Drug-Induced Long-QT Syndrome Associated With a Subclinical SCN5A Mutation

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Background—Subclinical mutations in genes associated with the congenital long-QT syndromes (LQTS) have been suggested as a risk factor for drug-induced LQTS and accompanying life-threatening arrhythmias. Recent studies have identified genetic variants of the cardiac K⁺ channel genes predisposing affected individuals to acquired LQTS. We have identified a novel Na⁺ channel mutation in an individual who exhibited drug-induced LQTS.

Methods and Results—An elderly Japanese woman with documented QT prolongation and torsade de pointes during treatment with the prokinetic drug cisapride underwent mutational analysis of LQTS-related genes. A novel missense mutation (L1825P) was identified within the C-terminus region of the cardiac Na⁺ channel (SCN5A). The L1825P channel heterologously expressed in tsA-201 cells showed Na⁺ current with slow decay and a prominent tetrodotoxin-sensitive noninactivating component, similar to the gain-of-function phenotype most commonly observed for SCN5A-associated congenital LQTS (LQT3). In addition, L1825P exhibited loss of function Na⁺ channel features characteristic of Brugada syndrome. Peak Na⁺ current density observed in cells expressing L1825P was significantly diminished, and the voltage dependence of activation and inactivation was shifted toward more positive and negative potentials, respectively.

Conclusions—This study demonstrates that subclinical mutations in the LQTS-related gene SCN5A may predispose certain individuals to drug-induced cardiac arrhythmias. (Circulation. 2002;106:1269-1274.)

Key Words: long-QT syndrome ■ drugs ■ genetics ■ torsade de pointes ■ ion channels

Congenital long-QT syndrome (LQTS) is a rare inherited disorder of cardiac repolarization that predisposes affected individuals to life-threatening arrhythmias. The molecular basis of LQTS is the prolongation of action potential duration attributable to defects in several ion channel genes encoding delayed rectifier K⁺ currents (I_K) or Na⁺ current (I Na). The cardiac Na⁺ channel α subunit gene SCN5A is responsible for a subgroup of LQTS (LQT3). Idiopathic ventricular fibrillation (IVF), or Brugada syndrome (BrS), is believed to be persistent Na⁺ current during the action potential plateau attributable to the defects in Na⁺ channel fast inactivation (gain of function) that delay repolarization. Pathophysiological mechanisms underlying BrS include diminished myocardial Na⁺ current (loss of function) and a resultant increase in transmural voltage gradient and ST-elevation on ECG.

Acquired LQTS, on the other hand, is a more prevalent disorder and is often caused by several drugs that preferentially block the rapid component of delayed rectifier K⁺ current (I_K). However, development of drug-induced LQTS is unpredictable and most likely occurs in a subset of susceptible individuals. It is therefore suggested that these individuals have a genetic substrate possibly involving cardiac ion channels that determine the susceptibility to life-threatening arrhythmias yet whose phenotype remains subclinical until drug exposure.

Recent studies indicate that drug-induced torsade de pointes (TdP) can be associated with silent mutations and common polymorphisms in K⁺ channel genes responsible for congenital LQTS. In this study we describe the first reported case of drug-induced LQTS associated with a novel SCN5A mutation (L1825P) and characterize its functional consequences. Despite the patient’s normal QT interval before exposure to cisapride and the anticipated normal sodium channel function, the heterologously expressed Na⁺ channel mutant L1825P exhibited severe abnormalities characteristic of both LQT3 and IVF/BrS. Our study suggests that...
subclinical mutations or polymorphisms in SCN5A may predispose to life-threatening drug-induced arrhythmias.

Methods

Patient

A 70-year-old woman was admitted to the hospital because of recurrent syncope. She had been treated with acebutolol (200 mg/d) and pirmenol (200 mg/d) for hypertension and frequent premature ventricular contractions, respectively. Her ECG while taking these medications exhibited complete right bundle-branch block (CRBBB) and a normal QT interval (QTc, 435 ms, Figure 1A). Soon after the addition of cisapride (5 mg/d) because of bowel transit dysfunction, she experienced general malaise and syncope. ECG telemetry on admission showed severe bradycardia (junctional rhythm, heart rate ~30 bpm) with QT prolongation (QTc, 480 ms) and repetitive TdP (Figure 1B). Time course of QT interval. Emergency pacing was performed (pacing rate, 80 bpm) at the first day. Marked QT prolongation was observed (QTc, 731 ms). It was shortened the next day after withdrawal of drugs (QTc, 594 ms). QT interval was normalized at the sixth day (QTc, 417 ms); D, ECG recordings on V2 lead during treadmill exercise test before exercise and at the maximum exercise are shown. Heart rate was increased from 66 to 93 bpm, and the QTc was prolonged from 408 to 457 ms.

