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]

Acknowledgments

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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.

Response of beat-by-beat QT variability to sympathetic stimulation in the LQT1 form of congenital long QT syndrome

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OBJECTIVES The purpose of this study was to test the hypothesis that the lability of beat-by-beat QT variability is prominent during sympathetic stimulation in LQT1 patients. We analyzed beat-by-beat QT variability using a newly developed program and applied cross-correlation methods in LQT1 patients before and after epinephrine infusion.

BACKGROUND Studies suggest that cardiac events associated with sympathetic stimulation are more common in the LQT1 form than the LQT2 and LQT3 forms of congenital long QT syndrome (LQTS). Although beat-by-beat alternation of T-wave morphology is observed in LQTS, its objective estimation is difficult because of complicated T-wave morphology.

METHODS Twelve-lead ECG was recorded under baseline conditions and during epinephrine infusion (0.1 μg/kg/min) in 14 LQT1 and five control patients. We measured beat-by-beat QT interval by a cross-correlation technique. Mean of successive changes in RR (ΔRR), QT (ΔQT), standard deviation of ΔRR (SD-ΔRR), ΔQT (SD-ΔQT), and QTI (QT/ RR) before and after epinephrine were compared between the two groups.

RESULTS No significant differences in any parameters were observed between the two groups under baseline conditions. ΔQT, SD-ΔQT, and QTI were increased in LQT1 but not in control patients during epinephrine (LQT1: ΔQT 2.3–4.2 ms, SD-ΔQT 2.2–4.1, QTI 0.10–0.22, $P < .005$ vs baseline; Control: ΔQT 2.5–2.4 ms, SD-ΔQT 1.9–2.1, QTI 0.08–0.09: P = NS vs baseline).

CONCLUSIONS Beat-by-beat QT variability analyzed by the cross-correlation method was greater in LQT1 patients during epinephrine infusion, suggesting sympathetic stimulation accentuates beat-by-beat alternation of repolarization in LQT1 patients.

KEYWORDS Long QT syndrome; Epinephrine; Sympathetic activity; QT interval; T-wave alternans

(Heart Rhythm 2005;2:149–154)

Introduction

The congenital long QT syndrome (LQTS) is a hereditary disorder associated with prolonged ventricular repolarization and the life-threatening polymorphic ventricular tachycardia torsades de pointes (TdP).[1,2] Genetic studies have shown that congenital LQTS is a primary electrical disease caused by mutation in specific ion channel genes.[3,4] Seven forms of congenital LQTS have been identified.

Among the seven forms, cardiac events associated with sympathetic stimulation are more common in the LQT1 form than in the other forms of congenital LQTS.

T-wave alternans (TWA), an ECG phenomenon characterized by beat-by-beat alternation of the morphology, amplitude, and/or polarity of the T wave, often is associated with congenital LQTS. TWA is an important prognostic indicator because it is commonly observed just preceding episodes of TdP.[5–7] Although beat-by-beat alternation of repolarization somewhere in the heart is presumed to underlie TWA, its objective estimation is difficult because of complicated T-wave morphology.

The present study used a novel method "cross-correlation technique" to assess beat-by-beat QT variability. The aim of the study was to test the hypothesis that the lability

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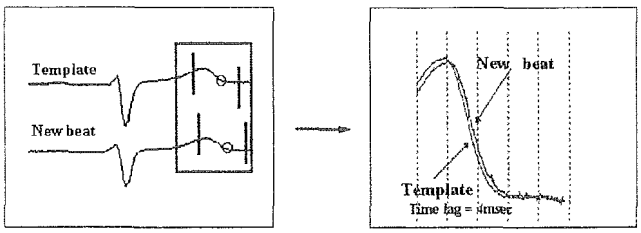

Figure 1 Algorithm of QT measurement by the cross-correlation method. See text for details.

of beat-by-beat QT variability is prominent during sympathetic stimulation in LQT1 patients in whom cardiac events often occur during sympathetic stimulation.

Methods

Study population

Fourteen LQT1 patients with *KCNQ1* mutation and five healthy volunteers used as controls were included in the study. The five healthy volunteers had no symptoms, and no abnormal T-wave morphologies were observed on 12-lead ECG. LQTS-affected individuals were noted based on the ECG diagnostic criteria of Keating et al.[8] The criteria include corrected QT (QTc) ≥470 ms in asymptomatic individuals and QTc >440 ms for men and >460 ms for women associated with one or more of the following: (1) stress-related syncope, (2) documented TdP, or (3) family history of early sudden cardiac death. Genotyping of LQTS was reviewed and approved by the Ethical Review Committee. Written informed consent was obtained from all patients.

Recording of standard 12-lead ECG

Standard 12-lead ECG was recorded using an FDX6521 (Fukuda Denshi Co., Tokyo, Japan) with the patient in the supine position without antiarrhythmic medications, including beta-blockers. ECG data were digitized using analog-to-digital converters at a sampling rate of 1,000 samples/second per channel.

ECG measurements

We measured QT interval beat by beat in the most stable lead to analyze T-wave morphology among precordial leads. The beat-by-beat changes of the QT interval were assessed during the latter half of T wave (Figure 1).

Specifically, the steps involved in analyzing a digitized ECG record included the following. (1) The operator selected a lead to analyze and the beginning and the end of the template T wave as an average of consecutive five beats. (2) The time of each R wave was identified using an automated peak detection algorithm. (3) For each of the other new beats, the time lags between the new beat and the template were calculated for comparison with the templates of QT morphology by a *cross-correlation method.* The templates were resampled as successive five beats before the newest analyzed beat.

We also analyzed beat-by-beat QT interval using a semi-automated digitizing program simultaneously. QT interval was defined as the time interval between QRS onset and the point at which the isoelectric line intersected a tangential line drawn at the maximal downslope of the T wave *(tangential method).*

Epinephrine administration

The epinephrine test was conducted as part of the clinical evaluation of LQTS.

A bolus injection of epinephrine 0.1 μg/kg was followed immediately by continuous infusion at 0.1 μg/kg/min. Twelve-lead ECG was recorded continuously during sinus rhythm under baseline conditions and usually for 5 minutes after start of epinephrine infusion. The effect of epinephrine on RR and QT intervals usually reached steady-state conditions 2 to 3 minutes after epinephrine was started. Epinephrine infusion for more than 5 minutes was avoided. ECG monitoring was continued for another 5 minutes after finishing epinephrine infusion to detect any occurrence of TdP. ECG data were collected under baseline conditions and at steady-state epinephrine effect 3 to 5 minutes after epinephrine was started.

Analyzed parameters

The following five ECG parameters were calculated from all RR and QT intervals recorded for 30 seconds during baseline conditions and at steady-state epinephrine conditions and then compared between the two groups (Figure 2): (1) ΔRR, the average of successive RR interval changes; (2) ΔQT, the average of successive QT interval changes; (3) SD-ΔRR, the standard deviation of RR interval; (4) SD-ΔQT, the standard deviation of the QT interval; and (5) QT index (QTI), the rate of change of QT interval

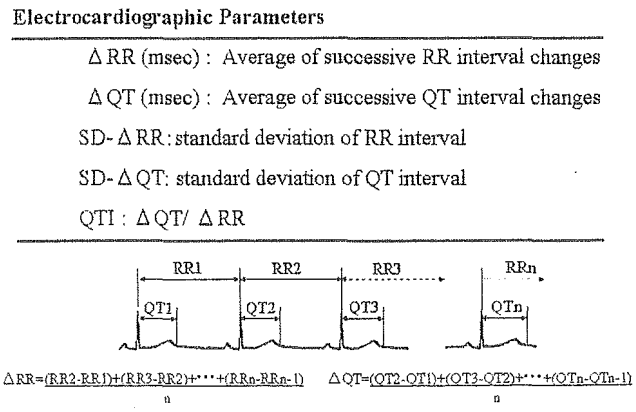

Figure 2 Five ECG parameters calculated in the present study. See text for details.

