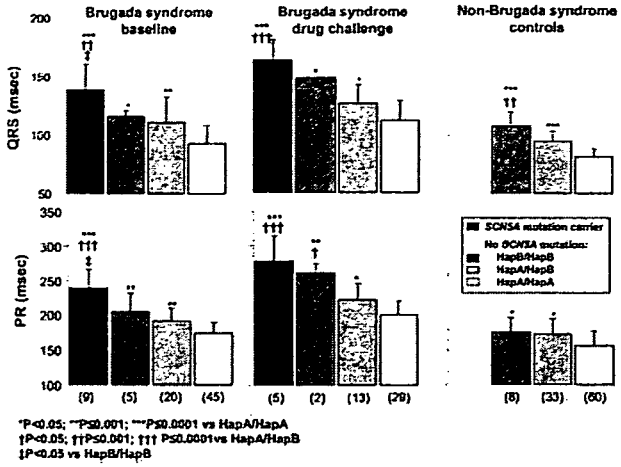

Figure 3. *SCN5A* promoter haplotype effects on durations of QRS_{V6} and PR_{II} in Brugada syndrome patients at baseline and after challenge with sodium channel blocking agents and in non–Brugada syndrome control subjects. Patient numbers are indicated in parentheses. Genotype effects on QRS_{V1} were similar to those on QRS_{V6} because of a high correlation between these 2 parameters (Pearson's coefficient, $r=0.96$). Data are presented as mean±SD. For Bonferroni-corrected significance levels for pairwise comparisons, refer to the Multiple Testing section in Patients and Methods.

In contrast, the *SCN5A* promoter haplotype we report here explained a remarkable proportion of variance in conduction parameters in the Japanese subjects studied (Table 3). Such associations could arise because the haplotypes studied are, in turn, in linkage disequilibrium with other functionally important variants in regulatory or other regions of the gene. However, in this case, the in vitro functional studies indicate that the effect is attributable to a variant within the haplotype block; at this point, the specific variant mediating this effect has not been identified.

A principal determinant of cardiac conduction in atrial and ventricular muscle is the sodium current; sodium channel blockers prolong PR and QRS durations, an effect also seen with loss of function mutations in *SCN5A*.[3] Critical degrees of conduction slowing represent a final common pathway to VF,[19] so dissection of the genetic determinants of cardiac conduction in the general population is a key step to understanding variable susceptibility to common arrhythmias resulting from conduction slowing, as in myocardial ischemia

TABLE 3. Variance Explained by the Haplotype Pair

	R^2, %		
	Control Subjects	Brugada Syndrome Baseline	Brugada Syndrome Drug Challenge
PR_{II}	12.2	28.4	33.0
QRS_{V1}	47.6	26.4	33.0
QRS_{V6}	48.5	24.9	36.2

or heart failure.[19] Thus, the data we present here implicate the *SCN5A* promoter variant HapB, which slowed conduction in normal subjects and exacerbated conduction slowing in those with Brugada syndrome, as a candidate modulator of variability in risk of SCD. Importantly, imposition of further depression of sodium channel function by administration of sodium channel blocking drugs further exacerbated conduction slowing in a gene-dose-dependent fashion. Studies in large numbers of subjects at risk for SCD are required to further establish the role of this and other regulatory region polymorphisms in modulating that risk.

Differences in disease penetrance and expression have been widely reported in the cardiac sodium and other channelopathies.[20–23] Relatives carrying an *SCN5A* mutation identical to that of the proband may be clinically unaffected,[20] and family members may display different phenotypes, eg, Brugada syndrome or conduction disease.[23] Genetic variants like the one presented here are obvious candidate modulators of this variability in phenotypic expression. Interindividual variability also has been noted in response to pharmacological challenge with sodium channel blockers in Brugada syndrome patients.[20,24] In some patients, even some carrying an *SCN5A* mutation, drug challenge fails to unmask a Brugada syndrome ECG. The significantly greater increases in PR and QRS durations with sodium channel blockade in HapB carriers thus identify variability in expression of the drug target, the sodium channel, as a key mediator of this variable drug effect. It is thus possible that other sodium channel blocker response phenotypes such as the increased mortality with sodium channel blockers in the CAST[2] was determined by variable sodium channel expression. DNA samples from that important clinical trial were not archived, so this question will remain unanswered. More generally, the data

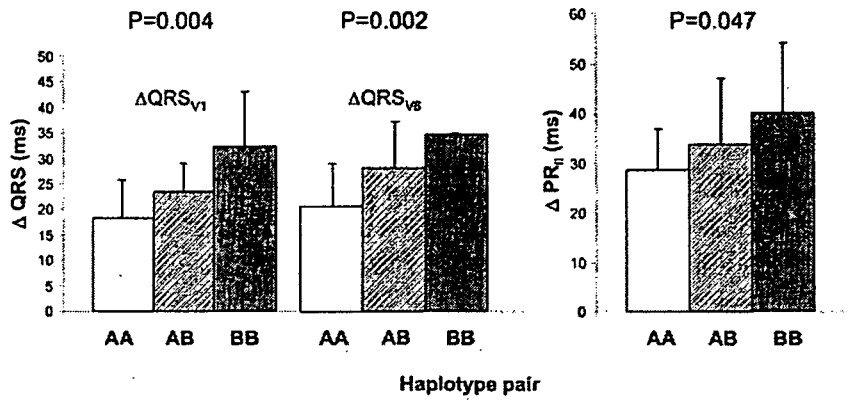

Figure 4. *SCN5A* promoter haplotype effects on extent of QRS (ΔQRS_{V1} and ΔQRS_{V6}) and PR (ΔPR_{II}) widening after sodium channel blockade. AA, n=29; AB, n=13; BB, n=2. Data are presented as mean±SD. The Bonferroni-corrected significance level is 0.002.

indicate that sodium channel function is additively suppressed by drug challenge, Brugada syndrome mutations, and the HapB regulatory variant. Although a strong reduction in reporter gene activity was observed for HapB compared with HapA in vitro, the extent to which this reduction translates proportionately into reduced sodium channel density in vivo is unknown.

