などに関してのアンケート調査を準備中である。さらに、本ネットワークにおける独立安全モニタリング委員会、イベント評価委員会の設定に関しても準備中である。

（2）自主臨床試験における安全性情報の院内収集システムの構築

治験においては既に行われている安全性情報の収集と同様に、自主臨床試験においても、被験者保護の観点から、安全性情報を行うことを検討した。今回は、自主臨床試験を行う研究者にとって負担にならないように、必要不可欠な安全性情報収集を行うこととした。SUSAR (suspected unexpected severe adverse reaction)のみを安全性情報として収集することとした。また、院内における安全性情報収集の流れは、別添の資料に示したような案を検討中である。また、安全性情報の報告書（別添）に関しても、必要不可欠なものに絞ったものを検討中である。

D. 考察

生活習慣病領域における全国規模の臨床研究ネットワークの構築は、様々な観点から必要とされている。既に、心筋梗塞症例を対象にした研究から、臨床研究に対する参加施設の姿勢、整備状況などの対応が把握できている。そのため、今後は、症例登録数の達成や臨床研究の質をあげるために、参加施設ごとのより詳細な解析を行い、心筋梗塞臨床研究ネットワークの構築が必要となる。

第三者審査機関の審査の効率化は、審査側、審査依頼側、事務局側のすべての立場から必要とされると考えられる。

E. 結論

本年度は、循環器疾患における臨床研究ネットワークを進める準備を行った。来年度は、臨床研究ネットワーク構築に向け、更に進める予定である。また、糖尿病、脳卒中などのさまざまなネットワークチームの検討を行う。また、自主臨床試験における安全性情報の院内収集システム構築の検討を開始した。来年度は、本システムに関して、如何に運営していくかという課題を検討する。

F. 健康危険情報

基盤整備事業の研究であり、健康危険に該当する情報はない。

G. 研究発表

1. 論文発表

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2. 学会発表

①発表者：演題名. 学会名、開催地、開催日、開催年.

朝倉 正紀：Potential Pharmacological Adjunctive Therapy for Acute Myocardial Infarction: Lessons from J-WIND Trials、日本循環器学会 Plenary Session、福岡、3月30日、2008年

H. 知的財産権の出願・登録状況（予定を含む）

1. 特許取得

該当なし

2. 実用新案登録

該当なし

Human atrial natriuretic peptide and nicorandil as adjuncts to reperfusion treatment for acute myocardial infarction (J-WIND): two randomised trials

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Summary

Background Patients who have acute myocardial infarction remain at major risk of cardiovascular events. We aimed to assess the effects of either human atrial natriuretic peptide or nicorandil on infarct size and cardiovascular outcome.

Methods We enrolled 1216 patients who had acute myocardial infarction and were undergoing reperfusion treatment in two prospective, single-blind trials at 65 hospitals in Japan. We randomly assigned 277 patients to receive intravenous atrial natriuretic peptide (0·025 μg/kg per min for 3 days) and 292 the same dose of placebo. 276 patients were assigned to receive intravenous nicorandil (0·067 mg/kg as a bolus, followed by 1·67 μg/kg per min as a 24-h continuous infusion), and 269 the same dose of placebo. Median follow-up was 2·7 (IQR 1·5–3·6) years for patients in the atrial natriuretic peptide trial and 2·5 (1·5–3·7) years for those in the nicorandil trial. Primary endpoints were infarct size (estimated from creatine kinase) and left ventricular ejection fraction (gauged by angiography of the left ventricle).

Findings 43 patients withdrew consent after randomisation, and 59 did not have acute myocardial infarction. We did not assess infarct size in 50 patients for whom we had fewer than six samples of blood. We did not have angiographs of left ventricles in 383 patients. Total creatine kinase was 66459·9 IU/mL per h in patients given atrial natriuretic peptide, compared with 77878·9 IU/mL per h in controls, with a ratio of 0·85 between these groups (95% CI 0·75–0·97, p=0·016), which indicated a reduction of 14·7% in infarct size (95% CI 3·0–24·9%). The left ventricular ejection fraction at 6–12 months increased in the atrial natriuretic peptide group (ratio 1·05, 95% CI 1·01–1·10, p=0·024). Total activity of creatine kinase did not differ between patients given nicorandil (70520·5 IU/mL per h) and controls (70852·7 IU/mL per h) (ratio 0·995, 95% CI 0·878–1·138, p=0·94). Intravenous nicorandil did not affect the size of the left ventricular ejection fraction, although oral administration of nicorandil during follow-up increased the left ventricular ejection fraction between the chronic and acute phases. 29 patients in the atrial natriuretic peptide group had severe hypotension, compared with one in the corresponding placebo group.

Interpretation Patients with acute myocardial infarction who were given atrial natriuretic peptide had lower infarct size, fewer reperfusion injuries, and better outcomes than controls. We believe that atrial natriuretic peptide could be a safe and effective adjunctive treatment in patients with acute myocardial infarction who receive percutaneous coronary intervention.

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See Comment page 1461

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Introduction

Despite availability of effective medical treatments, chronic heart failure remains a major cause of morbidity and mortality worldwide.[1-3] Ischaemic heart disease, in turn, is one of the main causes of chronic heart failure.[4] The most important treatment objectives are prevention of acute myocardial infarction, and, in individuals who have an acute myocardial infarction, reduction in infarct size and ischaemia or reperfusion injury.[5] Only a few medications have been shown to decrease ischaemia or reperfusion injury.[6-8]

Reperfusion of ischaemic myocardium reduces infarct size and improves left ventricular function, both of which contribute to better clinical outcomes in patients with acute myocardial infarction.[9-11] However, reperfusion can also cause tissue damage.[12] Several drugs have been trialled for the prevention or amelioration of such injuries, but results have not been consistently satisfactory.[13-15] Recently, human atrial natriuretic peptide and nicorandil have both been shown to be effective for reduction of myocardial damage after acute myocardial infarction in basic and clinical studies.[16-25] Atrial natriuretic peptide is a candidate for adjunctive treatment after acute myocardial infarction, because it has been shown to suppress the renin–angiotensin–aldosterone system and endothelin-1, both of which which modulate infarct size and cardiac remodelling.[19] Nicorandil is a combined adenosine triphosphate (ATP)-sensitive potassium channel opener and nitrate preparation that has also shown promise as an adjunctive treatment for acute myocardial infarction. In the clinical setting, however,

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the beneficial effects of atrial natriuretic peptide and nicorandil have only been tested in single-centre studies with small sample sizes.[20-25] The Japan working group studies on acute myocardial infarction for the reduction of necrotic damage by human atrial natriuretic peptide or nicorandil (J-WIND-ANP and J-WIND-KATP, respectively) aimed to assess the value of these drugs as adjuncts to percutaneous coronary intervention for patients with acute myocardial infarction.

Methods

Patients

We have described the protocols for the two trials previously.[26,27] In brief, we recruited patients to two independent, investigator-initiated, investigator-led, multicentre, prospective, randomised, single-blind, controlled trials at 65 hospitals. 27 hospitals participated in the atrial natriuretic peptide trial, and 38 separate hospitals in the nicorandil trial (table 1); the two studies were completely independent. We initially planned to include fewer hospitals, but we increased the number to promote enrolment of sufficient patients.

Eligibility criteria were age between 20 and 79 years; chest pain for more than 30 min; at least 0·1 mV of ST segment elevation in two adjacent ECG leads; admission to hospital within 12 h of the onset of symptoms; and one instance of acute myocardial infarction. Exclusion criteria were a history of myocardial infarction; left main trunk stenosis; severe liver or kidney dysfunction or both; suspected aortic dissection; previous coronary artery bypass grafting; and a history of drug allergy.

All patients gave written informed consent immediately after admission to hospital, and were asked to sign the same consent form again after 2 weeks when they had more time to decide. This system was applied on the recommendation of the institutional review boards. Only one patient, who was in the nicorandil group, withdrew their consent at their second opportunity. We enrolled patients from Oct 24, 2001, to Dec 13, 2005. The study protocol was approved by the institutional review boards and ethics committees of all participating hospitals, and was in accordance with the Declaration of Helsinki.