Table 1 Baseline ECG characteristics

	LQT1 (n = 14)	Control (n = 5)
Age	28 ± 20	29 ± 10
HR (bpm)	71 ± 10	68 ± 7
QT (ms)	454 ± 59*	387 ± 13
QTc (ms)	504 ± 76*	410 ± 36
$T_{peak-end}$ (ms)	102 ± 16	91 ± 19
QTD (ms)	71 ± 25*	45 ± 11

Values are reported as mean ± SD.

HR = heart rate; QTc = corrected QT interval; $T_{peak-end}$ = interval between T_{peak} and T_{end}; QTD = QT dispersion (maxQT-minQT).

*$P < .05$ vs control.

to RR interval, defined as the beat-by-beat value of ΔQT divided by ΔRR.

We examined the relationship between QT variability (ΔQT, SD-ΔQT) analyzed by cross-correlation methods and QT interval or heart rate before and after epinephrine infusion.

Statistical analysis

Data are expressed as mean ± SD. Paired and unpaired t-tests were used for couple observation. Correlation between continuous variables was tested by linear regression. For all tests, $P < .05$ was considered significant.

Results

Table 1 lists baseline ECG characteristics. No significant differences were observed regarding age and baseline heart rate between the LQT1 and control groups. The baseline QT and QTc intervals and QT dispersion, which were analyzed by the tangential method, were all significantly greater in the LQT1 group than in control group.

Beat-by-beat T wave variability before and after epinephrine

Figure 3 illustrates representative examples of superimposed QT complexes before and after epinephrine. The consecutive 10 beats of eight-lead ECGs were drawn temporally. In the control patient, no significant difference of beat-by-beat T-wave morphology was observed before and after epinephrine. In contrast, more significant beat-by-beat lability of the T wave was recognized after epinephrine in the LQT1 patient, although no significant change of beat-by-beat T-wave morphology was observed under baseline.

Beat-by-beat QT variability

The analyzed ECG leads were lead V_5 in three controls and six LQT1 patients, lead V_6 in two controls and five LQT1 patients, and lead V_2, V_3, and V_4 in each of the remaining LQT1 patients.

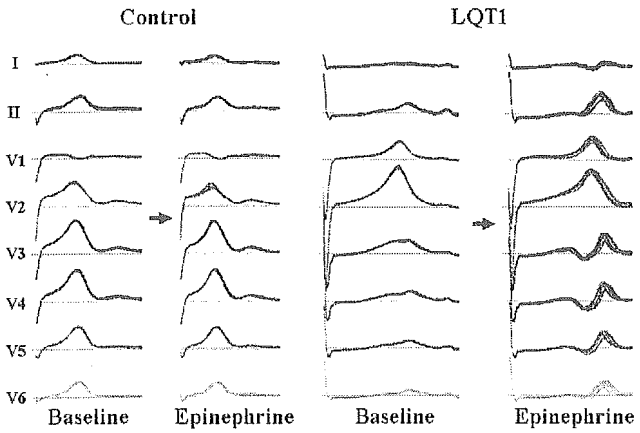

Figure 3 Representative example of superimposed QT complexes before and after epinephrine. The consecutive 10 beats of eight-lead ECGs are drawn temporally. In a control patient, no difference of beat-by-beat T-wave morphology is observed before and after epinephrine. However, more significant beat-by-beat alternans of T wave and change to biphasic T-wave pattern were observed after epinephrine in an LQT1 patient.

Figure 4 illustrates beat-by-beat change of the RR, QT, and the ΔRR and ΔQT in a control patient. The RR interval was decreased after bolus infusion of epinephrine, and remained decreased less than before epinephrine at the steady state condition. The ΔRR, which is heart rate variability, became small following the start of epinephrine. The QT interval was prolonged when the RR was decreased after bolus epinephrine, however the QT interval was slightly shortened compared before epinephrine at steady-state. The ΔQT was not changed before and after epinephrine infusion.

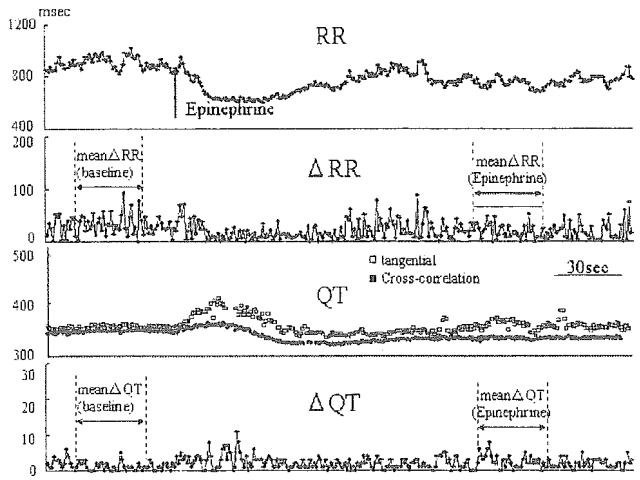

Figure 4 Beat-by-beat change of RR, QT, ΔRR, and ΔQT in a control patient. RR interval was decreased after bolus infusion of epinephrine and remained decreased less than before epinephrine at the steady-state condition. ΔRR became small after epinephrine was started. QT interval was prolonged when RR was decreased after bolus epinephrine but was slightly shortened compared with before epinephrine at the steady-state epinephrine effect. ΔQT was not changed before and after epinephrine infusion.

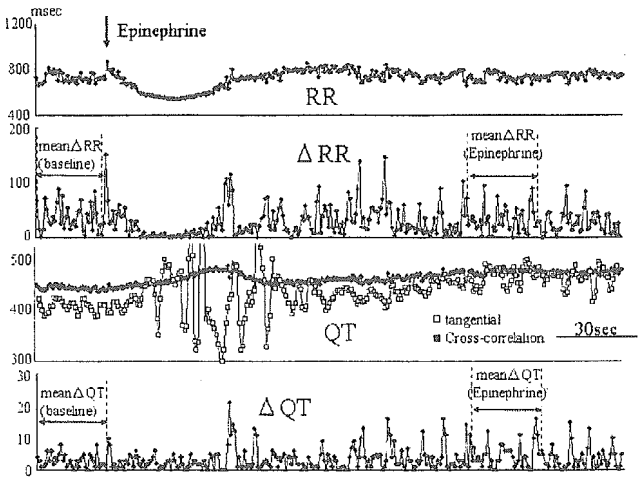

Figure 5 Beat-by-beat change of RR, QT, ΔRR, and ΔQT in an LQT1 patient. RR interval was decreased following bolus infusion of epinephrine and remained decreased at steady-state condition. ΔRR became small after bolus infusion of epinephrine. QT interval was prolonged after bolus infusion of epinephrine and remained prolonged at steady-state epinephrine effect compared with before epinephrine. Of note, ΔQT was significantly increased at steady-state epinephrine effect compared with before epinephrine.

Figure 5 illustrates beat-by-beat change of RR, QT, ΔRR, and ΔQT in an LQT1 patient. Similar to the control patient, the RR interval also was decreased following bolus infusion of epinephrine and remained decreased at steady-state epinephrine effect in the LQT1 patient. ΔRR became small after bolus infusion of epinephrine. QT interval was prolonged following the bolus infusion of epinephrine and remained prolonged at steady-state epinephrine effect compared before epinephrine. It is noteworthy that ΔQT was significantly increased at steady-state epinephrine effect compared with before epinephrine.

Table 2 lists composite data of the five ECG parameters before and at steady-state epinephrine effect in the LQT1 and control groups.