Brugada syndrome is endemic in Asia, where the disorder is also known as sudden unexplained nocturnal death syndrome[25]; in fact, the incidence is higher in Asia than in the United States and Europe.[26] Because HapB is common in Asians and absent in whites and has a large negative impact on cardiac conduction, a long-recognized feature of Brugada syndrome,[27] it may logically contribute to differences in Brugada syndrome incidence as a function of ethnicity. In this study, PR and QRS durations in individuals matched for haplotype were consistently longer in the Brugada syndrome group compared with control subjects; thus, the greatest conduction slowing was in those subjects with Brugada syndrome and the HapB/HapB genotype. Indeed, control HapB/HapB subjects had longer QRS durations than did those with manifest Brugada syndrome and the commoner HapA/HapA genotype. Thus, although the minor allele is quite common, it alone may give rise to one part of the spectrum of loss of sodium channel function that constitutes the Brugada syndrome. However, data at this stage do not indicate that HapB per se leads to Brugada syndrome.

More generally, the data fit nicely the concept that individuals vary in their ability to maintain sodium channel function to protect against the arrhythmia-prone substrate and identify HapB as a variant that contributes to such variable "antifibrillatory reserve."[10,28]

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Disclosures

Drs Shimizu and Miyamato are applying for a Japanese domestic patent based on this work. The other authors report no conflicts.

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CLINICAL PERSPECTIVE

The sodium current determines conduction velocity in the heart, and reducing sodium current predisposes to VF. Sodium channel blockers increased sudden death after MI in CAST, and at least some cases of the Brugada syndrome, in which structurally normal hearts are prone to VF, are due to loss of function mutations in the cardiac sodium channel gene *SCN5A*. Thus, variability in the synthesis of sodium channels could contribute to variable conduction velocity in heart and to VF susceptibility. This study represents an important first step to testing that hypothesis. A set of 6 DNA variants were identified in the *SCN5A* promoter, the region of the gene directing transcriptional activity. The variants are common but only in Asian subjects and are in tight linkage disequilibrium; ie, subjects have either wild-type sequences or all 6 variants, defining a haplotype block called HapB here. HapB sequences not only reduced transcriptional activity in vitro but also predicted slower conduction velocity, assessed by PR and QRS durations, in both Japanese control and Brugada syndrome subjects. The longest QRS durations were in Brugada syndrome patients homozygous for HapB (≈7%) challenged with sodium channel blockers. Indeed, normal subjects homozygous for HapB had longer QRS durations than Brugada syndrome patients homozygous for wild-type sequences. These data support the idea that common *SCN5A* promoter variants modulate conduction velocity and thus susceptibility to VF in response to challenges such as other arrhythmogenic mutations, sodium channel blocking drugs, or acute ischemia. In addition, HapB may contribute to the higher prevalence of Brugada syndrome in Asians.

A −786T>C polymorphism in the endothelial nitric oxide synthase gene reduces serum nitrite/nitrate levels from the heart due to an intracoronary injection of acetylcholine

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We identified a −786T>C polymorphism in the eNOS gene, and this polymorphism was strongly associated with coronary spasm. The present study aimed to elucidate whether the −786T>C polymorphism or acetylcholine (ACh)-induced coronary spasm affects serum nitrite/nitrate (NOx) levels. The study population comprised three groups: (i) 26 patients without coronary spasm in the left anterior descending coronary artery (LAD) with the T/T genotype (group A); (ii) 20 patients with coronary spasm in the LAD with the T/T genotype (group B); and (iii) 16 patients with coronary spasm in the LAD with the C/T genotype (group C). Paired blood samples were obtained from the coronary sinus (CS) and the aortic tract (Ao) before and after an intracoronary injection of ACh. Serum NOx and plasma lactate levels were measured. The delta NOx level was calculated as the serum concentration of NOx in the CS minus that in the Ao. We compared lactate extraction ratios (LERs) and delta NOx levels between the three groups. The LERs after the provocation test in groups A, B and C were 18.9 ± 2.4%, −0.5 ± 3.9% and −13.5 ± 4.2%, respectively. The LER in group C was significantly lower than in group B. The delta NOx levels after the provocation test in groups A, B and C were 11.5 ± 1.7 μmol/l, 10.4 ± 3.5 μmol/l and −2.1 ± 4.8 μmol/l, respectively. The delta NOx levels in group C were significantly lower ($P<0.05$). Although the NOx level was significantly increased after the provocation test in group A ($P<0.05$), the NOx level was significantly decreased after the provocation test in group C ($P=0.001$). In group B, the provocation test did not significantly change the delta NOx level. In conclusion, the −786T>C polymorphism reduces the NOx level from the heart due to an intracoronary injection of ACh, and thereby predisposes the patients to severe coronary spasm. *Pharmacogenetics and Genomics* 16:339–345 © 2006 Lippincott Williams & Wilkins.

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Keywords: acetylcholene, coronary vasospasm, endothelium-derived nitric oxide, genetic polymorphism

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Introduction

Coronary spasm plays an important role in the pathogenesis of not only variant angina, but also of ischemic heart diseases in general, including other forms of angina pectoris, acute myocardial infarction, and sudden death [1]; however, the precise mechanism of the pathogenesis remains to be elucidated.

We have shown that an intracoronary injection of acetylcholine (ACh) results in severe vasoconstriction in coronary spasm patients, whereas ACh causes coronary vasodilation in subjects with healthy coronary arteries [1–3]. ACh-induced vasodilation is mediated by NO released from the endothelium [4,5]. These results suggest that the endothelium in the coronary arteries of coronary spasm patients is dysfunctional and that, as a consequence, NO release in response to ACh is lessened. Indeed, we have previously shown that basal, ACh-stimulated and flow-dependent NO activities are decreased in both the coronary and brachial arteries of coronary spasm patients [6,7].