Procedures

An independent statistician generated our randomisation lists with a computer, by the permuted-block method. Within each centre, the block length was eight. Treatment allocations were concealed in opaque sealed envelopes until patients were enrolled. Physicians were not aware of the random assignments of patients until the follow-up stage; patients and those who analysed the data were unaware of the treatment assignment for the duration of the study. Both trials were designed as single-blind studies.

277 patients who were enrolled in the atrial natriuretic peptide trial were randomly assigned to receive an intravenous infusion of this drug after reperfusion treatment, at 0·025 μg/kg per min for 3 days, and 292 a placebo of 5% glucose solution by the same method. 276 patients in the other trial were randomly assigned to intravenous nicorandil, infused at 1·67 μg/kg per min for 24 h after bolus injection of nicorandil at a dose of 0·067 mg/kg, and 269 were assigned to 0·9% saline solution, by the same method. Previous studies have shown substantial cardiovascular protection with atrial natriuretic peptide and nicorandil at these doses.[20,22] Of the 276 patients assigned to receive nicorandil, 61 were given nicorandil orally, at the discretion of individual investigators, during the follow-up period.

We planned to stop the administration of treatment drugs in case of severe hypotension, which was defined as systolic blood pressure of less than 90 mm Hg, because of the vasodilator effect of these drugs. The study protocol did not restrict or specify any other diagnostic or therapeutic methods in the acute phase (2–8 weeks after acute myocardial infarction) or chronic phase (6–12 months).

We obtained data on baseline characteristics, emergent catheterisation, and medication at discharge after 1 month; data on follow-up catheterisation and medication after 6 months; and data on medication after 24 months. We also followed up all patients for cardiovascular events (ie, cardiac death, readmission to hospital due to heart failure, new onset of acute coronary syndrome, or revascularisation of new lesions) until the end of August, 2006. We took blood samples to measure concentrations of creatine kinase at a central laboratory, before the procedure and at 1, 3, 6, 9, 12, 18, 24, 36, 48, and 72 h after the onset of reperfusion.[14] We analysed total creatine kinase for all patients with at least six blood samples. We obtained right anterior oblique views with angiography of the left ventricle once in the acute phase (2–8 weeks), and once in the chronic phase (6–12 months).

Our primary endpoints were infarct size (which was estimated as the area under the concentration versus time curve for creatine kinase)[14] and ventricular ejection fraction (which was assessed by angiography of the left ventricle at 6–12 months after hospital admission).[15] The prespecified secondary endpoints were survival rate; cardiovascular events (such as cardiac death, readmission to hospital for heart failure, new onset of acute coronary syndrome, or revascularisation of new lesions); incidence of cardiac death or readmission to hospital for

	J-WIND-ANP study	J-WIND-KATP study
1–4 patients	7 hospitals	9 hospitals
5–9 patients	3 hospitals	13 hospitals
10–19 patients	7 hospitals	6 hospitals
More than 20 patients	10 hospitals	10 hospitals

Table 1: Distribution of patients between participating hospitals

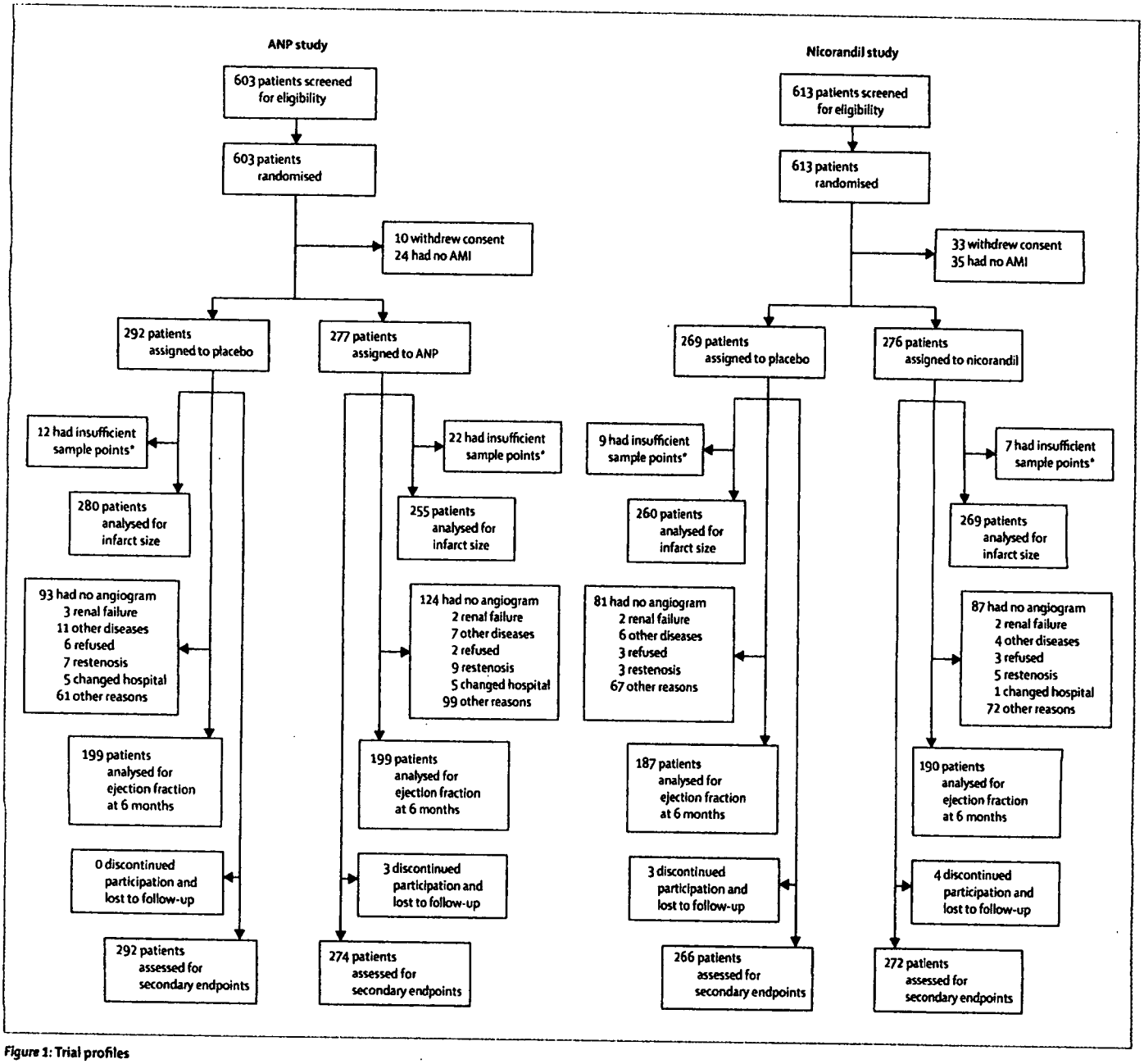

Figure 1: **Trial profiles**
ANP=atrial natriuretic peptide. AMI=acute myocardial infarction. *Fewer than six blood samples.

heart failure; or reperfusion injury before discharge from coronary care unit (such as malignant ventricular arrhythmia during reperfusion, recurrence of ST segment elevation, or worsening of chest pain). We also assessed infarct size, estimated by peak creatine kinase and troponin T;[28,29] left ventricular ejection fraction at acute phase; and end-diastolic or end-systolic volume index (assessed by angiography of the left ventricle). We looked at the effects of each drug on the primary endpoints in prespecified subgroups (sex, age, body-mass index, pre-angina, elapsed time between acute myocardial infarction and intervention, diabetes mellitus, hyperlipidaemia, smoking, and family history of acute myocardial infarction). We also did post-hoc analyses on the effect of chronic administration of nicorandil on the ejection fraction.

All data were collected by Koteisho-kyokai (Tokyo), an organisation established by the Japanese government in 2001–2003 and by NTT Data (Tokyo) in 2004–2006. Left ventricular ejection fraction and end-diastolic volume were measured by the area–length method, from angiography of the left ventricle. Two independent

interpreters, who were unaware of the treatment assigned to patients, measured left ventricular ejection fractions from the angiographs. We calculated the average value, unless the two investigators disagreed, in which case we referred to a third opinion.