No significant differences in ΔRR, ΔQT, SD-ΔRR, SD-ΔQT, and QTI were observed between the two groups under baseline conditions. Epinephrine increased ΔQT (2.3 ± 0.3 to 4.2 ± 2.3 ms, $P < .005$), SD-ΔQT (2.2 ± 1.9 to 4.1 ± 2.2 ms, $P < .005$), and QTI (0.10 ± 0.06 to 0.22 ± 0.16, $P < .005$) in LQT1 group but not in control group (ΔQT 2.5 ± 1.5 to 2.4 ± 0.5 ms, SD-ΔQT 1.9 ± 0.9 to 2.1 ± 0.6 ms, QTI 0.08 ± 0.02 to 0.09 ± 0.06, P = NS) (Figure 6).

ΔQT and SD-ΔQT showed significant correlation with QTc after epinephrine (r = 0.61; $P < .05$ and r = 0.65; $P < .05$, respectively) but not before epinephrine. On the other hand, the values were not correlated with heart rate either before or after epinephrine. No significant differences in the five ECG parameters were observed between patients with (n = 8) and patients without (n = 6) a history of syncope or cardiac arrest.

Discussion

Quantification of ventricular repolarization

Several methods have been proposed to quantify abnormalities of repolarization[9,10]; however, few of the techniques are suitable for routine clinical use. Thus, assessment of ventricular repolarization still is based largely on QT and QTc measurements and on qualitative description of morphologic alterations such as presence of notched, bifid, or biphasic T waves. A set of new morphologic ECG parameters proposed by Merri et al,[11] Benhorin et al,[12] and Priori et al[13] could provide a better description of repolarization and be more reproducible than QT interval duration, but these parameters have not yet obtained widespread application in clinical practice.

RT interval, the duration between the peak of R and T wave, was used to analyze the repolarization period to minimize the observer bias in manual acquisition of data.[14] Experimental studies[15,16] using arterially perfused canine left ventricular wedges suggest both the peak and the end of the T wave on the ECG are coincident with repolarization of epicardial and maximal M-cell action potentials, respectively, so that the interval between the T_{peak} and T_{end} reflects transmural dispersion of repolarization. The transmural dispersion of repolarization, the latter part of the T wave, is linked to ventricular arrhythmias such as TdP under long QT conditions. Therefore, the RT interval cannot detect the

Table 2 ECG parameters before and after epinephrine in LQT1 and control groups

	LQT1 (n = 14)		Control (n = 5)	
	Baseline	Epinephrine	Baseline	Epinephrine
ΔRR	33 ± 28	36 ± 39	39 ± 20	34 ± 25
ΔQT	2.3 ± 0.3	4.2 ± 2.3*,†	2.5 ± 1.5	2.4 ± 0.5
SD-ΔRR	23 ± 18	27 ± 27	30 ± 17	36 ± 27
SD-ΔQT	2.2 ± 1.9	4.1 ± 2.2*,†	1.9 ± 0.9	2.1 ± 0.6
QTI	0.10 ± 0.06	0.22 ± 0.16*,†	0.08 ± 0.02	0.09 ± 0.06

Values are reported as mean ± SD.
*$P < .05$ vs baseline.
†$P < .05$ vs control.

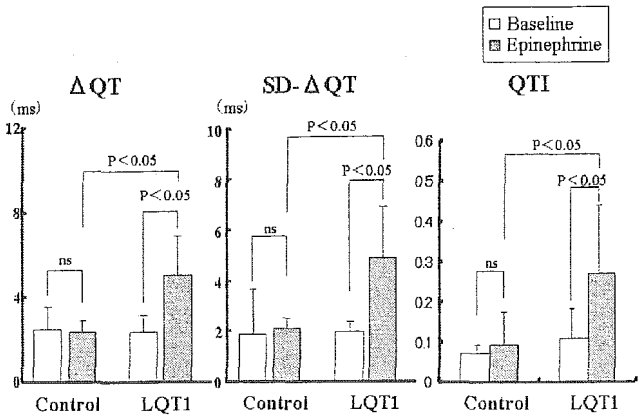

Figure 6 Comparison of ΔQT, SD-ΔQT, and QTI before and after epinephrine infusion between LQT1 and control groups. No significant differences in ΔQT, SD-ΔQT, and QTI were observed between the two groups under baseline conditions. Epinephrine increased ΔQT, SD-ΔQT, and QTI in the LQT1 group but not in the control group.

important part of the T wave and may underestimate a repolarization abnormality in patients with LQTS.

Our novel method, the cross-correlation method, analyzed beat-by-beat "time-lag" comparing the template of the latter part of the T wave, thus better description of QT interval could be assessed independently of complicated TU wave morphology. Beat-by-beat T-wave and QT variability measured by the cross-correlation method is not synonymous with T-wave alternans and could be analyzed more stably than with the standard tangential method even during epinephrine infusion in the two patient groups (Figures 4 and 5).

Variability of repolarization in LQT1 syndrome

Physical exercise and strong emotion precipitate syncope and sudden cardiac death in patients with congenital LQTS.[2] Experimental models[17,18] of LQTS and clinical studies[19] suggest catecholamine-enhanced early afterdepolarization and triggered activity play a pivotal role in the genesis of QT prolongation and TdP.

TWA is a well-known ECG phenomenon often associated with the development of cardiac arrhythmias,[20] particularly in the setting of acquired and congenital LQTS.[6,7] The phenomenon previously was described as QT interval variability or T-wave lability by the pronounced changes in T-wave morphology.[21,22] The mechanism underlying catecholamine-provoked T-wave lability is unclear. It also is clearly different from microvolt (μV-TWA). The μV-TWA shows no definite periodicity of the T-wave changes on surface ECG. Exercise-induced μV-TWA was not significantly different between genotype carriers and noncarriers in a study involving a large single kindred with LQTS.[21,23]

An experimental study by Shimizu and Antzelevitch[7] suggested that TWA observed at rapid rates under long QT conditions largely results from alternation of the M-cell action potential duration, leading to exaggeration of transmural dispersion of repolarization during alternating beats and thus the potential for development of TdP. Their data also suggested that unlike transient forms of TWA that damp out quickly and depend on electrical restitution factors, the steady-state electrical and mechanical alternans appears to largely result from beat-to-beat alternans of intracellular calcium cycling.

Our study showed that beat-by-beat QT variability was accentuated by epinephrine infusion only in LQT1 patients, indicating that variability of repolarization is made pronounced by sympathetic stimulation in patients of LQT1 but not in normal controls. Our result supports the clinical manifestation that life-threatening arrhythmia, such as TdP, often is observed under increased sympathetic activity in LQTS, especially in LQT1 patients.[23]

However, the numbers of families and individuals in the present study were small and limited the number of LQT1 patients. Therefore, our data may be limited to LQT1 and not applicable to LQTS patients with other genotypes.

Relationship among QT variability, QT interval, and heart rate

In the present study, QT variability after epinephrine was correlated with QTc interval after epinephrine but not with heart rate. The μV-TWA is a highly heart rate-dependent parameter and can be assessed invasively by arterial pacing or noninvasively by exercise. Heart rate threshold for induction of the μV-TWA was reported at 110 bpm in healthy adults.[24] No relationship between QT variability and heart rate after epinephrine was observed in this study, probably because of the lesser increase in heart rate in both groups.

Conclusion

Our data showed that beat-by-beat QT variability analyzed by the cross-correlation method was greater in LQT1 patients during sympathetic stimulation, suggesting that sympathetic stimulation accentuates beat-by-beat alternans of repolarization in the LQT1 syndrome.