In the endothelium of both animals and humans, the synthesis of nitric oxide (NO) from the amino acid

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L-arginine is catalysed by the endothelial nitric oxide synthase (eNOS) enzyme [4], and the resulting continuously generated NO serves to maintain basal vascular tone [4,5]. We previously reported that the –786T > C polymorphism in the 5′-flanking region of the eNOS gene was an independent risk factor for coronary spasm, as was smoking, although to a lesser degree [8,9]. As assessed by reporter gene assays, the –786C allele resulted in a significant reduction in eNOS gene promoter activity [8]. We also reported that the replication protein A1 represses the eNOS gene with the –786C allele, acting as a repressor of the eNOS gene transcription [10].

The present study aimed to elucidate whether an intracoronary injection of ACh increases NO production from the heart in coronary spasm patients and in non-coronary spasm patients; moreover, whether and how the –786T > C polymorphism of the eNOS gene affects NO levels from the heart.

Materials and methods

Study population

We analysed 64 subjects who were admitted consecutively at Kumamoto University Hospital. All subjects underwent coronary angiography with an intracoronary injection of ACh for evaluation of chest pain. Coronary spasm is defined as total or subtotal occlusion of the coronary artery associated with chest pain and/or ischemic electrocardiographical changes. After an intracoronary injection of isosorbide dinitrate (ISDN), the patients' coronary arteries appeared to be normal and exhibited no significant organic stenosis (< 50% luminal diameter).

Subsequently, we divided these subjects into three groups: (i) 26 T/T genotype patients who had no coronary spasm in the left coronary arteries (group A); (ii) 20 T/T genotype patients who had coronary spasm in the left coronary arteries after an intracoronary injection of ACh (group B); and (iii) 16 C/T genotype patients who had coronary spasm in the left coronary arteries after an intracoronary injection of ACh (group C). There were two C/T genotype patients without coronary spasm in the left coronary arteries. There were no patients with the C/C genotype.

When considering the clinical characteristics of the study patients, hypertension was operationally defined as a blood pressure > 145/90 mmHg, whereas diabetes mellitus was defined as fasting venous blood glucose levels ≥ 126 mg/dl or > 200 mg/dl on an oral glucose tolerance test. Cigarette smoking included current smokers and ex-smokers.

Written informed consent was obtained from all patients. The study was also in agreement with the guidelines of and approved by the ethics committee of Kumamoto University Graduate School of Medical Sciences.

Cardiac catheterization

All medications being taken by the study participants were discontinued at least 48 h prior to cardiac catheterization. Coronary arteriography was performed in the morning when the subjects were in a fasting state. After baseline arteriography of the left and right coronary arteries, an intracoronary injection of ACh was administrated, as previously described [3]. Two consecutive doses (50 and 100 μg) of ACh were injected, 4 min apart, into the left coronary artery; angiography was performed, and completed within 30 s of each injection. Then, 50 μg of ACh was injected into the right coronary artery and angiography was again performed 4 min apart. Finally, both left and right coronary arteriograms were taken after an intracoronary injection of 1 mg of ISDN. We evaluated the degree of organic stenosis after the injection of ISDN.

Blood sampling and assays

Paired blood samples were obtained from the aorta and the coronary sinus immediately following the injection of 100 μg of ACh into the left coronary artery and these samples were then immediately placed in an ice bath. After centrifuging of the blood sample for 10 min, the serum and plasma were packed in a freezer at –80°C for subsequent determination of nitrite/nitrate and lactate concentrations.

Screening for the –786T>C polymorphism of the eNOS gene by the allele-specific oligonucleotide method

The allele-specific oligonucleotide method was used to determine the presence of the –786T > C polymorphism. This method has been described previously [9]. In brief, polymerase chain fragments 236-bp in length, including the –786T/C site, were blotted in duplicate onto nylon membranes. Hybridization was accomplished with ^{32}P-radiolabelled oligonucleotides corresponding to either the –786T sequence (5′-GGG TCA GCC AGC CAG GGA A-3′: probe for the –786T sequence) or the –786C sequence (5′-GGG TCA GCC GGC CAG GGA A-3′: probe for the –786C sequence).

Measurement of plasma lactate concentration and serum nitrite/nitrate concentration

Plasma lactate concentrations were measured using an enzyme assay [12]. We determined the lactate extraction ratio (LER) as: 100 × [plasma lactate concentration at aorta (Ao)–plasma lactate concentration at coronary sinus (CS)]/lactate concentration at Ao. Serum nitrite/nitrate concentrations were measured using a flow injection autoanalyser (TCI-NOX1000, Tokyo Kasei Kogyo, Tokyo, Japan) which is based on the Griess Reaction methodology [11]. The samples were passed through a column containing copper-coated cadmium, which reduced all nitrate to nitrite; the nitrite was then detected by

reacting it with a Griess reagent; and, finally, nitrite/nitrate concentrations were then measured spectrophotometrically at 540 nm.

We analysed delta serum nitrite/nitrate (NOx) levels as: NOx concentration at the CS–NOx concentration at the Ao.

Statistical analysis

Continuous variables were compared using two-tailed unpaired *t*-tests. Categorical variables were compared by chi-square analysis with Fisher's exact probability test. LER and delta NOx levels were compared using two-tailed unpaired *t*-tests between the study groups. Comparison of delta NOx levels before and after an intracoronary injection of ACh was performed using the paired *t*-test. Linear regression analysis was used to correlate delta NOx level and LER. $P < 0.05$ was considered statistically significant.

Results

Clinical characteristics of the study population

The clinical characteristics of this study population are shown in Table 1. There were no significant differences between the three groups regarding age, gender, cigarette smoking, hypertension, diabetes mellitus, total cholesterol level, or body mass index.

Lactate extraction ratios

Before an intracoronary injection of ACh, LERs in groups A, B and C were 39.0 ± 2.3%, 36.1 ± 2.7% and 36.3 ± 3.1%, respectively (Fig. 1). There were no significant differences between the three groups. After an intracoronary injection of ACh, LERs in groups A, B and C were 19.0 ± 2%, −0.5 ± 4% and −13.5 ± 4%, respectively (Fig. 1). In the patients with the −786T/T genotype, LER was significantly lower in the patients with coronary spasm (group B) than in the patients without coronary spasm (group A) ($P < 0.0001$). In the coronary spasm patients, LER was significantly lower in the patients with the −786C/T genotype (group C) than in the patients with the −786T/T genotype (group B) ($P < 0.03$). The LERs in two patients with the −786C/T genotype, without coronary spasm, were 63.4% and 6.3% after an intracoronary injection of ACh.

