α (°)	90	90	90	90	90
β (°)	91.5	90	90	90	91.7
γ (°)	90	90	90	120	90
Beamline (detector)	BL41XU (ADSC Quantum 310R CCD detector)				
Wavelength (Å)	1.0	1.0	1.0	1.0	1.0
Resolution (Å)	50–2.15 (2.23–2.15)	50–2.50 (2.59–2.50)	50–3.20 (3.31–3.2)	50–3.80 (3.94–3.80)	50–2.70 (2.80–2.70)
No. of unique reflections	48664 (4428)	33288 (2925)	15097 (1437)	7169 (682)	26911 (2313)
R_{merge}†	0.081 (0.196)	0.089 (0.321)	0.091 (0.360)	0.117 (0.397)	0.085 (0.231)
$I/\sigma(I)$	9.8 (4.6)	10.3 (3.7)	10.9 (4.0)	8.4 (6.5)	10.1 (5.5)
Completeness (%)	98.1 (89.5)	98.6 (88.4)	99.5 (95.7)	99.8 (99.9)	95.9 (82.5)
Redundancy	3.3	6.5	7.0	19.2	3.4
No. of molecules in ASU	2	2	2	1	2
Matthews value (Å^3 Da^{-1})	2.4	2.4	2.5	3.1	2.7
Solvent content (%)	49	49	50	60	54

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith intensity measurement of reflection hkl and $\langle I(hkl)\rangle$ is its average.

Screen solution No. 46, but these crystals diffracted poorly. Subsequently, droplets were prepared by mixing 1 µl protein solution and 1 µl reservoir solution containing 15% PEG 8000, 0.1 *M* sodium cacodylate pH 6.5 and then equilibrated against 1 ml reservoir solution. Within a couple of weeks, using the hanging-drop method, improved tetragonal crystals (form 1-1; Fig. 1*a*) were obtained.

Orthorhombic crystals (form 1-2; Fig. 1*b*) were obtained using Additive Screen (Hampton Research, USA). The droplet was made by mixing 0.3 µl protein solution and 0.3 µl reservoir solution supplemented with one-fifth of the volume of 0.1 *M* cobalt(II) chloride (Additive Screen solution No. 4). The best crystals were obtained using the sitting-drop method after equilibration for 3 d against 0.1 ml of the same reservoir solution used to obtain form 1-1 crystals.

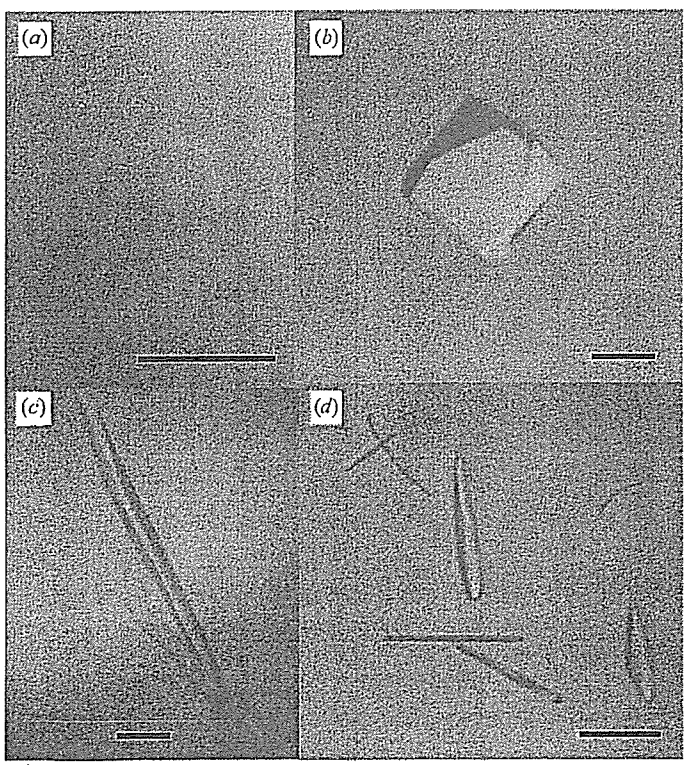

Figure 2
VAP2 crystals. (*a*) Form 2-2, (*b*) form 2-3, (*c*) form 2-4 and (*d*) form 2-5 crystals. The scale bars indicate 0.1 mm.