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Two Adults Requiring Implantable Defibrillators Because of Ventricular Tachycardia and Left Ventricular Dysfunction Caused by Presumed Kawasaki Disease

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Two Adults Requiring Implantable Defibrillators Because of Ventricular Tachycardia and Left Ventricular Dysfunction Caused by Presumed Kawasaki Disease

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There is an adult patient population in Japan with undiagnosed coronary artery lesions caused by Kawasaki disease (KD) occurring before 1967, the time at which KD was first described. Two adult patients presented with a low left ventricular (LV) ejection fraction and ventricular tachycardia (VT) caused by presumed KD. A 43-year-old man with rapid VT had a history of an acute febrile illness with desquamation of the fingertips at the age of 10 months. Coronary angiography (CAG) showed segmental stenosis of the right coronary artery (RCA) and occlusion of the left anterior descending artery with a giant aneurysm. The other patient was a 48-year-old man with a history of ischemic cardiomyopathy diagnosed after a previous myocardial infarction when he was 32 years old. He had segmental stenosis of the RCA on CAG. Non-sustained VT with transient unconsciousness was observed during 24-h Holter electrocardiography. Rapid VT with syncope was induced in both patients in the electrophysiologic studies and an implantable defibrillator was required to prevent sudden death. Physicians must be aware that VT can occur in older patients with LV dysfunction many years after KD. (*Circ J* 2005; **69:** 870–874)

Key Words: Coronary artery disease; Implantable defibrillator; Kawasaki disease; Left ventricular dysfunction; Non-sustained ventricular tachycardia

Kawasaki disease (KD) is an acute febrile infantile disease, first described in 1967[1] but there is an adult patient population in Japan with a history of acute KD and cardiac sequelae occurring before 1967[2] In most of these patients, the coronary artery lesions caused by KD were first recognized after an acute myocardial infarction or at autopsy for sudden death[3–8] We present 2 adult patients with ventricular tachycardia (VT) and left ventricular (LV) dysfunction caused by coronary artery lesions after presumed KD.

Case Reports

Case 1

In 1993 a 34-year-old man visited hospital because of headache. A 12-lead electrocardiography (ECG) revealed an abnormal Q wave in lead III and a QS pattern in V_1 and V_2. Coronary angiography (CAG) showed segmental stenosis of the right coronary artery (RCA) and occlusion of the left anterior descending artery (LAD) with calcification of a giant aneurysm. At the age of 10 months, he had had an episode of unexplained fever lasting 1 month with desquamation of the palms and fingertips. He was diagnosed in 1993 as having coronary artery lesions caused by KD. Multifocal premature ventricular contractions (PVC) were frequently observed in on 24-h Holter ECG. Furthermore, he had a low LV ejection fraction (LVEF). Beta-blocker was prescribed. At the age of 43 years, he visited another hospital because of fever associated with a common cold. An ECG revealed wide QRS tachycardia at a rate of 198 beats/min (Fig 1), as well as left axis deviation and right bundle-branch block. He was restored to normal sinus rhythm by direct conversion and was referred to us. Body length and body weight were 169 cm and 74 kg, respectively; blood pressure was 130/80 mmHg; total cholesterol was 228 mg/dl. At cardiac catheterization, the LV end-diastolic volume (LVEDV) and LVEF were 97 ml/m^2 and 41%, respectively. The CAG findings were similar to the previous imaging (Fig 2). Electron beam computed tomography showed the occlusion of the LAD with calcification of a giant aneurysm. He underwent coronary artery bypass grafting to the RCA and LAD. Amiodarone was prescribed. During electrophysiologic studies (EPS), 2 clinical and 2 non-clinical episodes of VT were induced in the left postero-septal wall of the left ventricle and a diastolic potential was recorded at the site. Radiofrequency catheter ablation was successful for 3 of the 4 foci. However, it was impossible to ablate the focus inducing rapid VT with syncope, so an implantable defibrillator (ICD) was inserted.

Case 2

In 1987, a 32-year-old man visited hospital because of general malaise. He was diagnosed as having ischemic cardiomyopathy after a previous myocardial infarction (MI). There was segmental stenosis of the right coronary artery on CAG but an almost normal left coronary artery. Multifocal PVC and couplets were detected on 24-h Holter ECG. He had experienced palpitations and transient unconsciousness 2 or 3 times since then. When 48 years old, he experienced presyncope with a cold sweat and an oppressive

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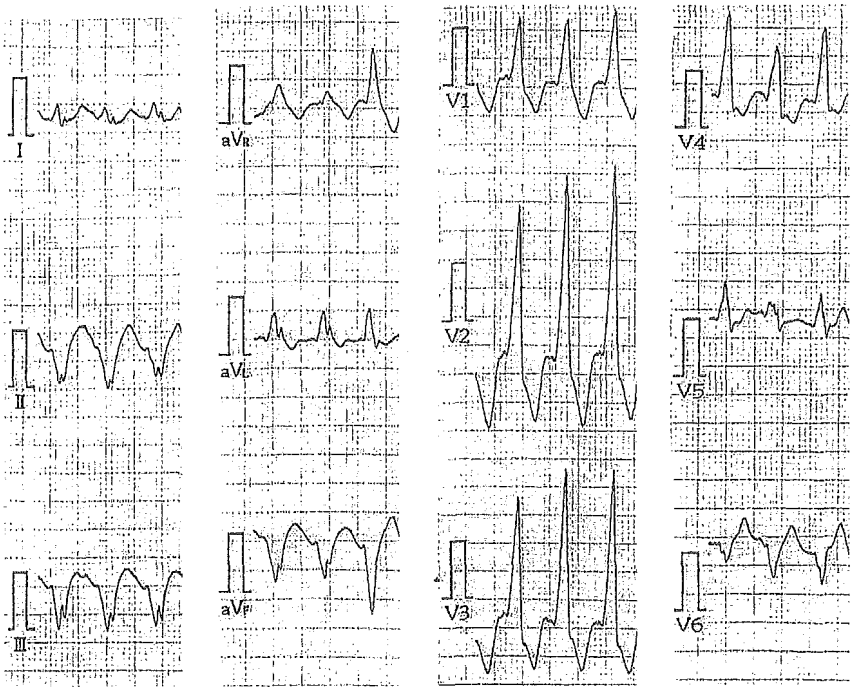

Fig 1. 12-lead ECG of wide QRS tachycardia of 198 beats/min. The ECG shows the left axis deviation and right bundle-branch block.

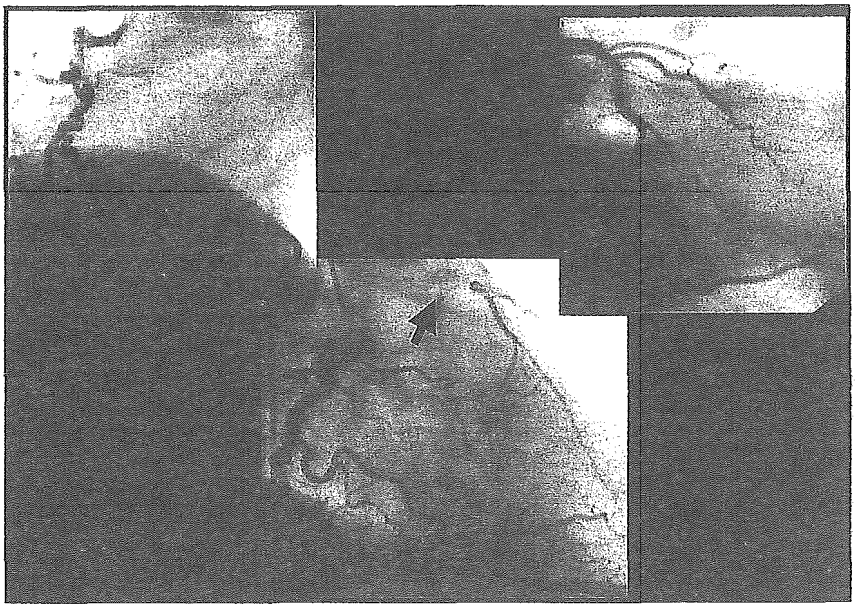

Fig 2. Case 1: Coronary angiogram shows segmental stenosis of the right coronary artery (Left). (Middle) The left anterior descending artery filled via collateral arteries from the right coronary artery (arrow shows a giant calcified aneurysm of the left anterior descending artery). (Right) The left anterior descending artery is occluded.

