Pronounced Shortening of QT Interval With Mexiletine Infusion Test in Patients With Type 3 Congenital Long QT Syndrome

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Background: Mexiletine is often used for medical therapy in LQT3 patients, however, the usefulness of mexiletine infusion test for LQT3 patients has not been reported. The aim of this study was to evaluate the usefulness of mexiletine infusion test for detecting LQT3 patients.

Methods and Results: We analyzed response in 12-lead electrocardiogram parameters measured in II or V5 to i.v. mexiletine infusion (2 mg/kg) during sinus rhythm among 31 genotype-positive LQT patients (29±18 years, 12 male). Change in QTc interval after mexiletine was compared between LQT3 (n=15, 24±21 years, 9 male) and other LQT patients (4 LQT1 and 12 LQT2; 34±14 years, 3 male). Baseline RR, QT, and QTc interval were not different between the 2 groups (981±182 vs. 1,023±192 ms; 550±94 vs. 524±75 ms; 556±66 vs. 520±62 ms, respectively). While QTc interval was shortened with mexiletine in both groups (P<0.0001 vs. baseline), degree of QTc shortening (ΔQTc) was significantly larger in LQT3 than in LQT1/LQT2 patients (99±39 vs. 48±32 ms; P=0.0004). The sensitivity, specificity and predictive accuracy of mexiletine infusion test for differentiating LQT3 from LQT1/LQT2 were 86.7%, 81.3% and 81.3%, respectively, and the optimal cut-off for ΔQTc was 69 ms on receiver operating characteristic analysis. No pro-arrhythmic event was observed.


Key Words: Diagnosis; Gene; Long QT syndrome; Mexiletine; Ventricular arrhythmia

Congenital long QT syndrome (LQTS), characterized by prolongation of the QT interval, T wave abnormalities, and torsade de pointes (TdP), is a genetic heart disorder that may cause sudden cardiac death. Congenital LQTS is clinically diagnosed by QT prolongation on standard 12-lead electrocardiogram (ECG), clinical history of syncope or cardiac arrest, and a family history of LQTS. Molecular genetics is now available to identify LQTS forms by mutations in genes encoding ion channels (KCNQ1, KCNH2, and SCN5A etc), and has enabled risk stratification and treatment of LQTS patients according to genotype. In congenital LQTS type 3 (LQT3), a gain-of-function mutation in SCN5A encoding the α subunit of cardiac voltage-dependent sodium channel may increase persistent (late) sodium current (late-I Na ) resulting in prolongation of action potential duration, leading to life-threatening ventricular arrhythmia.

Beta-blockers have been proved to be the first choice of pharmacological therapy in patients with congenital LQTS type 1 (LQT1) and type 2 (LQT2), but it has been believed to be less effective in LQT3 patients, who often experience cardiac events at rest or during sleep. In contrast, the late-I Na inhibitor, mexiletine, has been shown to be effective in shortening QT interval in
LQT3 rather than in LQT1 or LQT2, both clinically and experimentally. This indicates that mexiletine may be able to differentiate LQT3 from other major forms of LQTS such as LQT1 and LQT2. Therefore, the aim of this study was to investigate the usefulness of intra-venous injection of mexiletine for differentiating LQT3 from LQT1/LQT2.

Methods

Study Design and Subjects

The subjects consisted of genotype-positive LQT patients, including 4 with LQT1, 12 with LQT2, and 15 with LQT3. All the patients were informed about congenital LQTS and the importance of diagnosis, including drug infusion test, and mutual agreement was obtained regarding this study. Clinical characteristics are listed in Table 1. After baseline 12-lead ECG recording was completed, 2 mg/kg mexiletine was infused for 10 min during sinus rhythm. The 12-lead ECG was continuously monitored and recorded during infusion and for 5 min after infusion.

We analyzed change in 12-lead ECG parameters after mexiletine infusion in all patients, and compared them between LQT3 and other LQTS patients (LQT1 and LQT2), given that ECG response to mexiletine was similar between the LQT1 and LQT2 patients. All LQTS patients had neither oral β-blockers nor anti-arrhythmic agents at mexiletine infusion test.