3.1.2. X-ray analysis. For X-ray measurements, crystals of either crystal form were soaked in a solution containing 15% PEG 8000, 5% methanol, 20% xylitol and 0.1 *M* sodium cacodylate pH 6.5 for cryoprotection prior to flash-freezing. X-ray diffraction data were obtained by the oscillation method using beamline BL45XU and an oscillation angle of 0.75° per image. Data sets were collected using a CCD detector (Rigaku Jupiter) for crystal form 1-1 or an imaging-plate detector (Rigaku R-AXIS V) for crystal form 1-2. The unit-cell parameters and the data statistics for the two crystal forms are summarized in Table 1. The structures were determined at 2.5 Å resolution by the molecular-replacement method using the P-I SVMP acutolysin-C (PDB code 1qua) as a starting model (Takeda *et al.*, 2006). The coordinates and the structure factors have been deposited in the PDB (2erq for form 1-1 and 2ero for form 1-2 crystals).

3.2. VAP2 crystals

3.2.1. Crystallization. Five distinct crystal forms of VAP2 were analyzed by X-ray diffraction. The initial screening for VAP2 crystals was performed in the presence and absence of the inhibitor GM6001.

In the presence of GM6001, Crystal Screen solution No. 10 yielded crystals. With this as a starting condition, the pH of the mother liquor, the PEG concentration and molecular weight and the species and concentrations of salts and additives were optimized and four distinct crystal forms were obtained (forms 2-1, 2-2, 2-3 and 2-4). These four forms were only obtained in the presence of GM6001 and were never obtained in its absence. Monoclinic (form 2-1) and orthorhombic (form 2-2; Fig. 2*a*) forms were obtained by the sitting-drop method under identical conditions as follows: droplets were made by mixing 0.5 µl protein solution with 0.5 µl reservoir solution containing 30% PEG 8000, 0.1 *M* ammonium acetate, 0.1 *M* sodium cacodylate pH 6.5 and were equilibrated against 0.1 ml reservoir solution. Tetragonal form crystals (form 2-3; Fig. 2*b*) were obtained by adding a one-tenth volume of 1 *M* potassium chloride (Additive Screen solution No. 16)

to the mother liquor and using a reservoir solution containing 30% PEG 8000, 0.1 *M* ammonium acetate, 0.1 *M* sodium acetate pH 4.6 with the same drop and reservoir volumes described above. Hexagonal crystals (form 2-4; Fig. 2*c*) were obtained by the hanging-drop method using 1 ml of a reservoir solution containing 20% PEG 20 000, 0.2 *M* calcium acetate, 0.1 *M* sodium cacodylate pH 6.5. The droplet was made by mixing 1 µl protein solution and 1 µl reservoir solution supplemented with a one-fifth volume of 0.3 *M* glycyl-glycyl-glycine solution (Additive Screen solution No. 34).

In the absence of GM6001, crystals were obtained with Crystal Screen solution No. 46, but these crystals yielded poor diffraction data. To improve the quality of the crystals, several additives were screened. Monoclinic crystals (form 2-5; Fig. 2*d*) were obtained by adding a one-tenth volume of 40% *n*-propanol solution (Additive Screen solution No. 90) to the reservoir solution (final composition 4% *n*-propanol, 16.2% PEG 8000, 0.18 *M* calcium acetate, 0.09 *M* sodium cacodylate pH 6.5). A mixture of 0.5 µl protein solution and 0.5 µl reservoir solution was equilibrated against 0.1 ml reservoir solution. These form 2-5 crystals were only obtained in the absence of GM6001 and were never obtained in its presence.

3.2.2. X-ray analysis. The mother liquors of the form 2-2 and 2-3 crystals were suitable for freezing; all others were first cryoprotected. For form 2-1 and 2-4 crystals, 20% glycerol was added to the reservoir solution for cryoprotection. For form 2-1, the cryogenic solution was added gradually to the crystal droplet in order to avoid cracking induced by osmotic shock. Crystal form 2-5 was rinsed in a solution containing 15% PEG 8000, 5% methanol, 20% xylitol and 0.1 *M* sodium cacodylate pH 6.5 and then immediately flash-frozen at 100 K. Because these crystals were extremely thin and fragile, they were mounted in a LithoLoop, an etched Mylar film, to prevent bending of the crystal.

All diffraction data sets for the VAP2 crystals were acquired using the oscillation method and beamline BL41XU (the oscillation angle was 1.0° for all data sets) at a wavelength of 1.0 Å and data were collected using an ADSC Quantum 310R detector. The unit-cell parameters and statistics for the data sets are summarized in Table 2. The estimated number of molecules in the asymmetric unit for each crystal form was obtained by a preliminary molecular-replacement method using *MOLREP* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994) and the metalloproteinase (M) and cysteine-rich (C) domains of VAP1 (Takeda *et al.*, 2006) as the starting models. Structural analyses of these crystals along with the molecular-replacement phases are ongoing.