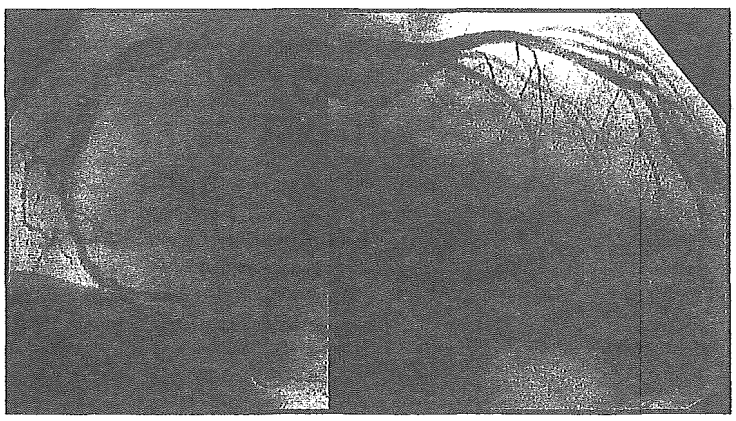

Fig 3. Case 2: Coronary angiogram shows segmental stenosis of the right coronary artery (Left) and an almost normal left anterior descending artery (Right).

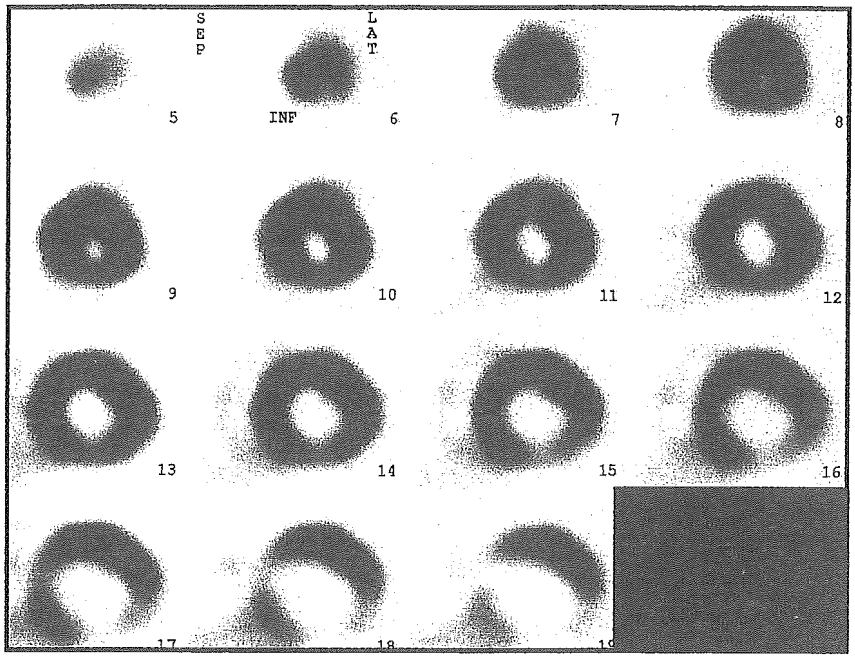

Fig 4. ^{99m}Tc-MIBI myocardial imaging at rest (short-axis view).

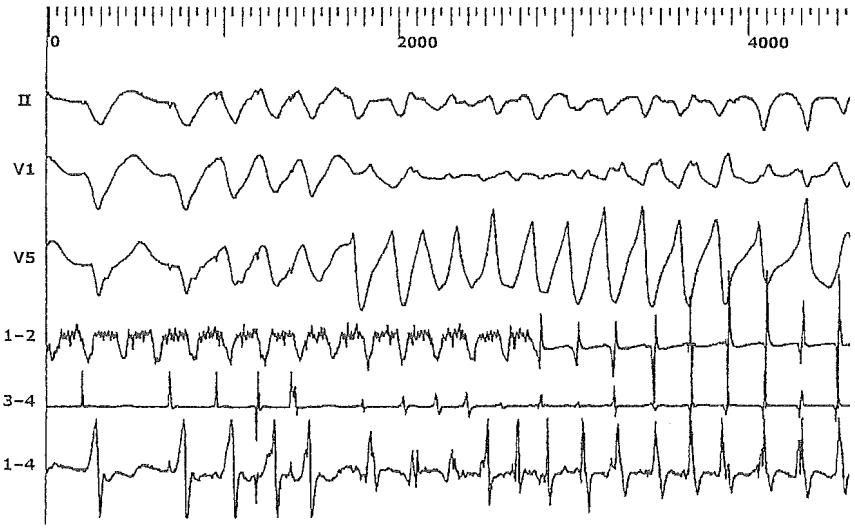

Fig 5. Rapid ventricular tachycardia (VT) with syncope during the electrophysiologic study. Polymorphic VT was induced by stimulation at the apex of the right ventricle, and it evolved into rapid VT with syncope.

sensation in his chest immediately after getting up during the night. Non-sustained VT (NSVT) including a 19-beat run was observed during 24-h Holter ECG and he was referred to us. Body length and body weight were 163 cm and 73 kg, respectively; blood pressure was 120/60 mmHg; total cholesterol was 196 mg/dl while taking 3-hydroxy-3-methyl-coenzyme. A reductase inhibitor. He had given up smoking when 32 years old. ECG revealed an abnormal Q wave in lead III, poor progression of the r wave in V_1 and V_2, and flat T waves in V_5 and V_6. An episode of acute KD was unknown. LVEDV and LVEF were 147 ml/m^2 and 32%, respectively, on left ventriculography. The CAG findings were almost the same previously (Fig 3). A perfusion defect in the inferior wall of the left ventricle was found on ^{99m}Tc-methoxy-isobutyl isomitrile myocardial imaging at rest (Fig 4). The histologic findings of a biopsy of the right ventricle did not correspond to that of any cardiomyopathy. Beta-blocker was prescribed. At EPS, monomorphic NSVT at a rate of 240 beats/min with left axis deviation and polymorphic VT of several beats run were induced by stimulation at the outflow tract of the right ventricle. They stopped spontaneously, and he had presyncope at the time. Furthermore, polymorphic VT was induced by stimulation at the apex of the right ventricle, and it evolved into rapid VT (Fig 5). He collapsed, but was restored to normal sinus rhythm by direct conversion. He had no abnormal potentials at the apex of the right ventricle in sinus rhythm; however, ICD implantation is planned to prevent sudden death.

Discussion

KD is an acute febrile disease affecting children, mainly those less than 5 years of age[1,9] Currently in Japan, approximately 6,000–8,000 patients develop KD each year. Its cause remains unknown, but it is a systemic vasculitis involving medium-sized vessels. Diagnosis is based on the major clinical features of acute KD, which include fever of at least 5 days duration, bilateral conjunctival injection, an erythematous reaction involving the lips and oral cavity,

polymorphous exanthema, cervical lymphadenopathy, erythema of the palms and soles and/or firm induration of the hands or feet in the early phases and desquamation of the fingers and toes in the post-inflammation period.[1,9] All these symptoms are self-limiting and not all occur in every patient. In addition, the severity of the symptoms varies and for these reasons the diagnosis of KD can be difficult. Acute systemic arteritis particularly affects the coronary arteries. In the 1970s, it was considered that approximately 20% of acute KD patients had cardiac sequelae immediately after the acute illness;[10] however, it was difficult to diagnose both acute KD and the development of coronary artery lesions at that time. Most patients with coronary artery lesions caused by KD are asymptomatic until acute myocardial infarction or sudden death occurs and there are probably many asymptomatic adult patients with coronary arterial lesions caused by KD who remain undiagnosed.