Serum nitrite/nitrate levels

Serum NOx levels in the aorta and the coronary sinus before and after the provocation test are shown in Table 2 There were no significant differences in the serum NOx levels between the three groups regarding each part or blood sampling time. Subsequently, we analysed the delta NOx level as: NOx at the CS–NOx concentration at the Ao.

Before the provocation test, the delta NOx levels in groups A, B and C were 7.6 ± 2.2 μmol/l, 12.9 ± 3.8 μmol/l and 14.8 ± 3.9 μmol/l, respectively (Fig. 1). There were no significant differences between the three groups. After the provocation test, the delta NOx levels in groups A, B and C were 11.5 ± 1.7 μmol/l, 10.4 ± 3.5 μmol/l and −2.1 ± 4.8 μmol/l, respectively (Fig. 1). The delta NOx levels in group C were significantly lower ($P < 0.05$). We compared the delta NOx levels before and after the provocation test in each group as shown in Fig. 2. Although the delta NOx level was significantly increased after the provocation test in group A ($P < 0.05$), the delta NOx level was significantly decreased after the provocation test in group C ($P < 0.001$). In group B, the provocation test did not significantly change the delta NOx level. In one of the two patients with the −786C/T genotype without coronary spasm, the delta NOx levels before and after the provocation test were 46.5 μmol/l and 38.1 μmol/l, respectively; the delta NOx levels of the other patient before and after the provocation test were 20.3 μmol/l and 18.4 μmol/l, respectively. The delta NOx levels in the two patients with the −786C/T genotype without coronary spasm basically decreased after the provocation test.

Correlation between delta NOx level and LER

Before the provocation test, the delta NOx level did not significantly correlate with LER ($r = -0.042$, P = NS) (Fig. 3). After the provocation test, the delta NOx level had a significant positive correlation with LER ($r = 0.346$, $P < 0.005$) (Fig. 3).

Discussion

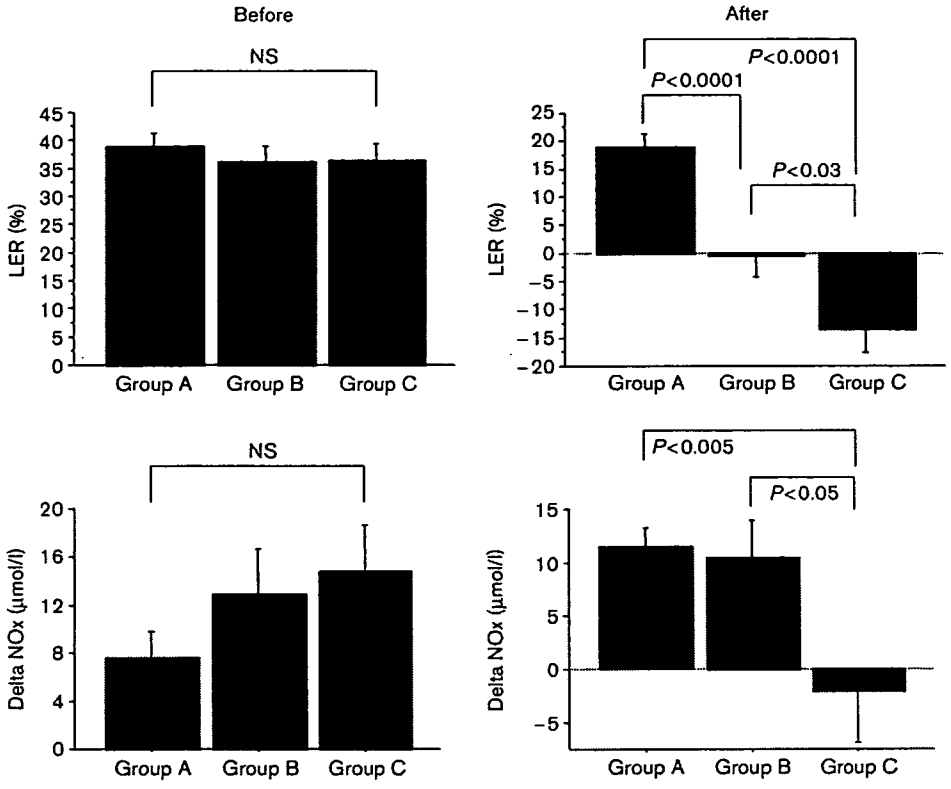
The vascular endothelium plays an important role in the regulation of regional blood flows by releasing an endothelium derived relaxing factor, a major component

Table 1 Clinical characteristics of the study subjects

	Group A: non-coronary spasm with −786T/T (n=26)	Group B: coronary spasm with −786T/T (n=20)	Group C: non-coronary spasm with −786C/T (n=16)	P
Age (years)	61 ± 13	64 ± 9	60 ± 15	NS
Men : women (ratio)	12 : 14	10 : 10	9 : 7	NS
Cigarette smoking, n (%)	10/26 (38)	10/20 (50)	10/16 (63)	NS
Hypertension, n (%)	8/26 (31)	8/20 (40)	4/16 (25)	NS
Diabetes mellitus, n (%)	1/26 (4)	4/20 (20)	4/26 (25)	NS
Total cholesterol (mg/dl)	184 ± 29	187 ± 24	184 ± 35	NS
Body mass index (kg/m^2)	24.0 ± 3.2	23.0 ± 1.5	23.5 ± 3.7	NS

Data are mean ± SD except where indicated.

Fig. 1



Lactate extraction ratio (LER) before (left) and after (right) an intracoronary injection of acetylcholine. Delta nitrite/nitrate (NOx) levels before (left) and after (right) an intracoronary injection of acetylcholine. The delta NOx indicates the difference in serum NOx level between the coronary sinus (CS) and the aorta (Ao) [delta NOx (CS–Ao)]. Values are expressed as the means ± SEM.