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B-Type Natriuretic Peptide Strongly Reflects Diastolic Wall Stress in Patients With Chronic Heart Failure

Comparison Between Systolic and Diastolic Heart Failure

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OBJECTIVES We explored the stimulus for B-type natriuretic peptide (BNP) secretion in the clinical setting of heart failure (HF).

BACKGROUND Increasingly, plasma BNP levels are being incorporated into the clinical assessment and management of systolic heart failure (SHF) as well as diastolic heart failure (DHF). However, heterogeneity in BNP levels among individuals with HF can cause some confusion in interpreting results.

METHODS In 160 consecutive patients presenting with HF, we measured plasma BNP levels and performed echocardiography and cardiac catheterization. Systolic and diastolic meridional wall stress was calculated from echocardiographic and hemodynamic data.

RESULTS Although plasma BNP had a significant correlation ($r^2 = 0.296$ [$p < 0.001$]) with left ventricular end-diastolic pressure (EDP) as previously reported, the correlation between plasma BNP and end-diastolic wall stress (EDWS) ($r^2 = 0.887$ [$p < 0.001$]) was more robust. In a subanalysis of 62 patients with DHF, a similar result was obtained ($r^2 = 0.143$ for EDP and $r^2 = 0.704$ for EDWS). In a comparison between SHF and DHF, the BNP level was significantly higher in SHF ($p < 0.001$). Although EDP did not show any difference, EDWS was significantly higher in SHF than in DHF ($p < 0.001$).

CONCLUSIONS The present study shows that plasma BNP levels reflect left ventricular EDWS more than any other parameter previously reported, not only in patients with SHF, but also in patients with DHF. The relationship of left ventricular EDWS to plasma BNP may provide a better fundamental understanding of the interindividual heterogeneity in BNP levels and their clinical utility in the diagnosis and management of HF. (J Am Coll Cardiol 2006;47:742–8)
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Plasma B-type natriuretic peptide (BNP) levels are reported not only to be a strong marker of left ventricular (LV) dysfunction, but also a marker to predict morbidity and mortality accurately in patients with chronic heart failure (HF) (1,2). Recently, BNP-guided therapy for chronic HF has been suggested. Troughton et al. (3) demonstrated that pharmacotherapy guided by BNP levels reduces cardiovascular events and delays time to first cardiovascular event compared with intensive clinically guided therapy. Recent reports also demonstrated the contribution of LV diastolic function to plasma BNP levels and the usefulness of BNP in the diagnosis of diastolic HF (4).

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However, heterogeneity in BNP levels among individuals with HF has been recognized, and it has caused some confusion in interpreting results (5). Previous human studies have suggested correlations between BNP levels and cardiac functional or dimensional indexes such as end-diastolic pressure (EDP), ejection fraction (EF), pulmonary capillary wedge pressure, and LV volume, none of which sufficiently explain the heterogeneity (6–9). Therefore, it is essential to determine the stimulus for BNP secretion in the clinical setting of HF. In vitro studies have clarified the mechanism of secretion and regulation of BNP precisely (10). Stretch of cardiomyocytes is reported to be the most important stimulus of BNP regulation (11). It is also believed that BNP in humans may be released from the heart in response to increased wall stress. However, there have been few human studies exploring a direct relationship between wall stress and BNP regulation (12). Vanderheyden et al. (13) have very recently demonstrated, for the first time, in 40 patients with aortic stenosis (AS), a significant correlation of BNP with LV end-diastolic wall stress (EDWS). In their study, however, subjects were limited to patients with AS. Hence, there now is a need for the same assessment in patients

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Abbreviations and Acronyms

AS	= aortic stenosis
BNP	= B-type natriuretic peptide
CHF	= congestive heart failure
DHF	= diastolic heart failure
EDP	= end-diastolic pressure
EDWS	= end-diastolic wall stress
EF	= ejection fraction
HF	= heart failure
LV	= left ventricle/ventricular
LVEDVI	= left ventricular end-diastolic volume index
LVMI	= left ventricular mass index
SHF	= systolic heart failure
SWS	= systolic wall stress

with HF of various etiologies. Accordingly, in the present study, we evaluated plasma BNP levels in 160 consecutive patients presenting with HF of various etiologies including diastolic HF.