The present 2 patients had coronary artery lesions and a low LVEF diagnosed when they were in their 30s. Both had segmental stenosis of the RCA, which is often found in patients with a history of KD and is considered typical of the lesions caused by the disease. It implies the development of several new small vessels, reflecting recanalization after coronary artery occlusion.[10,11] Because approximately two-thirds of patients with segmental stenosis or complete coronary occlusion are asymptomatic, coronary artery occlusion cannot be diagnosed without CAG;[12] and consequently, LV dysfunction after MI can exist unrecognized for many years in such patients. As a result, they are asymptomatic for many years after the episode of undiagnosed acute KD.[13] The 2 patients described here developed VT when in their 40s and it was most likely secondary to myocardial damage after a previous MI. We believe that VT develops with age, many years after the previous MI.

Giant aneurysms in the proximal portion of the coronary arteries are a characteristic coronary artery lesion caused by KD;[10] and they often develop late calcification on the outer surface. Case 1 had an occluded and calcified giant aneurysm of the LAD. Stenotic lesions and calcification involving the same segments in which the coronary aneurysms develop occur during the acute phase of KD. Furthermore, affected segments and almost normal segments may be found in the 1 patient.[14]

These 2 patients were born in the 1950s. The mother of Case 1 remembered the symptoms of his acute illness in infancy, which were consistent with acute KD. Any history of an acute illness in childhood was unknown in the other patient. However, we suspect that the characteristic coronary artery lesions and ischemic cardiomyopathy occurring in middle-age of individual without high risk of atherosclerosis signify a KD etiology.

Adults with ischemic cardiomyopathy, severely depressed LV function, and asymptomatic NSVT are at significant risk for future arrhythmic events.[15] Sudden death occasionally occurs in their 20s in patients with LV dysfunction and NSVT after KD.[16,17] In such cases there was probably an undiagnosed MI soon after the onset of KD and they then remained asymptomatic prior to their sudden death. We suspect that fatal arrhythmias in such patients are a late complication of MI,[13,18,19] which might occur earlier in KD patients with a low LVEF than in adults with LV dysfunction caused by atherosclerosis.

Treatment for KD patients with long-standing LV dysfunction after previous MI becomes more essential as they get older. An EPS and then antiarrhythmic treatment will be required to prevent sudden death. If critical VT is detected, catheter ablation or implantable cardioverter-defibrillator should be considered[20,21] because patients with severe coronary artery lesions caused by KD are likely to have cardiac events earlier than the normal population.

Conclusion

There is a population of adults in Japan with undiagnosed coronary arterial lesions caused by KD. They can be recognized by an episode of ventricular arrhythmia and a low LVEF, as well as by acute MI or sudden death. Detection and treatment of such KD patients is essential to prevent premature death.

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Effect of Protein Binding of Pilsicainide on the Pharmacokinetics

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Regular Article

Effect of Protein Binding of Pilsicainide on the Pharmacokinetics

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: To evaluate the effect of protein binding of pilsicainide on its clearance and the contribution of protein binding to optimized pilsicainide therapy, clinical laboratory and pharmacokinetic data were studied in 160 Japanese inpatients (Study 1) and 18 Japanese inpatients (Study 2). To determine the relation between protein concentration and the protein binding ratio of pilsicainide *in vitro*, the effect of human α_1-acid glycoprotein (AAG) and human albumin on the binding ratio was studied. The mean ratio of serum pilsicainide concentration to dose per body weight (C/D) increased with increases in the C-reactive protein (CRP) concentration in Study 1. The AAG level increased with increases in the CRP concentration and the binding ratio increases in the AAG concentration in the Study 2. The binding ratios increased with increased AAG and albumin concentrations; the AAG concentration relative to the ratio was particularly large *in vitro* study. These results suggest C/D is increased in patients with high CRP levels because of binding of pilsicainide to protein, resulting decreased clearance.

Key words: Pilsicainide; protein binding; C-reactive protein (CRP); α_1-acid glycoprotein (AAG)

Introduction

Pilsicainide hydrochloride, which was developed in Japan, is considered a class Ic antiarrhythmic agent[1] and is used widely for the treatment of supraventricular and ventricular arrhythmias. Several studies on the pharmacokinetics of pilsicainide in animals have shown wide interspecies variation.[2-4] In humans, pilsicainide is predominantly excreted in the urine in the unchanged form; less than 5% is metabolized to 2-hydroxymethylate. The elimination half-life ($t_{1/2}$) of pilsicainide is 4 to 9 hours, and approximately 30% is bounded to proteins. Because renal excretion is the major elimination route in humans, the $t_{1/2}$ is prolonged in patients with renal impairment with decreased creatinine clearance; therefore, it is important to adjust the dosage according renal function. The aim of this study was to evaluate the effect of protein binding of pilsicainide on its clearance and the contribution of protein binding to optimized pilsicainide therapy.

Material and Methods

Materials: Pilsicainide and 4-methylmexiletine were provided by the Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan) and Boehringer Ingelheim Co., Ltd. (Hyogo, Japan), respectively. Human albumin and human α_1-acid glycoprotein (AAG) were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other chemicals were of analytical grade and commercially available.

Subjects: Clinical laboratory and pharmacokinetic data were collected from 160 Japanese inpatients (Study 1) and 18 Japanese inpatients (Study 2). These patients received maintenance pilsicainide therapy (tid, 0700, 1200 and 1900 hours) as determined by their cardiologists for treatment of atrial fibrillation. Subjects in Study 1 were hospitalized at the National Cardiovascular Center (Osaka, Japan) between September 1998 and December 2003. Subjects in study 2 were hospitalized at Maizuru Kyosai Hospital (Kyoto, Japan) or Kyoritsu Hospital (Hyogo, Japan) between March 2004 and October 2004. Data of Study 1 were collected

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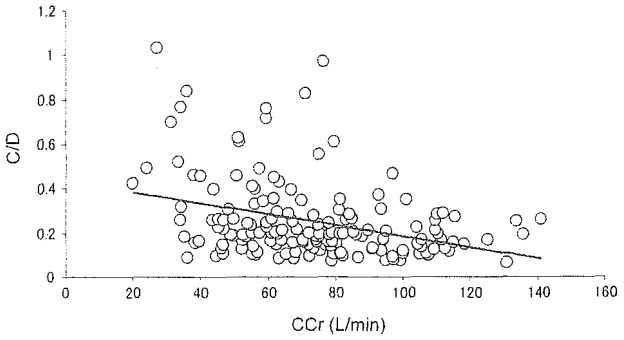

Fig. 1. Relation between creatinine clearance (CCr) and the ratio of serum pilsicainide trough concentration to dose per weight (C/D) in Study 1. The regression equation as determined by the least-square method is shown by the solid line ($y = 0.435 - 0.00251X$, $R^2 = 0.124$).

retrospectively; however, data of Study 2 were collected prospectively to verify the results of the Study 1. Because all subjects were inpatients, compliance was ensured by a nurse or pharmacist. Written informed consent was obtained from all subjects before enrollment in the study.

Blood sampling: To determine the serum concentration of pilsicainide and to obtain other laboratory data, blood samples were drawn at 0600 hours from an arm vein. Blood samples were centrifuged at 3000 rpm for 10 min. and serum samples were obtained. Both total and free fraction serum concentrations of pilsicainide were determined in Study 2, however, free fraction serum concentrations of pilsicainide were not able to determine in Study 1.

Preparation of sample solutions: To determine the relation between protein concentration and the protein binding ratio of pilsicainide *in vitro*, the effect of human AAG and human albumin on the binding ratio was studied using the 0.05 M phosphate buffer solution (pH 7.4). After the spiked samples were incubated for 60 min. at 37°C, the free fraction of the spiked samples was obtained by ultrafiltration.