Table 2 **Serum nitrite/nitrate levels (μmol/l) in aorta and coronary sinus before and after provocation test**

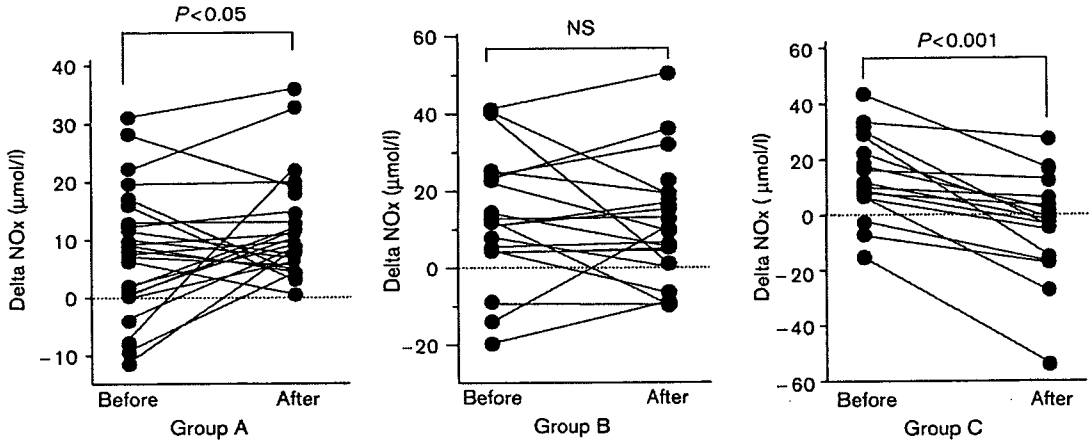
	Before		After	
	Aorta	Coronary sinus	Aorta	Coronary sinus
Group A: non-coronary spasm with −786T/T (n=26)	54.9±31.2	62.4±34.1	41.1±27.0	52.7±30.5
Group B: coronary spasm with −786T/T (n=20)	45.1±22.2	58.0±24.8	38.1±18.9	48.4±20.8
Group C: non-coronary spasm with −786C/T (n=16)	49.9±21.8	64.7±22.9	52.1±29.5	50.0±15.8

Data are mean±SD.

of which is endothelial NO [4,5]. An intracoronary injection of ACh results in severe vasoconstriction in coronary spasm patients, whereas ACh causes coronary vasodilation in subjects with healthy coronary arteries [1–3]. ACh-induced vasodilation is mediated by NO released from the endothelium [4,5]. Because NO is a labile substance with a short half-life and decomposes rapidly into nitrite and nitrate (NOx), its direct measurement has proved to be difficult [13]. It has been reported that increases in the serum NOx levels in rats treated with endotoxin were inhibited by the coadministration of NO synthase inhibitor nitro-L-argine methyl ester, suggesting that the NOx level reflects endogenous NO production [14].

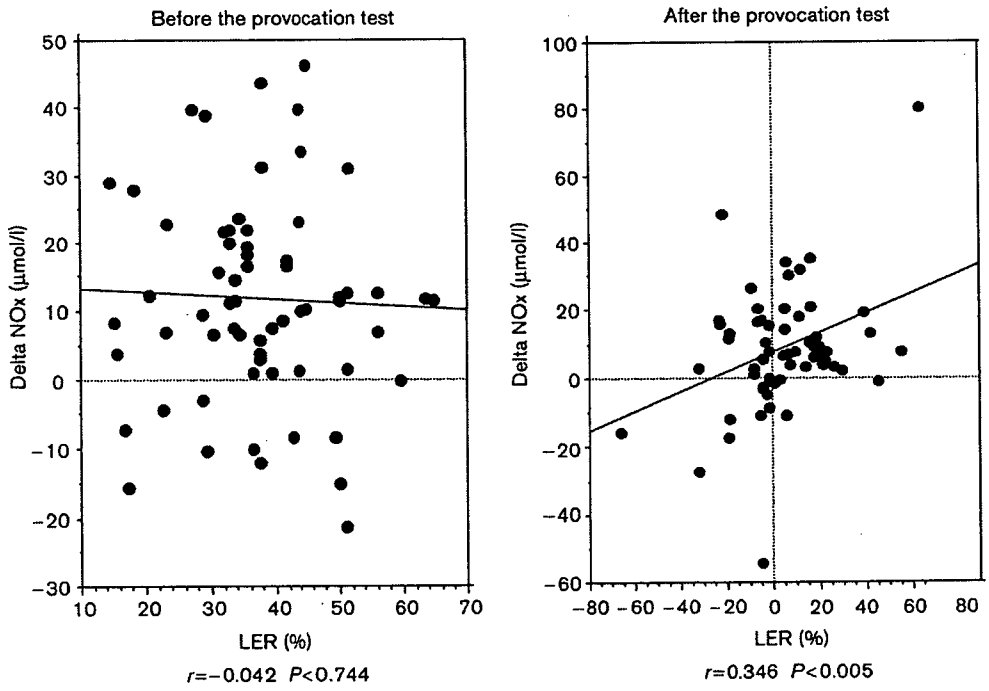
In the present study in coronary spasm patients, LER was significantly lower in the patients with the −786C/T genotype of the eNOS gene than in the patients with the −786T/T genotype after an intracoronary injection of ACh. The −786T > C polymorphism possibly causes coronary spasm and contributes to the severity. Naber *et al.* [15] reported that myocardial lactate uptake was reversed into net lactate production after an intracoronary injection of acetylcholine in subjects with the −786C allele. Our results on LER is in agreement with their report. As for possible actions to increase the severity of coronary spasm, the −786C > T polymorphism significantly reduced delta NOx levels in coronary spasm patients after the provocation test.

Fig. 2



Delta nitrite/nitrate (NOx) levels before and after an intracoronary injection of acetylcholine in groups A, B and C. The delta NOx indicates the difference in serum NOx level between the coronary sinus (CS) and the aorta (Ao) [delta NOx (CS–Ao)].

Fig. 3



Correlation between delta nitrite/nitrate (NOx) levels [coronary sinus (CS)–aorta (Ao)] and lactate extraction ratio (LER) before (left) and after (right) an intracoronary injection of acetylcholine.