Clinical findings and medications during the follow-up period were reported to a data and safety committee after registration. This committee, which consisted of three physicians and one statistician who did not participate in the trial, monitored all adverse events. Research nurses or doctors visited all participating hospitals to check that patients were registered, drugs were given, and data collected according to the protocol. Committee members did not provide any results to the steering committee, because discontinuation of the study was not recommended.

Statistical analysis

We calculated that a sample size of 300 patients would be needed in each group to detect a 20% reduction in the most important primary endpoint (total creatine kinase) with a statistical power of 80% at significance level of 0·05 (with a two-sided *t* test), accounting for dropout of some patients. We set equal sample sizes in both groups, because we expected to see almost the same reduction in infarct size with either treatment. Since creatine kinase and total creatine kinase are both log-normally distributed,[30] total creatine kinase was log-transformed before analysis. The left ventricular ejection fraction was also log-transformed before the analysis since the distribution was skewed.

Statistical analysis was done according to a prespecified analytical plan. Efficacy analysis was based on intention to treat. The primary efficacy analyses for total creatine kinase and left ventricular ejection fraction were done simply by *t* test. The estimated mean and differences on the log scale were transformed back to the original scale and were expressed as geometric means and ratios of geometric mean. If the calculated 95% CI for the ratio of the geometric mean did not cross the point of no effect (ie, 1) the difference between groups was regarded as significant. Furthermore, analysis of covariance for the two endpoints was used to estimate adjusted mean comparison, with effect of covariates and the interactions. We imputed missing data for patients by the predicted mean imputation method, with nonlinear regression. We applied multiple imputation techniques (with group means, Markov Chain Monte Carlo, Bayesian bootstrap, and last-observation-carried-forward methods) to assess the robustness and sensitivity of our conclusions.

Proportions were examined by Fisher's exact test. We examined time-to-event by the Kaplan-Meier method to estimate the survival for each group and then the differences in survival between groups by the log-rank test. The Cox proportional hazards model was used to assess baseline risk factors and an adjusted hazard ratio. The proportional hazards assumption was investigated graphically, with a test based on Schoenfeld residuals.[31,32]

All tests were two-sided, and a p value of less than 0·05 was regarded as significant. All analyses were done with SAS software (version 8.2). The trials are registered with Clinicaltrials.gov, numbers NCT00212056 and NCT00212030.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data at the end of the study, and had final responsibility for the decision to submit for publication.

Results

Figure 1 shows the trial profile. Table 2 shows baseline characteristics. Median follow-up was 2·7 (IQR 1·5–3·6) years in the atrial natriuretic peptide trial and 2·5 (1·5–3·7) years in the nicorandil trial. Table 3 shows

	Atrial natriuretic peptide study			Nicorandil study		
	ANP (n=277)	Control (n=292)	p	Nicorandil (n=276)	Control (n=269)	p
Age (years)	63·0 (10·4)	61·8 (10·7)	0·1652	61·1 (11·4)	63·7 (10·2)	0·0035
Sex (male)	211 (76·2%)	243 (83·2%)	0·0374	246 (89·1%)	220 (81·8%)	0·0153
Body-mass index	24·3 (3·5)	24·0 (2·9)	0·3733	24·2 (3·0)	23·4 (2·8)	0·0007
Killip classification (I, II, III, IV)	88·6%, 9·5%, 1·1%, 0·8%	90·3%, 7·5%, 1·4%, 0·7%	0·5274	91·1%, 8·2%, 0·4%, 0·4%	92·0%, 4·2%, 2·7%, 1·1%	0·7843
Pre-angina	105 (44·5%)	118 (46·1%)	0·7862	111 (44·6%)	111 (43·9%)	0·9284
Risk factors						
Hypertension	137 (56·1%)	162 (62·1%)	0·2046	127 (48·5%)	137 (53·9%)	0·2190
Diabetes mellitus	81 (33·8%)	86 (33·9%)	1·0000	104 (39·5%)	82 (32·9%)	0·1413
Hyperlipidaemia	127 (54·3%)	131 (50·6%)	0·4181	121 (46·7%)	114 (46·2%)	0·9291
Smoking	158 (63·7%)	175 (67·3%)	0·4022	178 (68·7%)	170 (66·1%)	0·5732

Data are number (%) or mean (SD), unless otherwise specified. ANP=atrial natriuretic peptide.

Table 2: Baseline characteristics on admission

	Atrial natriuretic peptide study		Nicorandil study	
	ANP (n=277)	Control (n=292)	Nicorandil (n=276)	Control (n=269)
Elapsed time (h)*	4·00 (3·00–6·00)	4·00 (2·50–6·00)	3·50 (2·50–5·00)	3·50 (2·50–5·00)
Infusion time (h)	1·00 (0·50–1·00)	1·00 (0·50–1·00)	0·70 (0·50–1·00)	0·75 (0·50–1·00)
IRA (LAD, LCx, RCA)	55·3%, 6·4%, 38·3%	52·3, 10·6, 37·1%	53·9, 7·4, 38·7%	44·5, 9·9, 45·6%
Stents	176 (63·5%)	193 (66·1%)	187 (67·8%)	183 (68·0%)
Rescue	64 (23·1%)	92 (31·5%)	94 (34·1%)	92 (34·2%)
Intra-aortic balloon pump	17 (6·1%)	14 (4·8%)	14 (5·1%)	15 (5·6%)
Final stenosis (<75%)	246 (93·5%)	266 (94·7%)	257 (96·6%)	255 (97·0%)
Final thrombolysis in myocardial infarction (0, 1, 2, 3)	3·9%, 1·9%, 5·0%, 89·1%	5·2%, 0·7%, 4·1%, 90·0%	3·7%, 0·7%, 5·2%, 90·3%	3·4%, 1·1%, 6·9%, 88·5%
Medications at 1 month				
ACE inhibitor	155 (57·8%)	173 (60·7%)	164 (61·0%)	163 (62·0%)
ARB	77 (28·7%)	99 (34·7%)	72 (26·8%)	69 (26·2%)
Spinorolactone	28 (10·4%)	33 (11·6%)	17 (6·3%)	22 (8·4%)
β blocker	112 (41·8%)	128 (44·9%)	110 (40·9%)	121 (46·0%)
Aspirin	225 (84·0%)	252 (88·4%)	251 (93·3%)	250 (95·1%)
Nitrates	81 (30·2%)	86 (30·2%)	50 (18·6%)	63 (24·0%)
Statins	129 (48·1%)	156 (54·7%)	126 (46·8%)	115 (43·7%)
Nicorandil	62 (23·1%)	52 (18·2%)	79 (29·4%)	34 (12·9%)
Medications at 6 months				
ACE inhibitor	103 (48·1%)	117 (44·8%)	120 (50·6%)	131 (53·9%)
ARB	69 (32·2%)	110 (42·1%)	68 (28·7%)	75 (30·9%)
Spinorolactone	26 (12·1%)	26 (10·0%)	11 (4·6%)	15 (6·2%)
β blocker	93 (43·5%)	118 (45·2%)	104 (43·9%)	113 (46·5%)
Aspirin	179 (83·6%)	233 (89·3%)	217 (91·6%)	229 (94·2%)
Nitrates	51 (23·8%)	63 (24·1%)	37 (15·6%)	49 (20·2%)
Statins	112 (52·3%)	150 (57·5%)	123 (51·9%)	118 (48·6%)
Nicorandil	46 (21·5%)	39 (14·9%)	55 (23·2%)	23 (9·5%)
Medications at 24 months				
ACE inhibitor	66 (47·5%)	63 (37·5%)	83 (52·5%)	75 (49·3%)
ARB	42 (30·2%)	72 (42·9%)	39 (24·7%)	43 (28·3%)
Spinorolactone	13 (9·4%)	21 (12·5%)	9 (5·7%)	4 (2·6%)
β blocker	57 (41·0%)	61 (36·3%)	77 (48·7%)	71 (46·7%)
Aspirin	113 (81·3%)	133 (79·2%)	143 (90·5%)	137 (90·1%)
Nitrates	29 (20·9%)	45 (26·8%)	23 (14·6%)	25 (16·4%)
Statins	66 (47·5%)	78 (46·4%)	81 (51·3%)	71 (46·7%)
Nicorandil	26 (18·7%)	26 (15·5%)	28 (17·7%)	11 (7·2%)

Data are median (IQR), number (%) or mean (SD), unless otherwise specified. ANP=atrial natriuretic peptide. IRA=infarct-related artery. LAD=left anterior descending coronary artery. LCx=left circumflex artery. RCA=right coronary artery. ARB=antiotensin receptor blocker. ACE=angiotensin-converting enzyme. *Period between acute myocardial infarction and start of intervention.