Figure 1. Electrocardiographic findings of the patient. A, ECG recording obtained from the proband during treatment with acebutolol and pirmenol before prescription of cisapride. Normal sinus rhythm (heart rate, 73 bpm) with complete right bundle-branch block was evident, and the QT interval was within normal limits (QTc, 435 ms). B, ECG telemetry on admission showed severe bradycardia after prescription of cisapride (junctional rhythm, heart rate ~30 bpm) with QT prolongation (QTc, 480 ms) and repetitive TdP. C, Time course of QT interval. Emergency pacing was performed (pacing rate, 80 bpm) at the first day. Marked QT prolongation was observed (QTc, 731 ms). It was shortened the next day after withdrawal of drugs (QTc, 594 ms). QT interval was normalized at the sixth day (QTc, 417 ms). D, ECG recordings on V2 lead during treadmill exercise test before exercise and at the maximum exercise are shown. ECG taken after 6 days of drug-free condition, equivalent to the baseline ECG, showed normal sinus rhythm with CRBBB and normal QT interval (QTc, 417 ms); therefore, the temporary pacing was terminated. Oral administration of mexiletine (300 mg/d) did not additionally change the QT interval (data not shown). The QT interval prolonged during exercise stress testing, contrary to the QT shortening most commonly observed for LQT3 patients (Figure 1D). Rate-dependent ST elevation was not observed. Cardiac catheterization showed normal left ventricular function and normal coronary arteries.

The proband’s paternal grandfather had sudden death of unknown cause, but the rest of her family members are asymptomatic. She has no offspring. Her sister showed normal QT interval (QTc, 440 ms) and was genetically unaffected (Figure 2A). The rest of her family members did not agree to additional examinations, including DNA diagnostics.
Molecular Genetics

Genomic DNA was extracted from peripheral blood leukocytes by a standard method. All exons of SCN5A were amplified by polymerase chain reaction (PCR) using primers designed by Wang et al.13 and analyzed by single-strand conformational polymorphism (SSCP), as previously reported.8 Genetic screening of KCNQ1, HERG, KCNE1, and KCNE2 was carried out as previously described.11,14 The PCR product showing an aberrant conformer was subcloned into pGEMTeasy (Promega), and multiple independent clones were sequenced using an ABI Prism 310 genetic analyzer (Applied Biosystems).

Site-Directed Mutagenesis and Electrophysiology

Wild-type (WT) human heart sodium channel α subunit (hH1) cDNA was subcloned into the pRcCMV plasmid (Invitrogen). Site-directed mutagenesis of hH1 was performed by an overlap-extension PCR strategy, as described.15 A 183-bp HincII/SacII fragment (No. 5290-5473 of hH1) of the mutant PCR fragment was subcloned back into the pRcCMV-WT plasmid to generate a mutant plasmid pRcCMV-L1825P. Correct assembly of the mutant was verified by restriction analysis, and the mutated regions generated by PCR were sequenced completely to identify clones without polymerase errors. The human cell line tsA-201 was transiently transfected with either pRcCMV-WT or pRcCMV-L1825P using standard calcium phosphate method in combination with a bicistronic plasmid pCD8-IRES-hCD81. Functional expression studies were performed on multiple independent recombinants. Results are presented as mean ± SEM, and statistical comparisons were made using the unpaired Student’s t test. Statistical significance was assumed for P<0.05. Cisapride and pirmenol were obtained from Welfide Co and Dainippon Pharmaceutical Inc, respectively.

Results

Molecular Genetics

Initial SSCP analysis was performed to screen for genetic variations in the LQTS-related K⁺ channel genes KCNQ1, HERG, KCNE1, and KCNE2. Because aberrant conformers were not detected in these genes, we screened SCN5A. An aberrant SSCP conformer was identified in exon 28 of the proband. DNA sequencing confirmed a T to C transition leading to amino acid substitution of proline for leucine1825 (L1825P; Figure 2B) located within the leading to amino acid substitution of proline for leucine1825 (L1825P; Figure 2B) located within the cardiac Na⁺ channel is illustrated with location of known mutations associated with either LQT3 (∙) or IVF/BrS (∙) and cardiac conduction defect (○) indicated. D, Amino acid sequence alignments of SCN5A with related Na⁺ channel sequences are shown in the lower panel. SCN5A indicates human heart; SCN1A, human brain type I; SCN2A, human brain type II; SCN3A, human brain type III; SCN4A, human skeletal muscle; rH1, rat heart; mH1, mouse heart; and Eel, eel electroplax.