We have reported that the −786T > C polymorphism enhanced the vasoconstriction response due to an intracoronary injection of ACh [9,16]. We suggested that reducing the ACh-induced NO production from the coronary endothelial cells in the patients with the −786T > C polymorphism causes significant

vasoconstriction. Although the ACh-induced NO is mainly generated by the endothelial cells, both endothelial cells and cardiomyocytes are thought to be potential sources of NO generation when a state of hypoxia exists in the heart. Node *et al.* [17] reported that NO production from the heart is increased in ischemic hearts, and after exertion, in patients with effort angina. These results suggest that hypoxia possibly accounts for an increase in NO production from the heart, including from coronary arterial endothelial cells and/or from cardiomyocytes. Han *et al.* [18] reported that hypoxic red blood cells (RBCs) generate HbFe(II)NO, and that the NO consumption rate therefore increases. The NO level is possibly reduced under the hypoxic condition because of an increase in the NO consumption rate of RBCs. In the present study, for non-coronary spasm patients with the –786T/T genotype (group A), NO was possibly generated from endothelial cells due to the intracoronary injection of ACh; furthermore, their coronary arteries did not produce coronary spasm. In coronary spasm patients with the –786T/T genotype (group B), an intracoronary injection of ACh caused coronary spasm. Although the NO consumption rate possibly increases in hypoxic RBCs, the total NO level in the serum was maintained at an overall high level in group B. The increase in NO production from the heart, including from the endothelial cells and/or from the cardiomyocytes, under an ischemic condition, immediately relaxed the coronary arteries. After an intracoronary injection of ACh, there was no significant difference in the delta NOx levels between groups A and B. Although the coronary spasm patients with the –786T/T genotype have high delta NOx levels before and after the provocation test, some of them possibly have coronary spasm for reasons other than the reduced NO production from the heart. In coronary spasm patients with the –786C allele (group C), reduced NO production from the endothelial cells due to the intracoronary injection of ACh caused coronary spasm, and an insufficient supply of NO production from the heart under this ischemic condition prolonged coronary spasm. An increase in the NO consumption rate in hypoxic RBCs possibly leads to a still more critical spasm state. Previously, we reported that the –786T > C polymorphism is strongly associated with coronary spasm and also with myocardial infarction without organic stenosis [19]; furthermore, we suggested that this polymorphism is possibly associated with the severity of coronary spasm. The –786T > C polymorphism reduced NO production from the heart, even in an ischemic condition, and predisposed the patients to a prolonged coronary spasm, leading to myocardial infarction without organic stenosis. Also, endothelial dysfunction and oxidative stress are known to be crucially involved in the pathogenesis of coronary spasm [20–24]. A decrease in NO production possibly increases oxidative stress and predisposes the patients with the –786C allele to coronary spasm.

There are some reports regarding systemic circulating NOx levels and the –786T > C polymorphism [10,25,26]. Although there is a low tendency for the systemic circulating NOx level in subjects with the –786C allele, there are few reports stating that it is clearly low. It is possible that there is not enough of a significant difference in the systemic circulating NOx level to classify this as being due to the genotype of the –786T > C polymorphism because of the influences of either meal and/or individual levels of oxidative stress. In the present study, an intracoronary injection of ACh significantly increased delta NOx levels in subjects without coronary spasm without the –786C allele, although it did not significantly change the delta NOx levels in subjects with coronary spasm without the –786C allele, and it significantly decreased the delta NOx level in subjects with coronary spasm with the –786C allele. There was a difference of sufficient magnitude in delta NOx levels before and after the provocation test to classify the genotype of the –786T > C polymorphism, even in coronary spasm patients. It is well known that NO plays a key role in the regulation of vascular tone [4,5,27,28] and has vasoprotective effects by scavenging superoxide radicals and suppressing platelet aggregation, leukocyte adhesion and smooth muscle cell proliferation [29–31]. A decrease in the delta NOx level possibly affects the cardiovascular system and leads to severe vasoconstriction. Furthermore, Tanus-Santos *et al.* [32] reported that the –786C allele decreases platelet-derived NO. The –786C allele may accelerate platelet aggregation and serve as a risk factor for cardiovascular disease. Indeed, it was reported that the –786C allele is associated with coronary spasm [8], myocardial infarction [19] and coronary organic stenosis [33].

In conclusion, the –786T > C polymorphism reduces NO production from the heart due to an intracoronary injection of ACh, and thus predisposes patients to a prolonged and more severe coronary spasm.

Study limitation

In the present study population, there were two non-coronary spasm patients with the –786C/T genotype and there were no patients with the –786C/C genotype, this is possibly because the study population was relatively small in size. However, we have previously reported that the frequencies of these patients are relatively low in the Japanese population [8,9,19]. In both patients with the –786C/T genotype without coronary spasm, delta NOx levels basically decreased after the provocation test. Even in the case of non-coronary spasm patients, the –786C allele possibly suppresses NO production from the heart, which is due to an intracoronary injection of ACh. Further studies in a larger population group, including many non-coronary spasm patients with the –786C allele and many patients with the –786C/C genotype, will be beneficial to further elucidate this topic.

NO is generated by NO synthase (NOS), which exists as a family of related but distinct isoforms, including neuronal (nNOS) [34,35], inducible (iNOS) [36,37], and endothelial (eNOS) [4] isoforms. It has been reported that eNOS is detected in the endothelial cells overlying normal human aortas, fatty streaks and advanced atherosclerotic lesions, whereas iNOS and nNOS are not detectable in normal vessels, although widespread production of these two isoforms has been found in early and advanced lesions associated with macrophages, endothelial cells and mesenchymal-appearing intimal cells [38]. In the present study, we did not distinguish which isoform of NOS produces NO from the heart before or after the provocation test.