Measurement of 12-Lead ECG

Measurement of the obtained 12-lead ECG parameters was manually performed (25 mm/s, 10 mm/mV). QT was defined as the interval between onset of QRS morphology and the point at which an isoelectic line intersected a tangential line drawn at the point of minimum dV/dt of a positive T wave or at the point of maximum dV/dt of a negative T wave. Secondary T wave or bifurcated T wave was included in the QT interval except when the second wave (positive U wave) was clearly separated from the T wave. QT interval was measured at 10-ms increments in the V5 lead by 2 electrophysiologists (M.F. and W.S.), and inter-observer variability was 3.2 ± 3.1 ms. If it was difficult to define QT interval in the V5 lead, then the V4 and limb lead II were used for measuring QT interval. Corrected QT (QTc) interval was used to correct the effects of heart rate. Bazett’s formula was applied as follows:

\[
QTc = \frac{QT \times RR^{1/2}}{RR}
\]

where RR is the interval between 2 continuous R waves.

Genetic Analysis

The protocol for genetic analysis was approved by the Institutional Ethics Committee and carried out according to the guidelines (M24-031-4). All patients provided informed consent before genetic analysis. Genomic DNA was isolated from whole blood using a DNA analyzer (QIAGEN GmbH, Hilden, Germany). Genetic screening for KCNQ1, KCNH2, and SCN5A was carried out using the direct sequencing method (ABI 3730 DNA Analyzer, Life Technologies, Carlsbad, CA, USA). cDNA sequence numbering was based on the GenBank reference sequence NM_000218, NM_000238 and AT038064 for KCNQ1, KCNH2, and SCN5A, respectively.

Statistical Analysis

All data are expressed as mean ± SD. In order to compare the ECG parameters before and after mexiletine infusion test, repeated-measure 2-way ANOVA was used (JMP ver. 8.0, SAS Institute). Differences in frequencies were analyzed with the chi-squared test, and 2-sided P<0.05 was considered statistically significant. Receiver operating characteristic (ROC) analysis was used for non-parametric data distribution; correlation between baseline QTc interval and ΔQTc was analyzed using Pearson’s product moment correlation coefficient.

Results

Baseline RR interval, QT interval and QTc interval were not different between the LQT3 and the LQT1/ LQT2 patients (Table 2). All of the patients with LQT1/LQT2 had a history of syncope and all of the LQT1 patients were VF survivors (Table 1). Two out of 4 LQT1 patients had mutation of A341V, reported as severe phenotype. On the other hand, LQT3 patients except for those with the specific mutations of SCN5A (E1784K, Y1795C) were symptomatic (Table 3).

Change in ECG Parameters With Mexiletine Infusion Test

Figure 1 shows the change in QTc interval in V5 after mexiletine infusion in LQT1, LQT2 and LQT3 patients, demonstrating that mexiletine shortened QTc interval more in LQT3 compared with LQT1 and LQT2. As shown in Table 2, intra-venous mexiletine significantly shortened the RR interval in both LQT3 and LQT1/LQT2 patients, however, the change in RR interval (ΔRR) was not different between the 2 groups. Although mexiletine significantly shortened the QT and QTc intervals in both groups (P<0.0001), the change in both QT (ΔQT) and QTc (ΔQTc) interval was significantly larger in the LQT3 than in LQT1/LQT2 patients (116±50 ms vs. 66±32 ms; P=0.0021, and 99±39 ms vs. 48±32 ms; P=0.0004, respectively; Table 2; Figure 2A). No significant difference was observed in the ΔRR, ΔQT and ΔQTc intervals between the LQT1 and LQT2 patients (data not shown). The sensitivity, specificity and predictive accuracy of ΔQTc for differentiating LQT3 from LQT1/LQT2 with mexiletine were 86.7%, 81.3%, and
interval, thus baseline ∆QTc/QTc tended to be larger in patients with mexiletine-sensitive mutations compared with those with mexiletine-insensitive mutations (0.19±0.06 vs. 0.11±0.05, P=0.055). Oral Mexiletine After Mexiletine Infusion Test
In the present study, 2 patients (patients 7 and 12; Table 3) out of 15 with LQT3 were treated with oral mexiletine after mexiletine infusion test, and ECG data after oral mexiletine were compared to those before mexiletine infusion test. Patient 7 was treated with 450 mg per day oral mexiletine. The RR, QT, and QTc interval were changed from 940 ms, 520 ms, and 536 ms to 1,000 ms, 460 ms, and 460 ms, respectively. Blood concentration of mexiletine was 0.60µg/mg at 1 month after initiation of oral mexiletine. Patient 12 was treated with 300 mg oral mexiletine and the RR, QT, and QTc interval were changed from 1,080 ms, 590 ms, and 568 ms to 1,160 ms, 560 ms, and 520 ms, respectively. Blood concentration of mexiletine was not available in patient 12. QT and QTc interval were shortened by oral mexiletine therapy in both patients.