Figure 2. Pedigree, the results of DNA sequencing, and the location of the mutation. A, Pedigree of the cisapride-induced long-QT syndrome. ECG and DNA were only available from the proband and her sister. Her sister’s Qtc interval was 440 ms, and she was genetically unaffected. SCD and AMI denote sudden cardiac death and acute myocardial infarction, respectively. Genetic and electrocardiographic examinations were not carried out for the family members marked with ND. Arrow indicates the proband. B, DNA sequences of clones representing normal (left) and mutant alleles (right) are shown. The mutant clone had a T to C transition. C, Predicted topology of the cardiac Na⁺ channel is illustrated with location of known mutations associated with either LQT3 (∙) or IVF/BrS (∙) and cardiac conduction defect (○) indicated. D, Amino acid sequence alignments of SCN5A with related Na⁺ channel sequences are shown in the lower panel. SCN5A indicates human heart; SCN1A, human brain type I; SCN2A, human brain type II; SCN3A, human brain type III; SCN4A, human skeletal muscle; rH1, rat heart; mH1, mouse heart; and Eel, eel electroplax.

**Figure 2.**

**A** Pedigree of the cisapride-induced long-QT syndrome. ECG and DNA were only available from the proband and her sister. Her sister’s Qtc interval was 440 ms, and she was genetically unaffected. SCD and AMI denote sudden cardiac death and acute myocardial infarction, respectively. Genetic and electrocardiographic examinations were not carried out for the family members marked with ND. Arrow indicates the proband. **B**, DNA sequences of clones representing normal (left) and mutant alleles (right) are shown. The mutant clone had a T to C transition. **C**, Predicted topology of the cardiac Na⁺ channel is illustrated with location of known mutations associated with either LQT3 (∙) or IVF/BrS (∙) and cardiac conduction defect (○) indicated. **D**, Amino acid sequence alignments of SCN5A with related Na⁺ channel sequences are shown in the lower panel. SCN5A indicates human heart; SCN1A, human brain type I; SCN2A, human brain type II; SCN3A, human brain type III; SCN4A, human skeletal muscle; rH1, rat heart; mH1, mouse heart; and Eel, eel electroplax.
Fraction of $P_{n,13}$ is shown (*$H_{11021}$) the difference between WT (open, $n=11005$) and L1825P (closed, $n=11001$) are illustrated in the lower panel. Statistical significance in activation and fast inactivation gating properties exhibited by L1825P will potentially result in greater reduction in Na$^+$ channel availability during excitation.

In addition to the dysfunction of activation and fast inactivation, some mutant Na$^+$ channels associated with IVF/BrS show defects in inactivation kinetics intermediate between fast and slow inactivation, referred to as $I_M$, which is now recognized as an important biophysical feature underlying certain Na$^+$ channelopathies. We analyzed the onset of slow inactivation by prolonging the prepulses to from 1 ms to 10 s followed by a brief repolarization to allow channels to recover from fast inactivation before the test pulse. Distinct from the BrS mutation T1620 mol/L18 or the LQT3/BrS mutant channel 1795insD$^{19}$ that have enhanced $I_M$, the time course of the onset of slow inactivation of L1825P was virtually identical to WT (Figure 4D).

Although L1825P channel shows slower open-state inactivation, a population of Na$^+$ channels enters an inactivated state without channel opening by a mechanism called closed-state inactivation. Closed-state inactivation greatly affects the Na$^+$ current through the Na$^+$ channel availability during excitation.

To test whether the $I_{Kr}$ blocker cisapride has direct effects on Na$^+$ currents, 1 mol/L cisapride, a sufficient concentration to block $I_{Kr}$, was applied to the bath solution. However, cisapride did not change the peak Na$^+$ current in either WT or L1825P channels (% control; WT, 99.8 ± 3.6%, n = 6; L1825P, 88.8 ± 4.3%, n = 8; NS). Cisapride failed to change the kinetics or the amplitude of the persistent Na$^+$ current in either WT or L1825P, confirming that cisapride-induced QT prolongation in the probed was mediated through mechanisms other than direct effects on cardiac Na$^+$ channels. We also examined the effect of the Na$^+$ channel blocker pirmenol, which the proband had been actively taking by the time she developed TdP. However, 10 mol/L pirmenol did not affect the persistent late current of L1825P, whereas it
blocked the peak Na current of L1825P to the extent comparable to WT (% control; WT, 95.7±4.0%, n=5; L1825P, 86.4±3.7%, n=4, NS). Therefore, it is unlikely that pirmenol had been reducing persistent Na current of L1825P, thereby protecting against QT prolongation before cisapride exposure.