METHODS

Patients. Among the patients referred to our National Cardiovascular Center Hospital between October 2003 and December 2004, we included in this study those admitted with congestive heart failure (CHF) consecutively. Patients who did not undergo LV catheterization or had renal dysfunction (serum creatinine >2.0 mg/dl) were excluded. A sample of 160 patients was obtained. For all participants, cardiac catheterization and echocardiograms were performed at a compensated CHF stage (before discharge), and plasma BNP was measured on the day before cardiac catheterization. The clinical characteristics of these patients are listed in Table 1.

BNP assay. Blood was collected into tubes containing EDTA, and plasma BNP was measured using a validated and commercially available immunoassay kit (Tosoh Co. Ltd., Japan).

Cardiac catheterization. Left ventricular pressure was recorded with a 5-F pigtail catheter connected to a fluid-filled transducer. Left ventricular volume and EF were determined with left ventriculography with contrast medium using Kennedy's formula.

Echocardiography. Echocardiographic examinations were performed with a Sonos 5500 machine equipped with a 2.5-MHz probe. M-mode images were obtained to measure left atrial and ventricular dimensions (14). The left ventricular mass index (LVMI) was estimated from the formula of Devereux et al. (15). The severity of mitral regurgitation was quantified on a semicontinuous scale from none (0) to moderately severe (3+). In patients with sinus rhythm, the pulsed Doppler transmitral flow velocity was recorded to measure a ratio of peak mitral E-wave velocity to peak mitral A-wave velocity (E/A ratio) and the deceleration time of the mitral E-wave velocity.

On the basis of hemodynamic and echocardiographic data, end-diastolic and systolic meridional wall stresses (WS) were calculated. These were obtained by using the formula: $WS = 0.334 \times P(LVID)/WT(1 + WT/LVID)$, where P = LV pressure (i.e., peak systolic pressure or EDP, which was obtained during cardiac catheterization), LVID = left ventricular internal dimension, and WT = wall thickness (16). In the present study, the posterior wall thickness was used to assess WT regardless of regional wall motion abnormalities. In the analysis of the interobserver reproducibility of the posterior wall thickness measurement in 48 patients with CHF, a high degree of the reproducibility was

Table 1. Patient Characteristics

	Total	SHF	DHF	p Value
n	160	98	62	
Women	31	25	40	0.052
Age, yrs	66.8 ± 1.0	66.3 ± 1.3	67.7 ± 1.6	0.485
BMI, kg/m^2	22.9 ± 0.3	22.8 ± 0.4	23.1 ± 0.4	0.684
NYHA functional class ≥2	32	37	24	0.138
HT	71	61	87	0.001
DM	35	36	34	0.946
HLP	53	49	58	0.338
AF	18	17	19	0.912
Etiology				
DCM	18	30	0	
ISCM or OMI	29	44	6	
HHD	26	9	53	
VHD	26	17	40	
Medications				
ACEI or ARB	70	77	57	0.013
Beta-blocker	51	54	46	0.397
Diuretics	60	71	42	0.001
BNP, pg/ml	282 ± 23	379 ± 33	129 ± 13	<0.001

Values are mean ± SEM or %.

ACEI = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin receptor blocker; BMI = body mass index; DCM = dilated cardiomyopathy; DHF = diastolic heart failure; DM = diabetes mellitus; HHD = hypertensive heart disease; HLP = hyperlipidemia; HT = hypertension; ISCM = ischemic cardiomyopathy; NYHA = New York Heart Association; OMI = old myocardial infarction; SHF = systolic heart failure; VHD = valvular heart disease.

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Table 2. Echocardiographic and Hemodynamic Parameters

	Total (n = 160)	SHF (n = 98)	DHF (n = 62)	p Value
FS, %	27 ± 1	20 ± 1	38 ± 1	<0.001
LVEDD, mm	57 ± 1	61 ± 1	50 ± 1	<0.001
LVMI, g/m^2	166 ± 4	179 ± 5	145 ± 6	<0.001
LAD, mm	45 ± 1	45 ± 1	44 ± 1	0.779
E/A	1.3 ± 0.1	1.5 ± 0.2	1.0 ± 0.1	0.024
EF, %	41.5 ± 1.1	32.0 ± 0.9	56.4 ± 0.5	<0.001
LVEDVI, ml/m^2	106 ± 4	125 ± 15	76 ± 2	<0.001
LVSP, mm Hg	134 ± 3	124 ± 3	151 ± 4	<0.001
LVEDP, mm Hg	14.9 ± 0.4	15.0 ± 0.6	14.8 ± 0.5	0.829

Values are mean ± SEM.