<table>
<thead>
<tr>
<th>Table 2. Response of ECG Parameters to Mexiletine</th>
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<tbody>
<tr>
<td>LQT3 (n=15)</td>
</tr>
<tr>
<td>RR interval (ms)</td>
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<tr>
<td>ΔRR (ms)</td>
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<tr>
<td>QT interval (ms)</td>
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<td>ΔQT (ms)</td>
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<tr>
<td>QTc interval (ms)</td>
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<td>ΔQTc (ms)</td>
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Data given as mean±SD *P<0.05 †vs. baseline; ‡vs. LQT1/LQT2. ECG, electrocardiography. Other abbreviations as in Table 1.

<table>
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<tr>
<th>Table 3. Mutation of SCN5A and ECG Response to Mexiletine Infusion in LQT3 Patients</th>
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<tbody>
<tr>
<td>Patient ID no.</td>
</tr>
<tr>
<td>1 E1784K Proband Asymptomatic</td>
</tr>
<tr>
<td>2 E1784K Proband Asymptomatic</td>
</tr>
<tr>
<td>3 E1784K Family of 2 Asymptomatic</td>
</tr>
<tr>
<td>4 E1784K Proband Asymptomatic</td>
</tr>
<tr>
<td>5 E1784K Family of 4 Asymptomatic</td>
</tr>
<tr>
<td>6 E1784K Family of 4 Asymptomatic</td>
</tr>
<tr>
<td>7 A1746T Proband Symptomatic</td>
</tr>
<tr>
<td>8 P1509-I1510 ins QKP Proband Asymptomatic</td>
</tr>
<tr>
<td>9 Y1795C Proband Asymptomatic</td>
</tr>
<tr>
<td>10 I1771M Proband Symptomatic</td>
</tr>
<tr>
<td>11 R1623Q Proband Symptomatic</td>
</tr>
<tr>
<td>12 V411M Proband Symptomatic</td>
</tr>
<tr>
<td>13 F1617/ fs/400 Proband Symptomatic</td>
</tr>
<tr>
<td>14 Q1507-P1509 del QKP Proband Symptomatic</td>
</tr>
<tr>
<td>15 A1186T Proband Symptomatic</td>
</tr>
</tbody>
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Patients 2, 3, and 4–6, were in the same family group, respectively. *Insensitive to mexiletine. Abbreviations as in Tables 1, 2.

81.3% respectively, when the best ΔQTc cut-off of 69 ms (calculated using ROC analysis) was used (Figure 2B).

Mutation Site-Specific Difference in QTc Shortening
Ten different mutations in the SCN5A gene were confirmed in the 15 LQT3 patients (Figure 3), in whom 6 asymptomatic patients had E1784K mutation (Table 3). The location of SCN5A mutations and the change in ECG parameters including ΔQTc with mexiletine infusion are shown in Figure 3A, Table 3. The extent of QTc shortening in response to mexiletine varied with location of SCN5A mutation. Therefore, we classified the SCN5A mutations as sensitive or insensitive to mexiletine infusion according to QTc interval after mexiletine (<500 ms or ≥500 ms), respectively. Three mutations, A1186T, R1623Q and I1771M, were defined as insensitive to mexiletine, whereas the remaining 7 mutations were sensitive. In patients with mexiletine-sensitive mutation, ΔQTc was significantly correlated with baseline QTc (r²=0.79, P=0.0074, Figure 3B). In contrast, patients with mexiletine-insensitive mutation had smaller ΔQTc even in the longer baseline QTc interval, thus baseline ΔQTc/QTc tended to be larger in patients with mexiletine-sensitive mutations compared with those with mexiletine-insensitive mutations (0.19±0.06 vs. 0.11±0.05, P=0.055).