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平成１９年度　刊行物・別刷

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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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

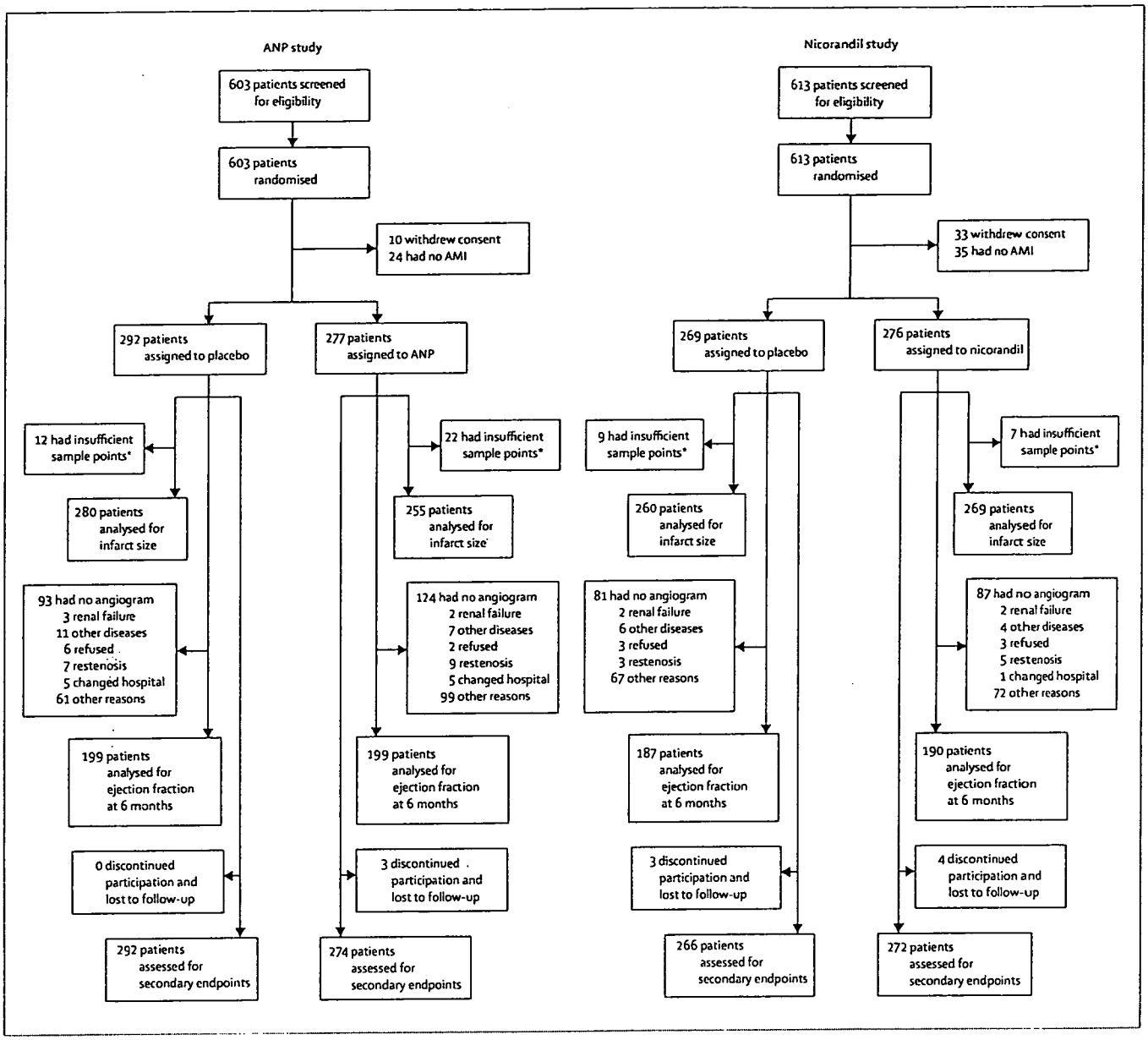

Figure 1: **Trial profiles**
ANP=atrial natriuretic peptide. AMI=acute myocardial infarction. *Fewer than six blood samples.

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,[10] 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)	66 459·9 (60 258·2–73 300·0)	77 878·9 (71 590·2–84 720·1)	0·016	70 520·5 (64 309·8–77 331·0)	70 852·7 (65 066·7–77 153·2)	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.[7,13] 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,[14] 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.