Table 3: Treatments and prescribed drugs

treatments and drugs throughout the study. Drugs used in the chronic stage did not differ between groups in either study, except that some patients in the nicorandil trial were given oral nicorandil during follow-up.

Table 4 and figure 2 show infarct size and left ventricular function at 2–8 weeks and 6–12 months in both studies. The ratio of total creatine kinase between the atrial natriuretic peptide and placebo groups was 0·85 (95% CI 0·75–0·97, p=0·0155); which indicates that atrial natriuretic peptide was associated with a reduction of 14·7% in infarct size. Subanalyses identified no factors that enhanced or reduced the influence of atrial natriuretic peptide on infarct size (figure 2). Nicorandil did not reduce infarct size compared with placebo, and no factors affected this finding. Treatment with atrial natriuretic peptide tended to increase the left ventricular ejection fraction (ratio 1·043, 95% CI 1·000–1·089, p=0·0525) at 2–8 weeks after the onset of acute myocardial infarction, and at 6–12 months (ratio 1·051, 95% CI 1·006–1·099, p=0·0236). By contrast, table 4 and figure 2 show that left ventricular ejection fraction did not differ in patients given nicorandil and controls at either 2–8 weeks or 6–12 months.

	J-WIND-ANP study			J-WIND-KATP study		
	Atrial natriuretic peptide	Control	p	Nicorandil	Control	p
Infarct size						
n	255	280		269	260	
Creatine kinase (area under curve) (IU/L h)	66459·9 (60258·2–73300·0)	77878·9 (71590·2–84720·1)	0·016	70520·5 (64309·8–77331·0)	70852·7 (65066·7–77153·2)	0·941
Peak creatine kinase (IU/L)	2487·5 (2217·6–2790·3)	2784·2 (2526·7–3067·9)	0·141	2557·1 (2306·1–2835·4)	2428·7 (2199·8–2681·5)	0·479
Troponin-T concentration (12–18 h) (ng/mL)	5·36 (4·76–6·03)	6·13 (5·55–6·79)	0·084	6·18 (5·51–6·93)	5·60 (4·97–6·32)	0·244
Troponin T (96 h) (ng/mL)	2·57 (2·25–2·94)	2·94 (2·64–3·27)	0·125	2·63 (2·36–2·94)	2·89 (2·61–3·19)	0·225
Left ventricle (2–8 weeks)						
n	187	207		168	170	
Median elapsed time (days)*	18·5 (IQR 15·0–27·0)	19·0 (IQR 16·0–25·0)		17·0 (IQR 14·0–23·0)	17·0 (IQR 14·0–24·0)	
Ejection fraction	43·0% (41·8–44·3)	41·3% (40·0–42·6)	0·053	42·0% (40·7–43·3)	41·6% (40·4–42·9)	0·680
End diastolic volume index (mL/m²)	98·8 (94·4–103·4)	102·3 (98·1–106·6)	0·272	111·2 (106·4–116·3)	105·9 (100·9–111·3)	0·147
End systolic volume index (mL/m²)	54·2 (51·2–57·4)	58·3 (55·5–61·4)	0·058	62·8 (59·2–66·6)	60·4 (57·0–64·1)	0·360
Left ventricle (6–12 months)						
n	155	199		190	187	
Median elapsed time (days)*	196·5 (IQR 180·5–230·5)	200·5 (IQR 183·0–226·0)		195·0 (IQR 180·0–231·0)	195·5 (IQR 183·0–232·0)	
Ejection fraction	44·7% (43·4–46·0)	42·5% (41·2–43·9)	0·024	42·5% (41·2–43·8)	43·2% (42·0–44·4)	0·460
End diastolic volume index (mL/m²)	100·6 (95·2–106·2)	100·9 (96·8–105·1)	0·930	109·8 (105·4–114·4)	105·7 (100·8–110·8)	0·230
End systolic volume index (mL/m²)	54·2 (50·6–58·0)	56·0 (53·1–58·9)	0·452	61·7 (58·4–65·2)	58·5 (55·1–62·1)	0·198

Data are mean (95% CI) or median (IQR). *Time between acute myocardial infarction and start of intervention.

Table 4: **Primary endpoints and other outcomes obtained by angiography of left ventricles**

Figure 3 shows reperfusion injuries, survival rates, and cardiovascular events. Reperfusion injuries were less common in the atrial natriuretic peptide group than in the placebo group (ratio 0·743, 95% CI 0·58–0·952, p=0·019). Although there were no differences between groups in either survival rates or the incidence of cardiovascular events, both cardiac death and readmission to hospital for heart failure were lower in patients given atrial natriuretic peptide than in controls (HR 0·267, 95% CI 0·089–0·799, p=0·0112). By contrast, cardiac death and readmission to hospital for heart failure were not significantly lower in patients given nicorandil than in controls (HR 0·799, 95% CI 0·307–1·973, p=0·5972). When nicorandil was given orally throughout the study after reperfusion treatment, the change of left ventricular ejection fraction increased substantially between the acute and chronic phase. The ejection fraction was 3·66% in the 61 patients who were given nicorandil orally, and 1·47% in the 241 patients who were not (difference 2·20, 95% CI 0·17–4·22, p=0·0338).

In the atrial natriuretic peptide trial, 29 patients given that drug had severe hypotension during the acute phase, compared with one control. In the other trial, three patients in the nicorandil group had severe hypotension, compared with no controls. No other severe adverse events were reported during the course of either study.

Discussion

We showed that adjunctive, acute-phase treatment with atrial natriuretic peptide after reperfusion therapy in patients with acute myocardial infarction reduced infarct size by 14·7%, increased the left ventricular ejection fraction during the chronic phase, and decreased the incidence of cardiac death and readmission to hospital because of heart failure. Intravenous treatment with nicorandil did not affect the primary endpoints, although patients who were given nicorandil orally had better cardiac function outcomes.

Interest in the cardioprotective effects of adenosine has increased, because of its variety of cardioprotective mechanisms. Unfortunately, in trials of adenosine, it only marginally improved infarct size and showed no clinical benefits.[7,33] We hypothesised that treatment with atrial natriuretic peptide and nicorandil in the acute phase might prove more effective than chronic-phase treatment for limitation of infarct size. The first window of ischaemic preconditioning is mediated by opening of the KATP channel,[34] which is the mechanism of action of nicorandil; and the second window is mediated by nitric oxide and activation of G kinase, which is the mechanism of action of atrial natriuretic peptide.

Before this clinical trial, we had tested whether atrial natriuretic peptide could limit infarct size in a canine model in which the left anterior coronary artery was ligated for 90 min, followed by 6 h of reperfusion. Treatment with atrial natriuretic peptide reduced infarct size by about 40% after reperfusion (unpublished data). Our results are consistent with the finding of Hayashi and coworkers[20] that infusion of atrial natriuretic peptide immediately after reperfusion in patients with their first anterior acute myocardial infarction increased left ventricular ejection fraction.