Oral Mexiletine After Mexiletine Infusion Test
In the present study, 2 patients (patients 7 and 12; Table 3) out of 15 with LQT3 were treated with oral mexiletine after mexiletine infusion test, and ECG data after oral mexiletine were compared to those before mexiletine infusion test. Patient 7 was treated with 450 mg per day oral mexiletine. The RR, QT, and QTc interval were changed from 940 ms, 590 ms, and 568 ms to 1,160 ms, 560 ms, and 520 ms, respectively. Blood concentration of mexiletine was not available in patient 12. QT and QTc interval were shortened by oral mexiletine therapy in both patients.
Mexiletine Infusion Test for LQT3

Complications of Mexiletine Infusion

There were no pro-arrhythmic complications including TdP, ventricular tachyarrhythmias, or premature ventricular contractions related to mexiletine infusion test.

Discussion

Main Findings

The main findings of the present study are that (1) mexiletine shortened QTc interval in the LQT3 patients more than in the LQT1, LQT2 patients, suggesting that mexiletine infusion test is a useful tool to distinguish LQT3 from LQT1 or LQT2; and (2) the difference in QTc shortening between mutation sites in response to mexiletine infusion was observed in LQT3 patients.

Usefulness of Mexiletine Infusion Test for LQT3

Clinical diagnosis of LQTS has been based on evaluation of the specific clinical setting with regard to history of syncope with sympathetic stimulation, cardiac events, and symptomatic
or asymptomatic history of family members. Normal range of QT interval on 12-lead ECG varies between the sexes and between children and adults. ECG diagnostic criteria involve QTc interval, T-wave morphology and QT dispersion, but concealed or low-penetrance LQTS is difficult to detect on 12-lead ECG at rest. Exercise stress testing such as treadmill or bicycle testing has been used for unmasking concealed or low-penetrance LQTS. Shimizu et al noted differential response in QTc interval on 12-lead ECG to epinephrine infusion test between LQT1, LQT2, and LQT3 patients. Although both exercise and catecholamine challenge test were useful tools to predict LQT1 and LQT2 syndrome before genetic testing, the identification of LQT3 syndrome remained difficult. Genetic testing to identify specific mutations in LQTS is now commercially available for clinical use, but there are some difficulties with regard to cost and time to diagnosis. The combination of exercise, epinephrine infusion test and mexiletine infusion test may facilitate effective clinical diagnosis of LQTS. In the present study, we focused on the usefulness of mexiletine infusion test to differentiate LQT3 from LQT1 or LQT2, which has been previously reported by Schwartz et al clinically, and by Shimizu et al experimentally. Schwartz et al first reported that Ina channel block with mexiletine shortened QT interval more in LQT3 than in LQT2 patients. Shimizu et al and Priori et al demonstrated differential response in action potential duration or QT interval to mexiletine between experimental LQT2 and LQT3 models. Furthermore, the QTc shortening with mexiletine infusion might be poor in the specific SCN5A mutations, as reported. The present study first demonstrated the usefulness of mexiletine infusion test to distinguish LQT3 from LQT1 or LQT2 quantitatively. The correct diagnosis of LQT3 is important because some LQT3 patients are at high risk of sudden cardiac death and need implantation of cardioverter defibrillator. Mexiletine has fewer side-effects, such as TdP, sinus bradycardia or atrioventricular block compared with β-blockers. In the present study, 6 of 15 LQT3 patients had bradycardia and 1 patient had 2:1 atrioventricular block. Beta-blockers can worsen bradycardia or atrioventricular block. RR interval was slightly but significantly decreased after mexiletine infusion in the present LQTS patients, probably due to reactive response to slight decrease of blood pressure. Therefore, the lower number of side-effects with mexiletine infusion test is another advantage of this test, to facilitate genetic testing in LQT3 syndrome.