Assay: Serum pilsicainide concentrations were determined by high-performance liquid chromatography (HPLC) with 4-methylmexiletine as an internal standard (IS). Briefly, pilsicainide was extracted with diethylether followed by evaporation of diethylether. The residue was reconstituted in the mobile phase before injection into the HPLC system. The HPLC system consisted of a reverse-phase column (Shim-pack, CLC-ODS, Shimadzu Corp., Kyoto, Japan) and an ultraviolet absorbance detector set at 210 nm. The mobile phase consisted of a mixture of 0.044 M phosphate buffer (pH 2.6) and 0.5% triethylamine and acetonitrile (70:30 by volume), and the flow rate was 1.2 mL/min. Retention times of the IS and pilsicainide were 11.5 and 5.0 min., respectively. The minimum measurable concentration in this system was 50 ng/mL in 0.5 mL serum. Inter- and intra-day variations were less than 5.0%. The free fraction of the serum and the spiked samples were obtained by ultrafiltration with a disposable ultrafilter (Kurabo Industries, LTD., Osaka, Japan). The AAG serum concentration was measured by an immunodiffusion plate system (MBL plate, MBL Corp., Nagoya, Japan).

Table 1. Patient characteristics and clinical laboratory data in Groups 1 and 2 in Study 1.

	Group 1	Group 2	P value
N	105	55	
Age (yr)	64.6 ± 12.6	66.3 ± 11.1	NS
Body weight (kg)	56.7 ± 11.6	60.2 ± 12.4	NS
Total protein (g/dL)	6.78 ± 0.63	6.84 ± 0.55	NS
Albumin (g/dL)	3.92 ± 0.34	3.89 ± 0.41	NS
SCr (mg/dL)	0.86 ± 0.26	0.86 ± 0.28	NS
CCr (mL/min)	70.7 ± 21.2	78.1 ± 30.6	NS
BUN (mg/dL)	17.5 ± 5.28	17.6 ± 6.06	NS
CRP (mg/dL)	0.405 ± 0.742	2.18 ± 2.98	< 0.001

Group 1 consisted of subjects with ratios of measured C/D to estimated C/D was < 1.
Group 2 consisted of subjects with ratios was > 1.
C/D, the ratio of serum pilsicainide concentration to dose per weight; SCr, serum creatinine; CCr, creatinine crearance; BUN, blood urea nitrogen; CRP, C-reactive protein.
Data are mean ± SD.

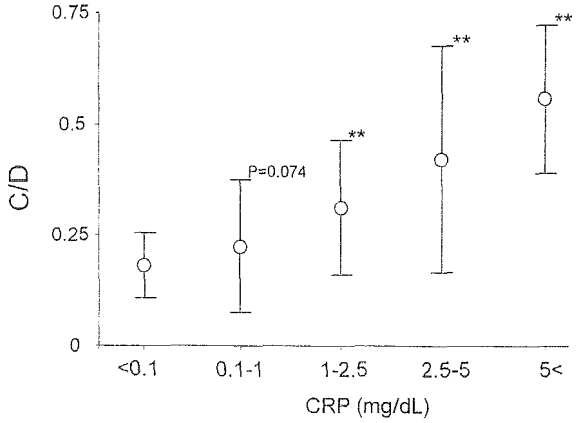

Fig. 2. Relation between C-reactive protein (CRP) levels and the ratio of serum pilsicainide concentration to dose per weight (C/D). **, $P < 0.01$ vs. CRP value < 0.1 mg/dL. Each point represents mean ± SD.

Pharmacokinetic analysis: Because all subjects were given fixed maintenance doses of pilsicainide for at least 1 week, it was assumed that their serum concentrations had reached steady state. All blood sampling was performed at the same time of day (i.e., 0600 hours). Therefore, to evaluate the pharmacokinetics, the ratio of serum pilsicainide concentration to dose per body weight (C/D) was used in this study. Creatinine clearance (CCr) was calculated from serum creatinine levels, age and weight according to the equation of

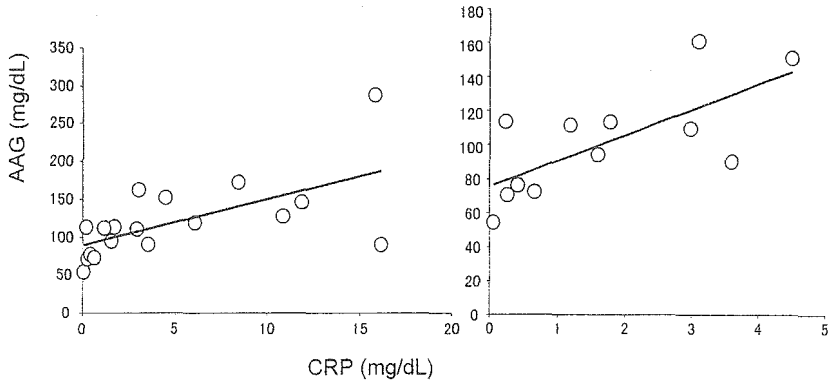

Fig. 3. Relation between serum levels of C-reactive protein (CRP) and α_1-acid glycoprotein (AAG). The regression equation as determined by the least-squares method are shown by solid lines (left panel, $y=6.06x+89.4$, $R^2=0.380$, right panel, $y=15.1x+75.4$, $R^2=0.499$).

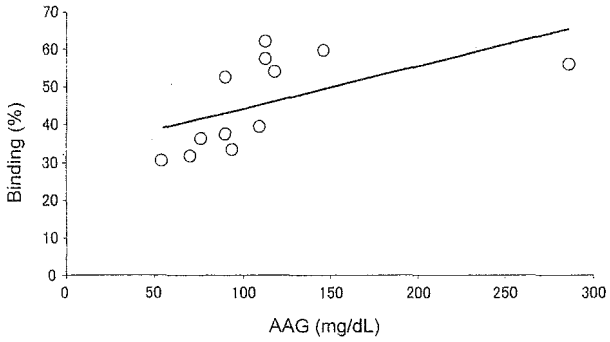

Fig. 4. Relation between serum α_1-acid glycoprotein (AAG) and the binding ratio of pilsicainide in serum. The regression equation as determined by the least-squares method is shown by the solid line ($y=0.113x+33.0$, $R^2=0.314$).

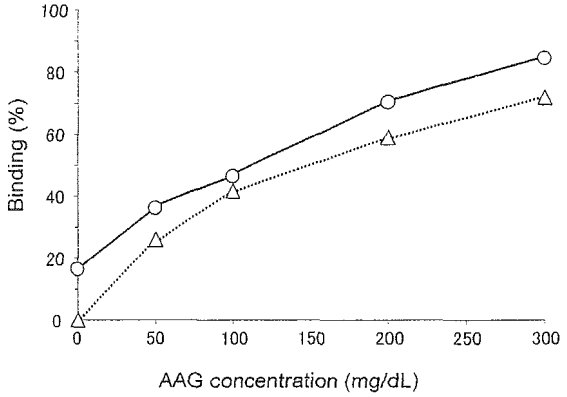

Fig. 5. Relation between α_1-acid glycoprotein (AAG) and the binding ratio of pilsicainide in samples containing of 4.0 g/dL (open circles) or 0 g/dL albumin (open triangles). Each point represents the mean of three experiments.

Cockcroft and Gault.[7]

Statistical analysis: Data are presented as mean ± standard deviation (SD). Statistical analysis was performed with Students t-test, and significance was set at $P<0.05$.