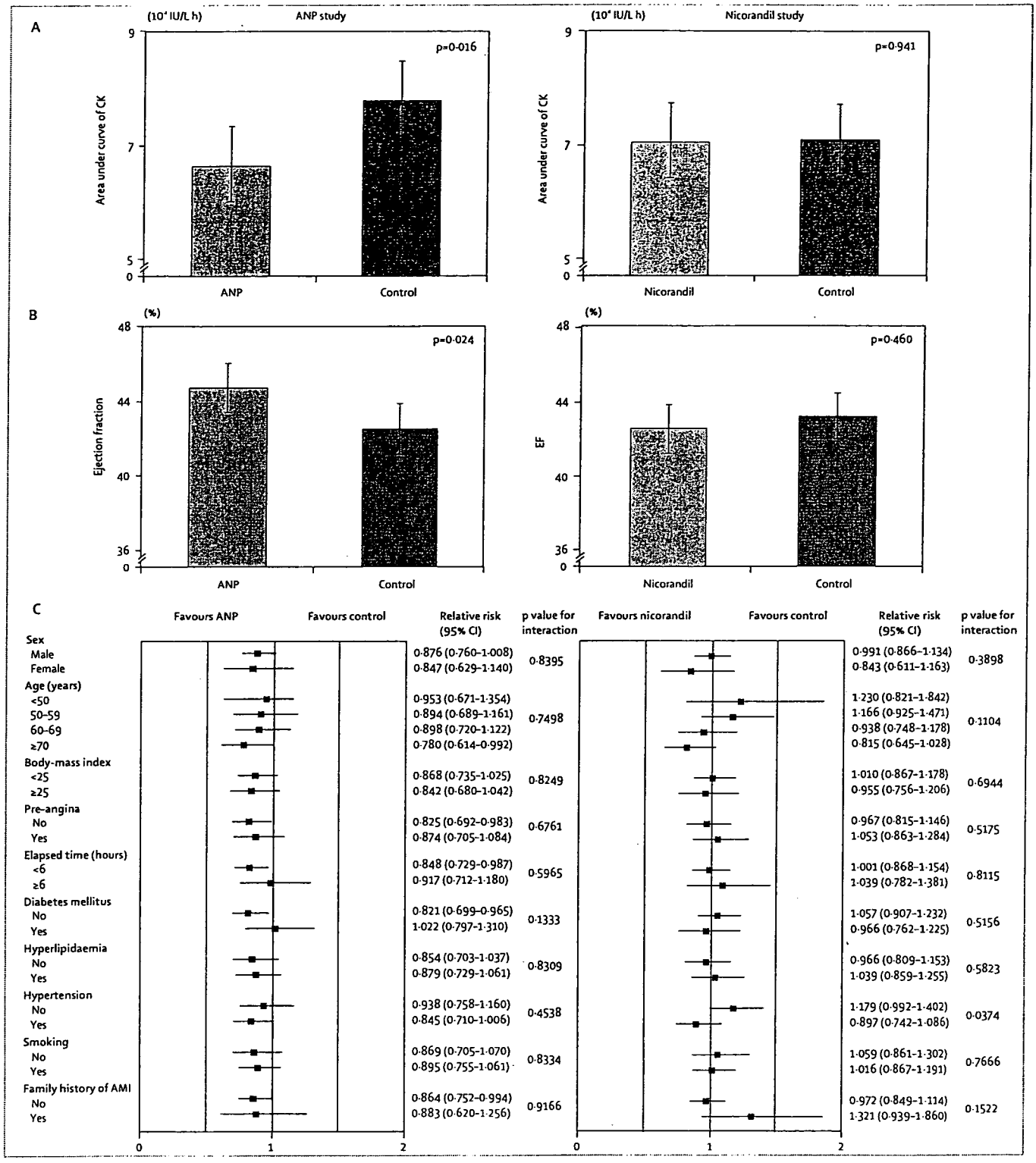

Figure 2: Primary endpoints and subgroup analyses
CK=creatine kinase. AMI=acute myocardial infarction. ANP=atrial natriuretic peptide. Panel A shows area under curve of creatine kinase concentration versus time. Panel B represents left ventricular ejection fraction measured at 6–12 months.

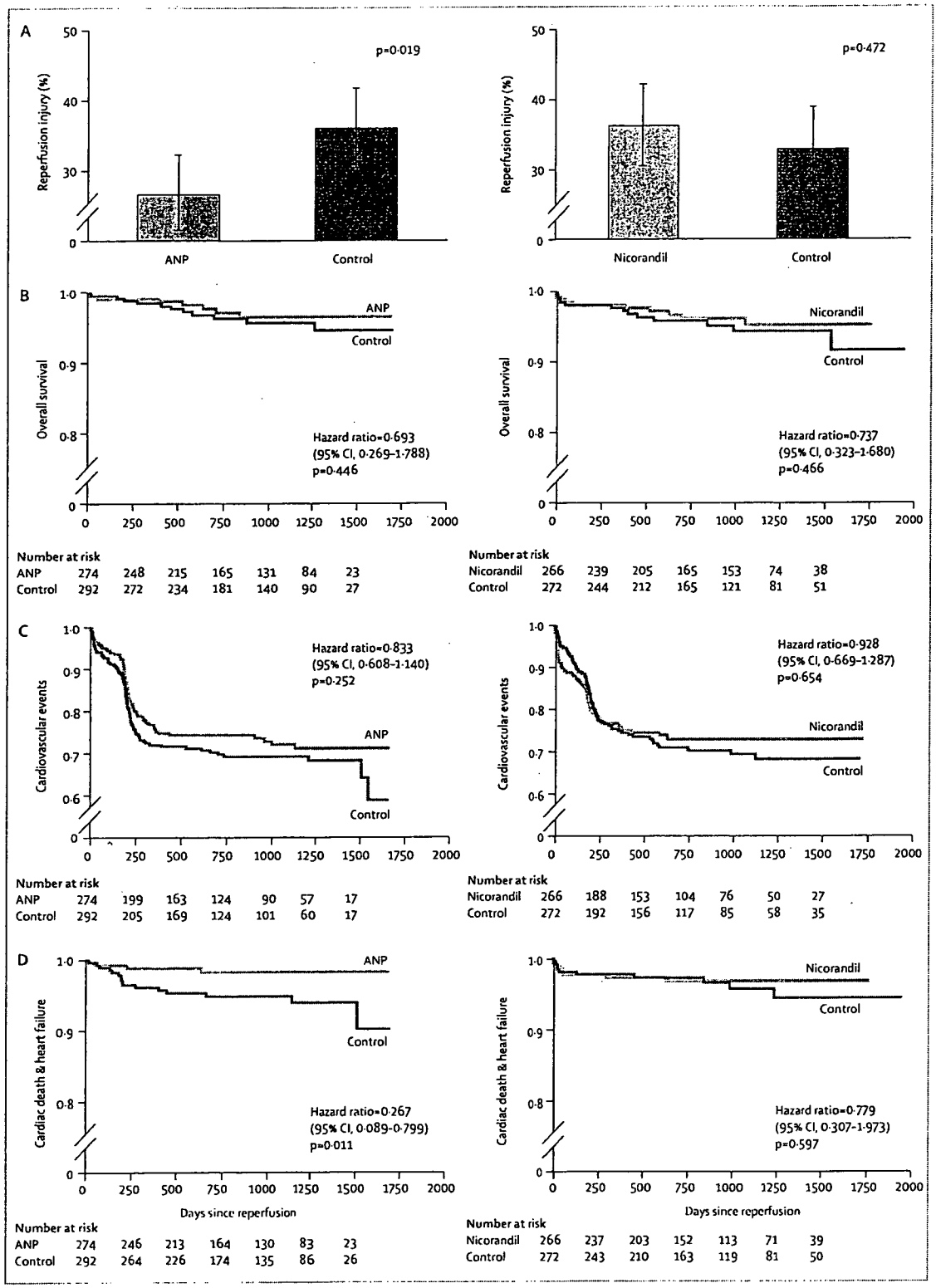

Figure 3: Secondary endpoints and other subanalyses
ANP=atrial natriuretic peptide.

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[35] showed that nicorandil could reduce the incidence of unstable angina in patients with stable angina.

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

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

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