EF = ejection fraction; E/A = ratio of peak mitral E-wave velocity to peak mitral A-wave velocity; FS = fractional shortening; LAD = left atrial dimension; LVEDD = left ventricular end-diastolic dimension; LVEDP = left ventricular end-diastolic pressure; LVEDVI = left ventricular end-diastolic volume index; LVMI = left ventricular mass index; LVSP = left ventricular peak systolic pressure. Other abbreviations as in Table 1.

found with an intraclass correlation coefficient value 0.830 (95% confidence interval 0.609 to 0.925), and absolute difference was small (mean ± SD; 0.01 ± 1.16 mm). Also, adequate M-mode images were not available in three patients, and they were excluded in the present study.

Statistical analysis. Comparisons between groups were made using chi-square analysis for proportions and unpaired Student *t* tests for continuous variables. Linearity of a relationship between two variables was assessed by linear regression analysis; $p < 0.05$ was considered significant. Results were expressed as mean ± SEM.

RESULTS

Patient characteristics. Clinical characteristics of the group of 160 patients are summarized in Table 1. Mean age was 66.8 ± 1.0 years (range 20 to 87 years), and 31% of the patients were women. In all, 98 patients had HF symptoms with an LV EF of ≤50%. These comprised the systolic heart failure group (SHF). The diastolic heart failure group (DHF) was comprised of 62 patients with preserved systolic function (LV EF >50%). Mean age and body mass index did not differ significantly between SHF and DHF groups, while there was a trend of more female patients in DHF. A history of hypertension and etiologies of dilated cardiomyopathy and ischemic cardiomyopathy/old myocardial infarction were more prevalent in SHF. Patients with SHF were more likely to be taking angiotensin-converting enzyme inhibitors or angiotensin receptor blockers and diuretics.

Geometric and functional parameters obtained by echocardiography or cardiac catheterization are shown in Table 2. In total patients, mean EF was 41.5 ± 1.1% (range 13% to 66%), and mean LVMI and LV end-diastolic volume index (LVEDVI) were 166 ± 4 g/m^2 and 106 ± 4 ml/m^2, respectively.

Correlations of plasma BNP to echocardiographic and hemodynamic parameters. Scatter plots of plasma BNP levels (dependent variable) against some echocardiographic and hemodynamic parameters (independent) are shown in Figure 1. There were strong correlations between LV EF, LVEDVI or LV end-systolic volume index, or LV EDP and plasma BNP (coefficient of correlation; $r^2 = 0.325$, 0.343, 0.421, and 0.328, respectively). There were weak correlations with parameters of transmitral Doppler flow $r^2 = 0.201$ and 0.101 for E/A and deceleration time, respectively. In contrast, LVMI and left atrial diameter did not show significant correlations with BNP levels. Although LV systolic wall stress (SWS) calculated by echocardiographic and hemodynamic parameters showed a modest correlation ($r^2 = 0.277$), a correlation of BNP with LV EDWS was much more robust ($r^2 = 0.887$).

Although age, gender, and atrial fibrillation were not significantly associated, body mass index (BMI) and New York Heart Association functional class ≥II were associated with BNP levels ($p < 0.001$ in both).

Comparison between SHF and DHF. Plasma BNP levels were significantly higher in SHF than in DHF (median [interquartile range]; 267 [136 to 583] and 105 [64 to 146] pg/ml, respectively, $p < 0.001$); however, EDP levels did not show any differences as shown in Figure 2 and Table 2. Other parameters such as SWS, EDWS, LV end-diastolic dimension, LVMI, LVEDVI, and LV peak systolic pressure were significantly higher in SHF than in DHF ($p < 0.001$). Scatter plots in patients with SHF and DHF are demonstrated in Figures 3A and 3B and Figures 3C and 3D, respectively. End-diastolic wall stress showed a better correlation with BNP ($r^2 = 0.704$) than EDP ($r^2 = 0.143$) in DHF as well as in SHF.

Subanalysis in patients without local wall motion abnormality. It is conceivable that this estimation of wall stress did not accurately reflect the entire non-uniform LV wall stress in patients with regional asynergy in LV wall motion or with variation in segmental LV wall thickness. In the present study, 83% of patients with ischemic cardiomyopathy or old myocardial infarction and 28% with dilated cardiomyopathy had regional wall motion abnormalities. Therefore, a subanalysis was performed for patients without local wall motion abnormality (n = 105). As a result, an even stronger correlation was obtained as shown ($r^2 = 0.919$). A correlation in patients with regional wall motion abnormality (n = 55) was still strong ($r^2 = 0.820$).