**Discussion**

Drug-induced LQTS is a prevalent life-threatening disorder with uncertain etiologies but shares substantial clinical features with congenital LQTS. It has been inferred that a subset of individuals with normal or borderline QT-interval may carry subclinical mutations in LQTS disease genes and are susceptible to life-threatening arrhythmias on drug exposure. Our study suggests that Na channel mutations can predispose apparently stable patients to life-threatening arrhythmias when treated with agents that inhibit K channels.

Rodent et al hypothesized repolarization reserve as a potential mechanism underlying susceptibility to drug-induced LQTS. The cardiac action potential is orchestrated by a fine balance between inward and outward currents expressed in myocardial cells. The action potential duration is prolonged by either an increase of inward currents or a decrease of outward currents. Because the outward currents consist of multiple distinct K currents, such as I , I , or I , K channel dysfunction attributable to mutations or polymorphisms could be potentially compensated by other K currents, and thereby the repolarization is tolerated (repolarization reserve). To extrapolate this hypothesis, it is assumed that repolarization reserve allows the dysfunction of the L1825P mutation to be tolerated until the time of drug exposure. However, administration of cisapride blocked I , and exhausted the repolarization reserve, leading to manifest the action potential prolongation and TdP. There are several lines of evidence supporting the existence of functional interplay between Na and outward K currents in the in vivo myocardium. Using a canine perfused ventricular wedge model, Shimizu et al have shown that mexiletine reduced transmural dispersion of repolarization and prevented TdP in the LQT2 model as well as in LQT3. Moreover, K channel opener nicorandil prevents TdP in congenital or acquired models of LQT1, LQT2, and LQT3. The functional association between gain of function of I and loss of function of I attributable to cisapride may be responsible for the manifestation of drug-induced QT prolongation in the present case.

Moreover, QT-prolonging drugs usually require multiple risk factors, such as hypokalemia, female sex, or slow heart rate to manifest life-threatening arrhythmias. In the present case, bradycardia, presumably induced by acebutolol, was the additional risk factor to trigger QT prolongation and TdP. Another explanation is that the contribution to persistent Na current of L1825P to the cardiac action potential duration may be relatively small, because the persistent current is offset by the concomitant loss of function phenotype (Figures A, 4A, 4D, and 4E). Alternatively, biophysical dysfunctions of the L1825P allele could be functionally compensated by the WT allele. Furthermore, manifestation of LQTS is variable and seems to be determined in genotype-specific, mutation-specific, or individual-specific manners, and up to 75% of the
gene carriers show normal QT interval. It is speculated that this patient could be a congenital LQTS with an SCN5A mutation that exhibits reduced penetrance. Moreover, contribution of other genes, such as P450 3A4, a major metabolizing enzyme of cisapride, remains to be examined.

Mutations in SCN5A result in multiple arrhythmic syndromes, including LQT3, IVF/BrS, an inherited cardiac conduction defect, and sudden infant death syndrome, constituting a spectrum of disease entities termed cardiac Na+ channelopathies. Heterologously expressed L1825P channels exhibit biophysical properties strikingly similar to an SCN5A mutation 1795insD found in an unusual LQT3 family, in which affected individuals exhibited rate-dependent QT prolongation and ST elevation. The L1825P and 1795insD mutations, both located at the C-terminal of the Na+ channel, share the following biophysical properties: (1) prominent persistent late Na+ current; (2) negative shift of steady-state inactivation; and (3) decreased current density. These results suggest that LQT3 and IVF/BrS are closely related Na+ channelopathies with overlapping phenotypes and that L1825P is another example of a mutation that can explain this clinical overlap. Nonetheless, clinical manifestations of these 2 mutations are not identical. Exercise-induced ST elevation observed in 1795insD was not evident in L1825P despite the fact that both channels exhibited BrS-like properties. This phenotypic difference in ST elevation may be attributed to the difference in the kinetics of I_{Na}, the physiological mechanism for the rate-dependent ST elevation in 1795insD, but it was not evident in L1825P.

In summary, we propose that subclinical mutations in the LQTS-related gene SCN3A may predispose the subset of individuals to life-threatening arrhythmias during drug therapy.

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