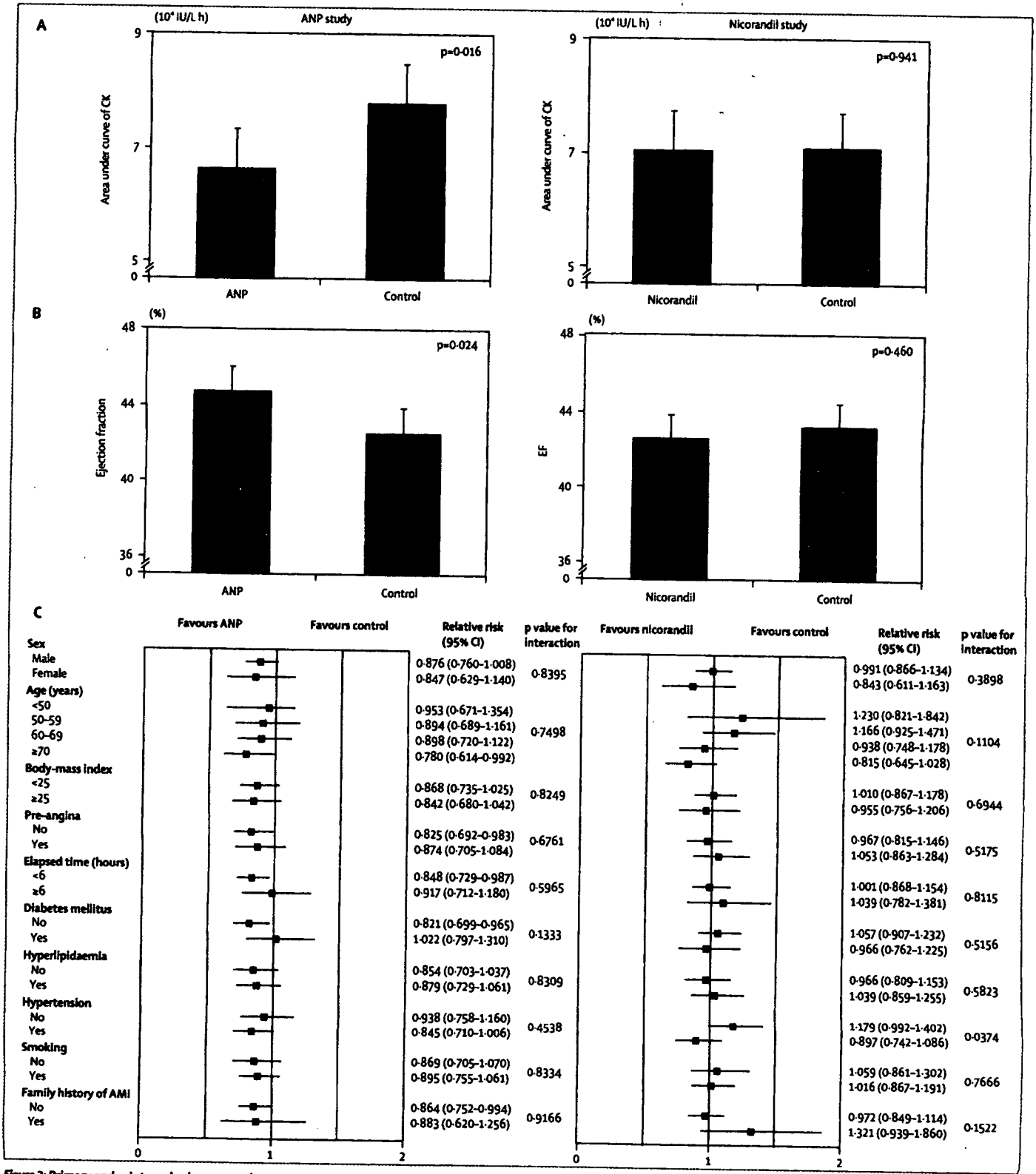

Figure 2: **Primary endpoints and subgroup analyses**
CK=creatine kinase. AMI=acute myocardial infarction. ANP=atrial natriuretic peptide. Panel A shows area under curve of creatine kinase concentration versus time. Panel B represents left ventricular ejection fraction measured at 6–12 months.

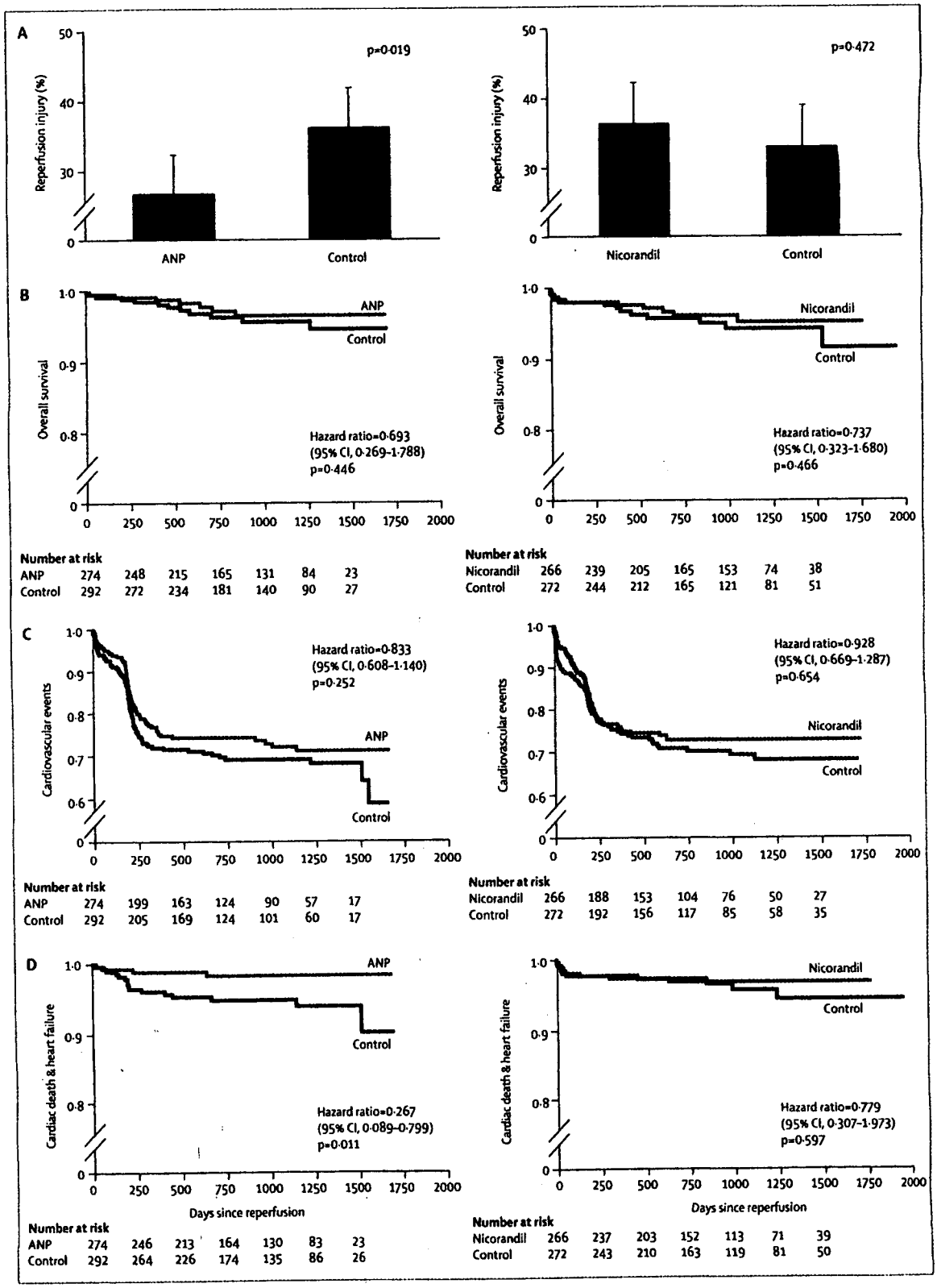

Figure 3: **Secondary endpoints and other subanalyses**
ANP=atrial natriuretic peptide.

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[15] 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.

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Conflict of interest statement

We declare that we have no conflict of interest.