DISCUSSION

Heterogeneity of BNP levels among individuals with HF can cause some confusion in interpreting results. It has been unclear why some patients with LV EF <35% have BNP levels in the normal range whereas others exhibit extremely elevated levels, and why some patients with isolated diastolic dysfunction (i.e., with normal EF) show a similar increase of plasma BNP as do the patients with severe systolic dysfunction. One of the answers to the question has been the change of EDP levels in the LV (6). Another recent report has demonstrated that heterogeneity of BNP levels in patients with systolic HF reflects the severity of diastolic abnormality, right ventricular function, and mitral regurgi-

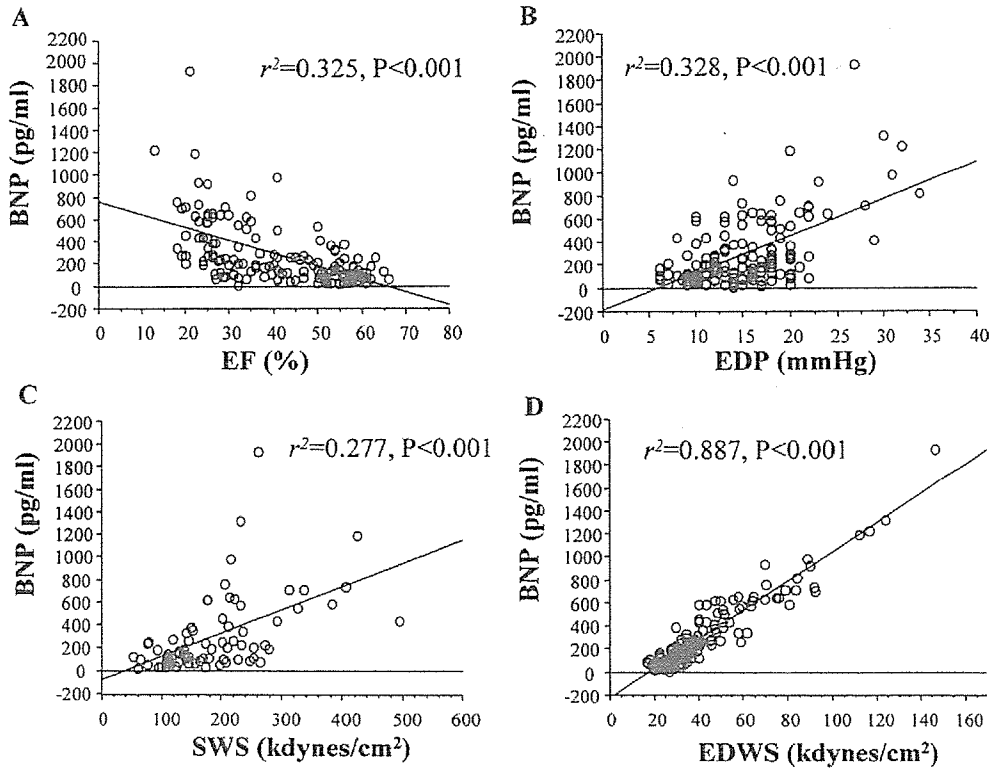

Figure 1. Correlation between B-type natriuretic peptide (BNP) and left ventricular functional parameters in all 160 patients. **(A)** Left ventricular ejection fraction (EF) (%). **(B)** End-diastolic pressure (EDP) (mm Hg). **(C)** End-systolic wall stress (SWS) (kdynes/cm^2). **(D)** End-diastolic wall stress (EDWS) (kdynes/cm^2).