Results

The relation between CCr and C/D in Study 1 was shown in **Fig. 1**. Comparisons of patient characteristics and clinical laboratory data between Groups 1 and 2 in Study 1 are shown in the **Table 1**. The C/D of Group 1 was greater than the value calculated by the regression equation determined between C/D and CCr, and that of Group 2 was less than the calculated value. No significant differences were observed in patient characteristics and clinical laboratory data with the exception of C-reactive protein (CRP) levels; the mean CRP level for Group 2 was significantly greater than that for Group 1. C/D relative to CRP concentration in Study 1 is shown in **Fig. 2**, mean C/D increased with increases in the CRP concentration. The relation between the CRP concentration and AAG level in serum in Study 2 is shown in **Fig. 3**, the AAG level increased with increases

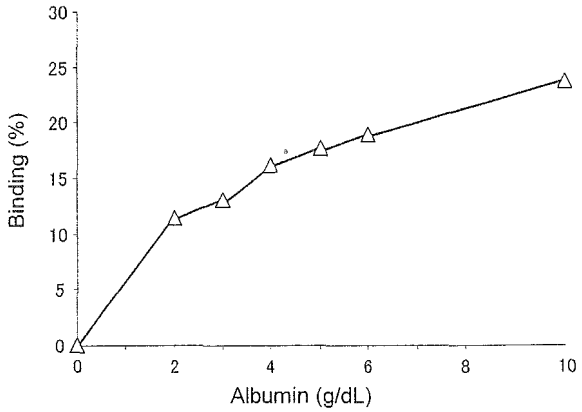

Fig. 6. Relation between albumin and the binding ratio of pilsicainide. Each point represents the mean of three experiments.

in the CRP concentration. The relation between the AAG concentration and the protein binding ratio of pilsicainide to protein in serum in Study 2 is shown in **Fig. 4**, the binding ratio increased with increases in the

AAG concentration. Relations between the AAG concentration and the binding ratio and between albumin concentration and the binding ratio are shown in **Fig. 5** and **6**, respectively. The binding ratios increased with increased AAG and albumin concentrations; the AAG concentration relative to the ratio was particularly large.

Discussion

Pilsicainide is widely used in Japan to treat supraventricular and ventricular arrhythmias. However, some adverse effects of this drug are believed to be related to excessively elevated serum concentrations. Pilsicainide has a relatively narrow therapeutic window. Therefore, determination of pilsicainide serum concentrations and appropriate dosage adjustments are essential to optimize pilsicainide therapy. Because pilsicainide appears to be primarily eliminated by the kidney, appropriate dosage should also be determined according to renal function. In study1, a significant association was identified between CCr and C/D; however, individual variation was large. Therefore, it is suggested C/D value is effected by the other factors except renal function. On the other hand, C/D values were increased in patients with higher CRP values. The binding ratio of pilsicainide to protein predominantly to AAG was approximately 30% in serum. CRP and AAG are proteins included in acute phase reactions. Pilsicainide bound to both AAG and to albumin *in vitro*. However, the affinity for AAG was higher than the affinity for albumin. In study 2, a significant association between CRP and AAG was identified. A significant association between AAG and pilsicainide binding was also identified. Thus these data in Study 2 and *in vitro* suggest that measured C/D values in patients with higher CRP levels were increased than the C/D calculated by their CCr in Study 1. These results suggest that C/D is increased in patients with high CRP levels because of binding of pilsicainide to protein, resulting decreased clearance.

In conclusion, adjustment of pilsicainide dosage requires monitoring not only of individual renal function but also of CRP value.

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Pharmacokinetic Characteristics of Amiodarone in Long-Term Oral Therapy in Japanese Population

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To evaluate the pharmacokinetic properties and an optimum dose schedule of amiodarone in long-term oral therapy, serum concentrations of amiodarone and its metabolite, desethylamiodarone, were monitored from 345 Japanese inpatients who received amiodarone therapy for a variety of cardiac arrhythmias. Serum amiodarone and desethylamiodarone concentrations were determined by high performance liquid chromatography system. It was observed that the amiodarone and desethylamiodarone concentrations gradually increased with time. The frequency distribution in the amiodarone clearance of 245 subjects, who received fixed maintenance amiodarone therapy for at least 6 months, was nearly a unimodal one. The variation in the ratio of desetylamiodarone to amiodarone concentration in serum was very small. Although no differences in age, dose, dose duration, amiodarone or desethyamiodarone concentration or ratio were observed between men and women: however, the mean amiodarone clearance of women was significantly higer than that of men. The laboratory data were mostly within normal values and no significant relations were observed between serum amiodarone concentration and clinical laboratory data. These results suggest that the individual variation in pharmacokinetics of amiodarone is comparatively small, which might be sufficient to decide that the maintenance dose was the same one (200 mg/d) in long-term oral amiodarone therapy.

Key words amiodarone; long-term therapy; pharmacokinetics; Japanese

Amiodarone is an important antiarrhythmic agent in the prevention of sustained ventricular tachycardia and fibrillation. Until recently, however, concerns about potentially dangerous non-cardiac side effects and its complex pharmacokinetics have limited the use of amiodarone to the most drug-resistant and high risk subjects. Nonetheless, amiodarone appears to significantly improve in patients after myocardial infarction, and amiodarone has begun to be accepted as a first-line therapy as opposed to a last-chance drug. Amiodarone's increase in popularity is exhibited by its rapidly growing use in the management of refractory atrial arrhythmias.[1,2]

On the other hand, amiodarone is predominantly metabolized to desethylamiodarone, which is the active metabolite, by cytochrome P450(CYP)3A4 and CYP2C8, and desethylamiodarone is further metabolized by CYP3A4.[3,4] It has unique pharmacokinetic properties, with 55 d constituting a typical half-life, and pharmacokinetic interactions with various therapeutic agents,[5—7] including warfarin,[8—10] phenytoin,[11—13] flecainide[14—16] and cyclosporine.[17,18] With the growing number of patients maintained on long-term amiodarone therapy, it is more important to evaluate interindividual variability and characteristics in detail; however, few reports of the pharmacokinetics of amiodarone in long-term therapy have been published,[19] especially, no report was published using the Japanese population.

The aim of this study was to evaluate an optimum dose schedule and pharmacokinetic properties of amiodarone in long-term oral therapy in the Japanese population.

Subjects Data were collected from 345 Japanese inpatients who received amiodarone therapy for a variety of cardiac arrhythmias at the National Cardiovascular Center between September 1998 and December 2003. Patients received loading doses from 200 to 400 mg/d for 2 weeks (bid, 0700 and 1900 h), as determined by their cardiologist on the clinical response, without attempts to achieve a specific serum concentration, and received daily maintenance doses from 100 to 200 mg (bid, 0700 and 1900 h). The most common long-term maintenance dose was 200 mg/d. Because all subjects were inpatients, compliance was ensured through administration by a nurse or pharmacist.

Blood Sampling To determine the serum concentration of amiodarone and desethylamiodarone and laboratory examination data, blood samples were drawn at 0700 h from an arm vein. Blood samples were centrifuged at 3000 rpm for 10 min, and serum samples were obtained. Written informed consent was obtained from all subjects before participation in this study.

Assay Serum amiodarone and desethylamiodarone were determined by an HPLC system using amitriptyline as an internal standard (IS).[20] In brief, amiodarone and desethylamiodarone were extracted with diethylether followed by evaporation. The residue was reconstituted in methanol before injection into the HPLC system. The HPLC system consisted of a reverse-phase column (Shim-pack, CLC-ODS, Shimadzu Corp., Kyoto, Japan), and an ultraviolet absorbance detector operated at 242 nm. The mobile phase consisted of a mixture of methanol, water, and 28% ammonia water (91 : 8.8 : 0.2 by volume), and the flow rate was 1.5 ml/min. Retention times of the IS, desethylamiodarone and amiodarone were 6.6, 11.7, and 19.2 min, respectively. The minimum measurable concentration was 50 ng/ml when 0.5 ml of serum was used. Inter- and intraday variations were less than 5.0%.

Pharmacokinetic Analysis It is well known that amiodarone has a long half-life as does desethylamiodarone, which is an inhibitor of metabolism of amiodarone through

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