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Original Article

Impact of Adenosine Receptor Signaling and Metabolism on Pathophysiology in Patients with Chronic Heart Failure

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Adenosine is well known to be a cardioprotective substance in ischemic heart disease. However, the modulation of adenosine receptors and the production and degradation of endogenous adenosine in chronic heart failure (CHF) are not fully understood. We analyzed the gene expression patterns of adenosine-related genes in human failing and nonfailing myocardium using DNA microarray analysis and quantitative real time–polymerase chain reaction (RT-PCR). DNA microarray analysis revealed that the gene expression of adenosine A2a, A2b, and A3 receptors (A2aR, A2bR, and A3R) as well as that of adenosine deaminase (ADA) decreased in failing myocardium. The down-regulation of these genes was verified by quantitative RT-PCR. We also measured the activities of these adenosine metabolism–related enzymes in failing myocardium and cardiac adenosine levels in patients with CHF. In CHF patients, we observed the decreased enzyme activity of ADA and the elevation of cardiac adenosine levels in CHF patients. To enhance the signaling of adenosine receptors, we increased plasma adenosine levels using dipyridamole, which decreased the severity of CHF. The gene expression of A2aR, A2bR, A3R, and ADA was decreased in the failing hearts, and this decrease may impair adenosine-related signal transduction. The activities of adenosine-related enzymes were altered, thus increasing the myocardial adenosine levels; this increase may compensate for the impairment of adenosine-related signal transduction in patients with CHF. The impairment of adenosine-related signal transmission contributes to the pathophysiology of CHF. (*Hypertens Res* 2007; 30: 781–787)

***Key Words*: DNA microarray, adenosine, single nucleotide polymorphism, heart failure, adenosine deaminase, adenosine A2a receptor**

Introduction

Chronic heart failure (CHF) represents the common characteristics secondary to various cardiac diseases, such as systemic hypertension, dilated cardiomyopathy, hypertrophic cardiomyopathy, ischemic heart disease, valvular heart disease, and myocarditis (*1*). Interestingly, catecholamine, angiotensin, aldosterone, and cytokines are known to be involved in the pathophysiology of CHF (*2–5*), as evidenced

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Table1. Patient Characteristics

Case	Age (years old)	Sex	Diagnosis	Operation	LAD (mm)	LVDd (mm)	EF (%)	MR	ANP (ng/mL)	BNP (ng/mL)
01	53	M	ICM	Batista	31	88	24	IV	25	90
02	45	M	DCM	Batista	63	81	39	IV	85	217
03	72	M	DCM	Batista	52	71	14	III	86	201
04	58	F	ICM	Dor	44	76	24	I	NA	NA
05	57	M	HCM	Dor	54	52	44	III	20	80
06	69	M	DCM	Batista	49	86	15	IV	100	465
07	40	M	AR	Dor	44	76	38	I	39	200
08	75	M	ICM	Dor	28	48	35	II	37	150
09	32	M	DCM	Batista	54	81	26	IV	170	403
10	51	F	Myocarditis	Dor	26	68	35	IV	70	196
11	54	M	ICM	Dor	47	64	27	I	84	302
12	58	M	Myocarditis	Dor	48	77	18	III	800	2,710

LAD, left atrial diameter; LVDd, diastolic left ventricular diameter; EF, ejection fraction; MR, severity of mitral regurgitation; ANP, the concentration of plasma atrial natriuretic peptide (ng/mL); BNP, the concentration of plasma brain natriuretic peptide; M, male; F, female; ICM, ischemic cardiomyopathy; DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; AR, aortic valve regurgitation; NA, not available.

by the fact that β-adrenoceptor antagonists, angiotensin-converting enzyme (ACE) inhibitors, and aldosterone receptor antagonists are widely accepted as drugs for CHF (*6*, *7*). Adenosine has biological effects on various tissues (*8–10*). Since several lines of evidence (*9*, *10*) support the idea that adenosine is cardioprotective against deleterious sequels in CHF as well as ischemic heart disease, it is intriguing and important to analyze the adenosine receptor– or adenosine metabolism–related genes using DNA microarray analysis. Adenosine is known to be an endogenous nucleoside acting as a cardioprotective substance that modulates numerous physiological processes, including the regulation of coronary blood flow (*9*, *10*). Adenosine is produced or degraded by several enzymes, including 5′-nucleotidase, adenosine deaminase (ADA), and adenosine kinase (AK). Adenosine elicits its physiological actions by binding to four specific receptors: A1, A2a, A2b, and A3. A1 and A3 receptors are coupled through Gi protein to adenylate cyclase inhibition, while A2a and A2b receptors are coupled to adenylate cyclase activation through Gs protein. However, the adenosine metabolism and its receptor-mediated signaling in patients with CHF remain unclear.

In the present study, we first examined gene expression in failing and nonfailing myocardium by focusing on adenosine-related genes using DNA microarray analysis followed by quantitative real time–polymerase chain reaction (RT-PCR). Then, to examine whether or not the consequences of the altered gene expression are related to the pathophysiology of human CHF, we also measured cardiac adenosine levels and the activities of adenosine-related enzymes. Finally, we tested whether or not increased adenosine levels using dipyridamole, an adenosine uptake inhibitor, improves the pathophysiology of patients with CHF.

Table 2. The Comparison of Gene Expressions between the Nonfailing and Failing Hearts

Gene name	Fold change
Adenosine receptors	
A1 receptor	1.51±0.32
A2a receptor	0.29±0.04
A2b receptor	0.75±0.07
A3 receptor	0.61±0.04
Adenosine-related enzymes	
Adenosine deaminase	0.52±0.03
Adenosine kinase	1.14±0.16

Methods

RNA Samples from Human Heart Tissues

Tissue samples of human failing heart were obtained from 12 patients (average age 55 years [range 32–75 years]; 10 males and 2 females) who had undergone partial left ventriculectomy (the Batista or Dor procedure) for end-stage heart failure at Hayama Heart Center. All heart tissues were stored in RNA Later (Ambion, Austin, USA). Because of the difficulty of acquiring nonfailing heart tissues in Japan, we obtained total RNAs of nonfailing myocardium of Mongolian people from BioChain Institute Inc. (Hayward, USA). The collection and use of tissue were approved by independent ethics committees of the National Cardiovascular Center at Osaka University and of the Hayama Heart Center.

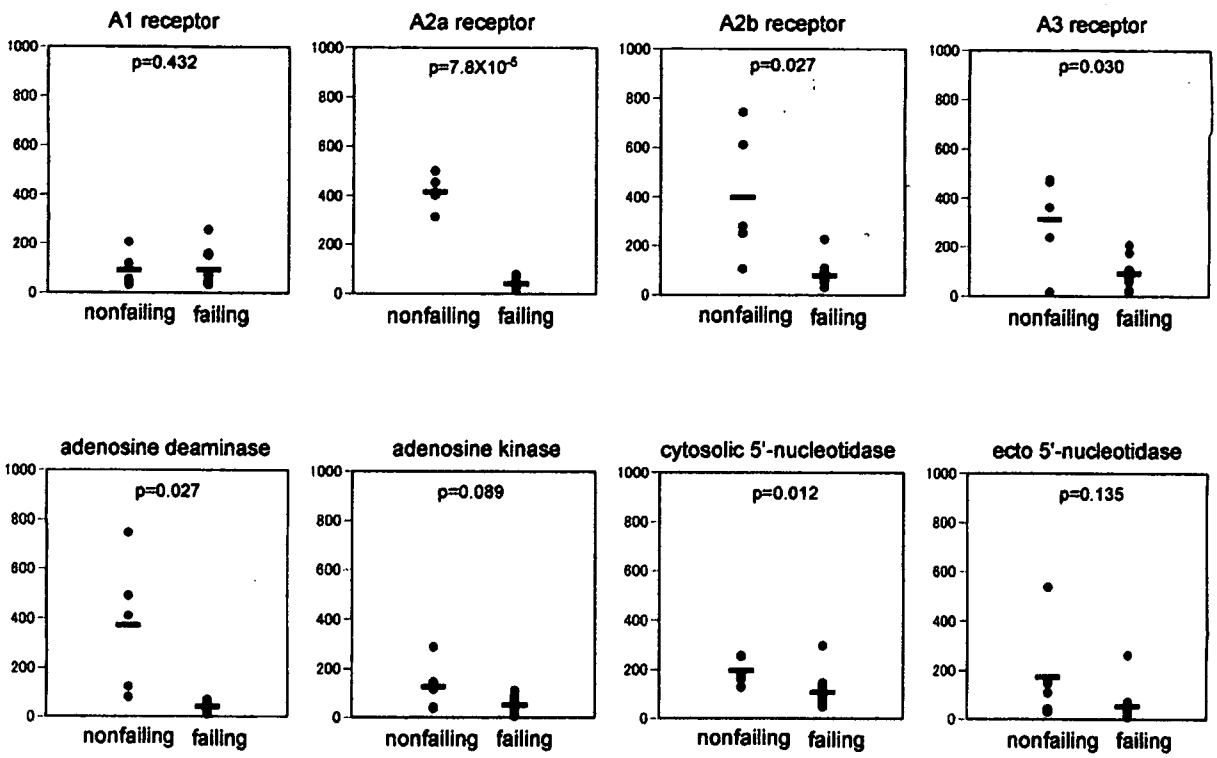

Fig. 1. *Quantitative real-time RT-PCR of genes related to adenosine. Expression of eight genes related to adenosine were verified by quantitative real-time RT-PCR. The expression levels of A2aR, A2bR, ADA, and cytosolic 5′-nucleotidase were significantly down-regulated in failing hearts compared to nonfailing hearts. The levels of gene expression of the other four genes did not differ significantly between failing and nonfailing hearts. The relative expression levels are normalized by GAPDH expression as 100.*