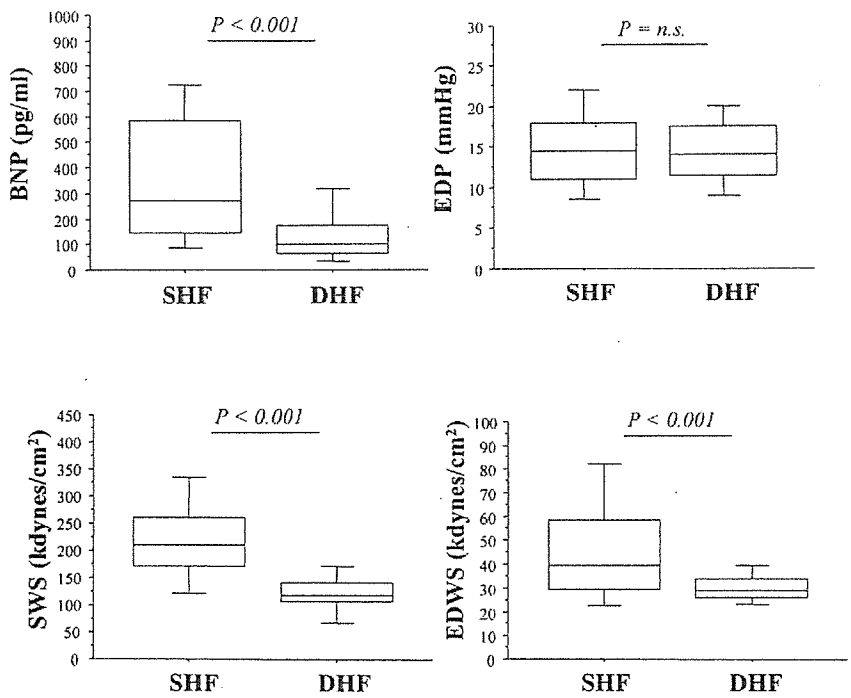

Figure 2. Differences of B-type natriuretic peptide (BNP) and left ventricular functional parameters between systolic heart failure (SHF) (n = 98) and diastolic heart failure (DHF) (n = 62). The **box** defines the interquartile range with the median indicated by the **crossbar**. The **error bars** indicate the 10th and 90th percentiles. EDP = end-diastolic pressure (mm Hg); EDVI = end-diastolic volume index (ml/m^2); EDWS = end-diastolic wall stress (kdynes/cm^2); SWS = end-systolic wall stress (kdynes/cm^2).

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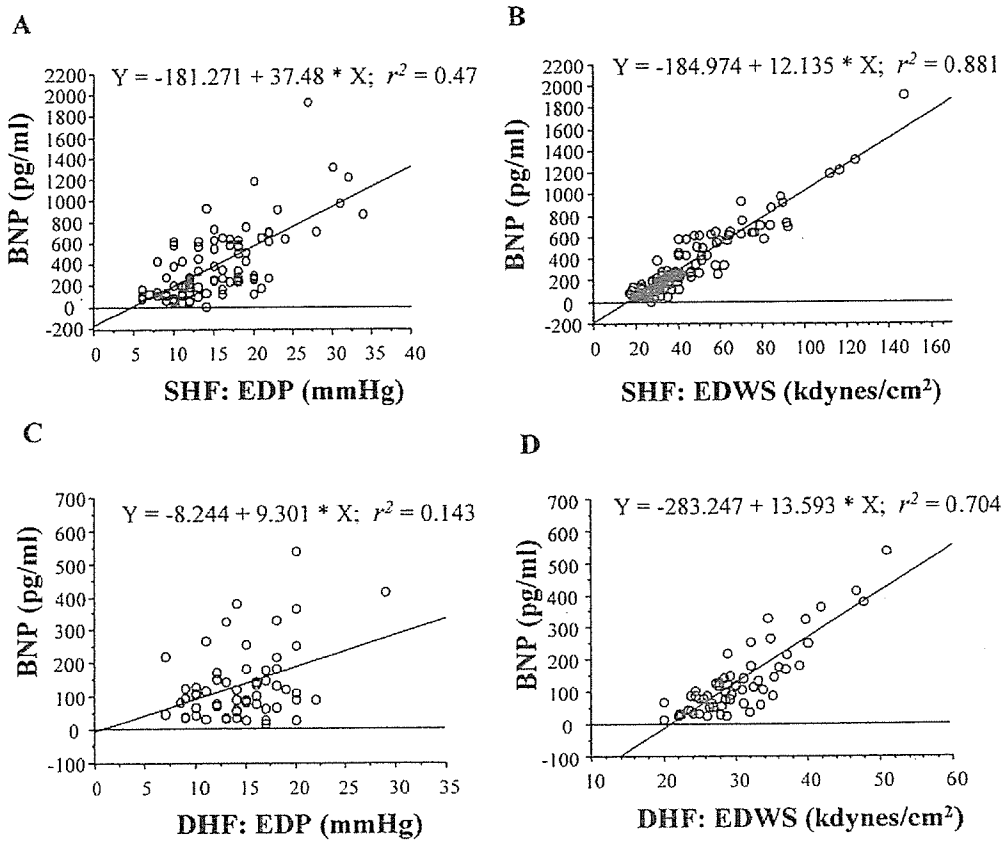

Figure 3. Correlation between B-type natriuretic peptide (BNP) and left ventricular functional parameters in 98 patients with systolic heart failure (SHF) **(A and B)** and in 62 patients with diastolic heart failure (DHF) **(C and D)**; **(A and C)** end-diastolic pressure (EDP) (mm Hg) and **(B and D)** end-diastolic wall stress (EDWS) (kdynes/cm^2).