Mutation Site-Specific Differences in QT Shortening
Mexiletine shortens QTc interval in LQT3 rather than in LQT1 or LQT2 because of its suppression of late-I Na , which may depend on the gating state of the sodium channel and the binding of local anesthetics. Although both exercise and catecholamine challenge test were useful tools to predict LQT1 and LQT2 syndrome before genetic testing, the identification of LQT3 syndrome remained difficult. Genetic testing to identify specific mutations in LQTS is now commercially available for clinical use, but there are some difficulties with regard to cost and time to diagnosis. The combination of exercise, epinephrine infusion test and mexiletine infusion test may facilitate effective clinical diagnosis of LQTS. In the present study, we focused on the usefulness of mexiletine infusion test to differentiate LQT3 from LQT1 or LQT2, which has been previously reported by Schwartz et al clinically, and by Shimizu et al experimentally. Schwartz et al first reported that Ina channel block with mexiletine shortened QT interval more in LQT3 than in LQT2 patients. Shimizu et al and Priori et al demonstrated differential response in action potential duration or QT interval to mexiletine between experimental LQT2 and LQT3 models. Furthermore, the QTc shortening with mexiletine infusion might be poor in the specific SCN5A mutations, as reported. The present study first demonstrated the usefulness of mexiletine infusion test to distinguish LQT3 from LQT1 or LQT2 quantitatively. The correct diagnosis of LQT3 is important because some LQT3 patients are at high risk of sudden cardiac death and need implantation of cardioverter defibrillator. Mexiletine has fewer side-effects, such as TdP, sinus bradycardia or atrioventricular block compared with β-blockers. In the present study, 6 of 15 LQT3 patients had bradycardia and 1 patient had 2:1 atrioventricular block. Beta-blockers can worsen bradycardia or atrioventricular block. RR interval was slightly but significantly decreased after mexiletine infusion in the present LQTS patients, probably due to reactive response to slight decrease of blood pressure. Therefore, the lower number of side-effects with mexiletine infusion test is another advantage of this test, to facilitate genetic testing in LQT3 syndrome.

Figure 3. (A) Topology of the α-subunit of the Nav1.5 cardiac sodium channel and localization of the 10 long QT syndrome type 3 (LQT3) mutations in the present patients. Underline, mexiletine-insensitive mutations. (B) Relationship between baseline corrected QT (QTc) interval and change in QTc interval (ΔQTc) with mexiletine. (●) Mexiletine-sensitive mutations; (○) mexiletine-insensitive mutations.
inactivation was not altered in the mexiletine-insensitive mutants, I1771M\textsuperscript{26} and R1623Q.\textsuperscript{37} On the other hand, mexiletine is known to bind to the sodium channel during the inactivated state.\textsuperscript{38} These findings suggest that the negative shift in V1/2 of steady-state inactivation in E1784K and other mexiletine-sensitive mutant channels may be attributable to the greater shortening of QT interval in response to mexiletine infusion.\textsuperscript{29} Thus, such functional heterogeneity of Na channels may account for the heterogeneous response of QT interval to mexiletine therapy in LQT3 patients.

**Study Limitations**

First, this study included a small number of patients with genotype-positive LQT3s and therefore a selection bias may have been present. This study was also a cross-sectional study by a single center. Second, the reproducibility of the response of ECG parameters to mexiletine infusion was not evaluated. Third, the usefulness of mexiletine infusion was evaluated in this study, therefore, the chronic effect of oral mexiletine therapy on QT interval remains unknown.

**Conclusions**

Mexiletine infusion test with a ΔQTc cut-off of 69 ms was a safe and useful method to facilitate the genetic testing of LQT3 patients.

**Acknowledgments**

The authors thank Naotaka Ohta, Toshiko Shibata, Hiromi Fujiyama, Miyuki Hozan and Akihiro Fujiwara for excellent technical support in this study. R1623Q is effective in reducing dispersion of repolarization and preventing torsade des points in LQT2 and LQT3 models of the long-QT syndrome.\textsuperscript{39} Circulation 2001; 103: 855.

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