RNA Isolation and DNA Microarray Hybridization

Total RNA was extracted from 12 failing human heart tissues (Table 1) by TRIzol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's protocol. The integrity of the RNA was verified with an RNA 6000 Nano LabChip Kit with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA). DNA microarray analysis was performed according to the Affymetrix GeneChip expression analysis protocol. Biotinylated cRNA was generated and was applied to Affymetrix oligonucleotide array GeneChip Human Genome U95 sets (Affymetrix, Santa Clara, USA). Expression differences between the nonfailing and failing hearts were analyzed by MAS ver 4.0 (Affymetrix).

Quantitative Real-Time RT-PCR

Eight RT-PCR products ADA, AK, cytosolic and ecto 5′-nucleotidase, and adenosine receptors (A1, A2a, A2b, and A3) from 5 nonfailing heart tissues and 12 failing heart tissues were used to confirm the DNA microarray data by quantitative real-time RT-PCR using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, USA). The respective primers used in this study were designed according to the sequences available in GenBank using Primer Express Software (Applied Biosystems). We used GAPDH as the internal control gene because it showed similar expression levels in nonfailing and failing heart samples.

Measurements of Adenosine-Related Enzyme Activities and Plasma Adenosine Levels

The preparation of the myocardium for the measurement of adenosine-related enzymes, *i.e.*, 5′-nucleotidase, ADA, and AK, was reported previously (*10*). Failing myocardium were obtained from 12 patients who had undergone cardiac biopsy. Blood was sampled from either the ascending aorta near the ostium of the coronary artery or the coronary sinus vein using an NIH catheter. Plasma adenosine levels were determined by radioimmunoassay as previously reported (*11*).

Dipyridamole Treatment

Twenty-one patients judged to be in functional classification II or III of the New York Heart Association (NYHA) were

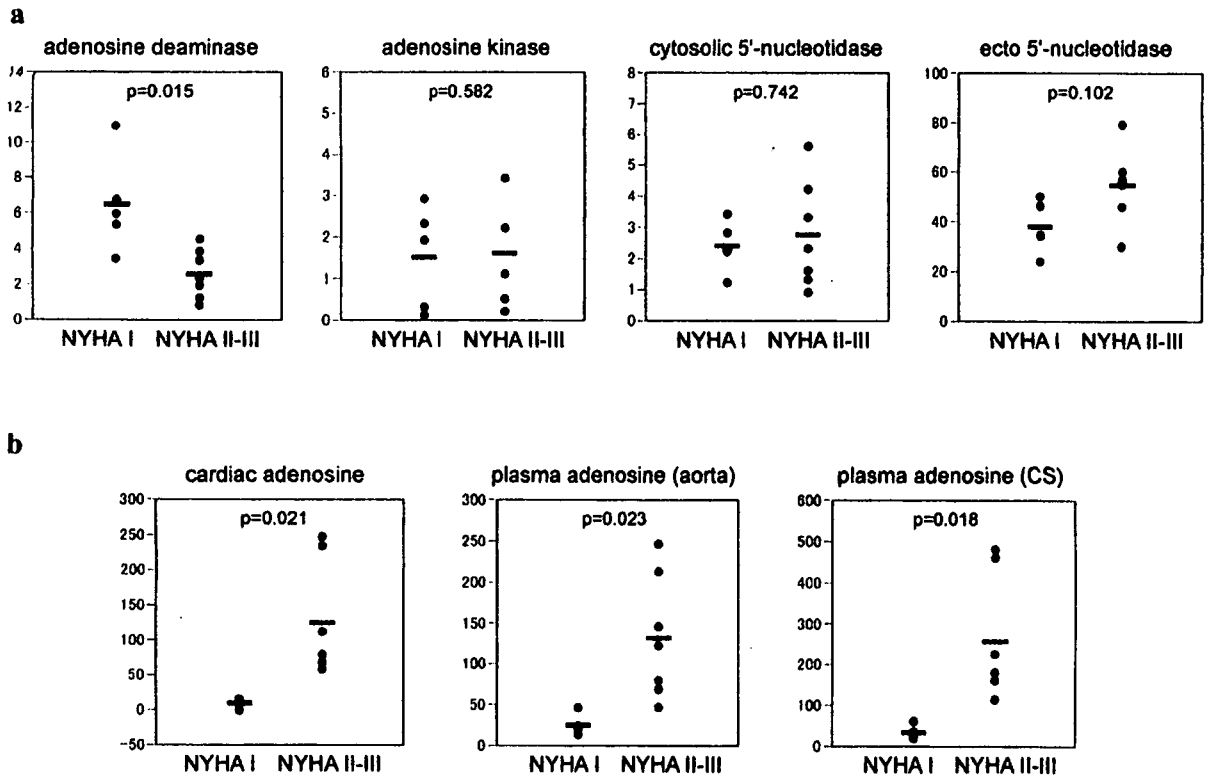

Fig. 2. *Enzyme activities of adenosine-related enzymes and cardiac adenosine levels. a: The enzyme activity of ADA was repressed in patients with NYHA II-III compared to those with NYHA I. The enzyme activity of ecto 5′-nucleotidase was elevated in patients with NYHA II-III compared to those with NYHA I. The activities of AK and cytosolic 5′-nucleotidase did not differ between patients with NYHA I and those with NYHA II-III. The unit of activity of each enzyme is nmol/kg protein/min. b: Cardiac adenosine levels were elevated in patients with NYHA II-III compared to those with NYHA I. The unit of plasma adenosine level is nmol/L.*

examined. There were 11 patients with dilated cardiomyopathy, 5 patients with ischemic cardiomyopathy, 3 patients with valvular heart disease, and 2 patients with hypertensive heart disease. We administered dipyridamole at 75 mg/day ($n=8$) or 300 mg/day ($n=6$) for 6 months. At the onset and again 6 months after the onset of administration we assessed NYHA classification, ejection fraction (EF), and fractional shortening (FS) using echocardiography, and we measured maximal oxygen uptake using an ergometer.

Statistical Analysis

Statistical analyses were performed using ANOVA when the data were compared among the groups. When ANOVA reached a significant level, we compared pairs of data using the Bonferroni test. The values are expressed as means±SD, with $p<0.05$ considered significant.

Results

Expression of Adenosine-Related Genes in Human Failing Hearts

We examined the expression levels of six adenosine-related genes in failing myocardium using DNA microarray analysis. Table 2 shows that ADA expression was down-regulated in the failing hearts compared to the nonfailing hearts. Interestingly, the adenosine receptors were modulated in the myocardium of patients with CHF. Most of all, the expression of A2a receptors was markedly down-regulated in the failing myocardium to less than one-third the level in the nonfailing hearts.