tation in addition to LV EF, age, and renal function (7). The present study demonstrates the significance of LV EDWS in the regulation of BNP in patients with HF in general. This was true not only in patients with SHF but also with DHF. Although correlation analysis suggested a relationship between other parameters of LV geometry and function including EDP and plasma BNP levels, the correlation between LV EDWS and BNP was the most robust (r^2 = 0.887). Many studies including ours have shown that BNP levels correlate well with changes in filling pressures during tailored therapy (6,17), while O'Neill et al. (18) recently reported that plasma BNP might not correlate closely with changes in intracardiac filling pressures. In any case, plasma BNP levels are not uniform across different patients with the same LVEDP (i.e., interindividual heterogeneity), and this may be because BNP is determined more by EDWS than by filling pressure. Left ventricular EDWS might account for the wide variations that they observed in patients with HF.

The present result suggests that LV EDWS may regulate BNP secretion in humans. Indeed, experiments using cultured neonatal rat ventricular cells showed that cardiac myocytes are able to respond to mechanical stretch by increasing BNP secretion and gene expression (11). Wiese et al. (19), using isolated human myocardium, have also demonstrated that, while the isometric contraction mode did not have any influence on BNP expression, diastolic overstretch increased BNP gene expression in a time-dependent manner. This implies that diastolic stretch (i.e., preload rather than afterload) seems to be the mechanical factor responsible for the induction of BNP expression and may be the reason that in the present study LV EDWS shows a better correlation with the plasma BNP levels than does LV SWS. Furthermore, in vitro studies have implicated the contributions of local paracrine and autocrine factors in the stretch-induced BNP activation (11). Local angiotensin II was shown to play a critical role in the development of stretch-induced cardiac hypertrophy and to at least partly regulate mechanical load-induced BNP expression. Recently, in addition to stimuli such as myocyte stretching and neurohumoral activation, acute myocardial hypoxia has been reported to increase cardiac BNP gene transcription and raise the plasma proBNP concentration in an animal study (20). This mechanism may explain the increase in plasma BNP in patients with acute coronary syndromes and myocardial infarction (21). In the present study, because such patients with acute ischemia were not included, the correlation between LV EDWS and plasma BNP might actually be stronger.

Myocardial wall stress is one of the primary determinants of myocardial oxygen consumption (22). Cardiac decompensation is thought to result when the feedback loop that normalizes wall stress to abnormal loading of the heart dysfunctions. The increased wall stress may act directly or indirectly via cellular mediators such as angiotensin, endothelin, inflammatory cytokines, reactive oxygen species, and matrix metalloproteinase to orchestrate a variety of molecular and cellular remodeling events determining the structural and functional properties of the myocardium and, ultimately, the rate of disease progression (23–27). Therefore, usefulness of plasma BNP levels in predicting morbidity and mortality accurately in patients with chronic HF may be explained by the relationship between the LV EDWS and BNP. Many other factors, such as age, gender, body mass, genetics, etc., are also known to affect plasma BNP levels. However, the demonstration of the link between the hemodynamics (LV EDWS) and neurohormonal factor (BNP) may support the usefulness of BNP-guided treatment of HF. Although more randomized studies are needed, pharmacotherapy guided by BNP levels is intriguing and promising (3).

There are several methods to estimate the wall stress, and we used a formula based on M-mode echocardiographic variables (16). This method may have several limitations. For example, when there is regional asynergy in LV wall motion and variation in local LV wall thickness, the estimate may not reflect the entire non-uniform LV wall stress correctly. To test this possibility, we analyzed the data of the patients without LV asynergy demonstrated by echocardiogram and LV ventriculography. We obtained an even better correlation. Interestingly, a correlation in patients with a local wall motion abnormality was still strong (r^2 = 0.820). There are several other limitations to our study. Echocardiography and blood sampling were typically performed the day before cardiac catheterization. This time lag could have influenced the results. A further limitation is that the study population was composed of the patients who were in stable condition and could tolerate LV cardiac catheterization; thus, patients who could not bear cardiac catheterization (e.g., patients with New York Heart Association functional class IV HF) were excluded.

In the present study, we demonstrated that plasma BNP levels strongly reflect EDWS in the LV more than any other parameter previously reported. In addition, EDWS accurately accounts for the increase in plasma BNP levels even in patients with diastolic HF. The relationship of LV EDWS to plasma BNP may give a better understanding to the interindividual heterogeneity of plasma BNP levels and its clinical utility in the diagnosis and management of HF.

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