We performed quantitative RT-PCR of these genes to confirm the expression patterns of these transcripts related to adenosine from DNA microarray analysis. The results showed that the mRNA levels of A2a receptor, A2b receptor, A3 receptor, ADA, and cytosolic 5′-nucleotidase were down-regulated in the failing hearts compared with the nonfailing

Table 3. The before and after Dipyridamole Treatment of CHF

	Pre-medication	6-month treatment	*p* value
Control group ($n=7$, average 66 years old, 6 male)			
LAD (mm)	47.7±10.5	47.8±10.6	n.s.
LVDd (mm)	58.6±7.1	58.2±7.5	n.s.
LVDs (mm)	49.7±9.0	49.9±9.1	n.s.
FS (%)	16.6±6.6	15.0±6.2	n.s.
EF (%)	34.6±8.4	34.0±9.7	n.s.
BNP (ng/mL)	211.4±172.5	227.3±178.9	n.s.
NYHA(I/II/III/IV)	0/3/4/0	0/3/4/0	n.s
Vo_2 (mL/kg/min)	16.9±5.3	17.5±5.4	n.s.
Workload (Mets)	5.0±1.3	5.2±1.2	n.s.
Dypiridamol group ($n=14$, average 66 years old, 10 male)			
LAD (mm)	49.5±6.8	46±6.6	n.s.
LVDd (mm)	58.9±11.9	54.8±11.9	n.s.
LVDs (mm)	50.8±12.1	45.1±12.4	n.s.
FS (%)	14.4±5.1	18.7±6.1	0.02
EF (%)	34.1±9.9	45.4±10.5	0.01
BNP (ng/mL)	236.8±154.0	105.8±125.1	0.02
NYHA(I/II/III/IV)	0/1/13/0	1/7/6/0	0.001
Vo_2 (mL/kg/min)	16.5±3.9	20.4±4.2	0.052
Workload (Mets)	5.6±1.4	6.4±1.2	n.s.

LAD, left atrial diameter; LVDd and LVDs, diastolic and systolic left ventricular diameters, respectively; FS, fractional shortenening; EF, ejection fraction; MR, severity of mitral regurgitation; BNP, the concentration of plasma brain natriuretic peptide; Vo_2, oxygen consumption. Values are expressed as the individual number or mean±SD. *p* values are obtained by the comparison between the conditions of pre-medication and 6 months medication.

hearts (Fig. 1). The expression of the A1 receptor, adenosine kinase, and ecto 5′-nucleotidase did not differ between the failing and the nonfailing hearts.

Enzyme Activity Assay and Adenosine Level

We examined the enzyme activities of the adenosine-related enzyme and the cardiac adenosine level to examine whether or not the altered gene expression reflects the change in adenosine metabolism in patients with CHF. We observed that ADA activity was lower in patients with NYHA II-III than in patients with NYHA I (Fig. 2), while cytosolic 5′-nucleotidase activity was unchanged. Cardiac adenosine levels were higher in patients with NYHA II-III than in those with NYHA I. Together, these results suggest that adenosine plays an important role in the pathophysiology of CHF.

An Adenosine Potentiator as a Therapy Target for CHF

In 21 patients with CHF, we administered dipyridamole at either 75 or 300 mg daily in 14 patients with CHF for 6 months. Table 3 shows the clinical data on the control and dipyridamole groups. Echocardiography showed that dipyridamole increased cardiac functions such as EF and fractional shortening. Dipyridamole decreased plasma brain natriuretic peptide (BNP) level in patients with CHF. This indicates that the enhancement of plasma adenosine levels compensates for the down-regulation of adenosine receptors and improves the pathophysiology of CHF.

Discussion

Impact of the Present Study on the Pathophysiology of CHF

Despite the recent advances in our knowledge of CHF, the complex pathophysiological events of CHF, especially at the molecular and genetic levels, remain to be fully elucidated (*12*). Microarray analysis has been expected to respond to the questions surrounding this complexity. In the present study, we demonstrated the expression of the genes related to adenosine in failing and nonfailing human heart tissues using microarray analysis and quantitative real-time RT-PCR. We clarified the downregulation of the A2a receptor, the A2b receptor, the A3 receptor, cytosolic 5′-nucleotide, and ADA genes. We also revealed the elevation of cardiac adenosine levels in CHF patients with NYHA II-III compared to the patients with NYHA I. Finally we suggested that the enhancement of adenosine level improves cardiac functions in patients with CHF. This report revealed that adenosine is involved in the pathophysiology of CHF, and the augmenta-

tion of endogenous adenosine can be a novel treatment for CHF.

The Down-Regulation of Adenosine Receptors and the Pathophysiology of CHF

Since adenosine is known to be cardioprotective, the down-regulation of adenosine receptors as shown in the present study is speculated to be a cause of CHF. Indeed, the activation of A2a and A2b receptors increases myocardial contractility *via* cyclic AMP-independent pathways (*13*) and increases coronary blood flow *via* K_{ATP} channel-dependent mechanisms (*14*). Since decreases in myocardial contractility or abnormal coronary perfusion are thought to be potential causes of CHF, these functional abnormalities of A2a and A2b receptors may be responsible for CHF. Furthermore, the lack of A2a and A2b receptor function facilitates platelet aggregation and leukocyte activation, both of which damage the myocardium and coronary microcirculation (*15*).

Importantly, it is reported that A2a receptors are up-regulated in the peripheral circulating cells of patients with end-stage CHF compared with control subjects (*16*). The difference between the results of that study and those of ours remains unknown, but may be attributable to the differences in 1) the severity of CHF, 2) the causes of CHF, and 3) sampling sites for assessing A2a receptor expression. First, Varani *et al.* investigated patients with very severe CHF who have had heart transplants, and we investigated moderate to severe CHF patients who have undergone either the Batista or Dor operation. Secondly, the causes of CHF in the present study were mainly non-ischemic heart diseases such as dilated cardiomyopathy, whereas Varani *et al.* did not discuss the causes of CHF in their patients. Finally, the regulation of A2a receptor in lymphocytes may differ from that in cardiomyocytes and the up-regulation of A2a receptor in lymphocytes as reported by Varani *et al.* does not necessarily indicate the upregulation of A2aR in the myocardia seen in the present study (*16*).

We also observed the down-regulation of A3 receptors in the myocardia of CHF patients. Since the activation of A3 receptors also provides cardioprotection (*17*), the down-regulation of A3 receptor expression may contribute to the severity of CHF.

Using rat myocardial infarction (MI) models, we recently reported that long-term stimulation of A2b receptors attenuates cardiac fibrosis in non-infarcted myocardium and improves cardiac function (*18*). These results suggest that the down-regulation of A2b receptors might be involved in cardiac fibrosis in patients with CHF.

The Down-Regulation of ADA and the Pathophysiology of CHF

What are the roles of ADA expression down-regulation and of the modulated activities of ADA? The reduced activity of cardiac adenosine deaminase in the myocardia of patients with CHF enhances intracellular adenosine levels by inhibiting adenosine degradation. Enhanced adenosine is released extracellularly and acts on adenosine receptors of various other cells in the heart and vessels. Since adenosine is believed to be cardioprotective, the changes are thought to compensatee for the down-regulation of adenosine receptors and the pathophysiology of CHF. Although the expression level of cytosolic 5′-nucleotidase decreased, the activities of ecto and cytosolic 5′-nucleotidase were not modulated, and the adenosine degradation capability *via* ADA decreased without modulation of the adenosine production capability. We do not attempt to clarify the reason why cytosolic 5′-nucleotidase activity was not changed despite the decreased expression level of cytosolic 5′-nucleotidase. One possibility is that cytosolic 5′-nucleotidase is phosphorylated and activated by neurohumoral factors such as angiotensin II, preventing any change in the activity of cytosolic 5′-nucleotidase. As a whole, we found that cardiac adenosine levels are elevated in patients with CHF, and that further increases in adenosine levels by dipyridamole administration restored cardiac function. Since the patients with CHF (NYHA II-III) were enrolled in this study, it is unclear whether or not this result can be applied to patients with more severe CHF. Our observation that the plasma adenosine level was high in patients with NYHA class III or IV implied that dipyridamole treatment might improve cardiac dysfunction in patients with class IV CHF.

Conclusions

We found that the gene expression of the A2a receptor, the A2b receptor, the A3 receptor, ADA and cytosolic 5′-nucleotidase was down-regulated in human failing myocardia. This result implies that the downregulation of adenosine plays an important role in causing heart failure by impairing adenosine signal transduction. We also found elevated cardiac adenosine levels and the repression of ADA enzyme in patients with severe heart failure. The enhancement of adenosine levels by dipyridamole improved cardiac functions in a small population of patients with CHF. These results from our basic and clinical research imply that adenosine therapy might be a promising approach to treat CHF, although we need to perform either medium- or large-scale trials to confirm this.

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