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ORIGINAL RESEARCH ARTICLE

Therapeutic Efficacy of Mexiletine for Long QT Syndrome Type 2: Evidence From Human Induced Pluripotent Stem Cell–Derived Cardiomyocytes, Transgenic Rabbits, and Patients

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BACKGROUND: Despite major advances in the clinical management of long QT syndrome, some patients are not fully protected by beta-blocker therapy. Mexiletine is a well-known sodium channel blocker, with proven efficacy in patients with sodium channel–mediated long QT syndrome type 3. Our aim was to evaluate the efficacy of mexiletine in long QT syndrome type 2 (LQT2) using cardiomyocytes derived from patient-specific human induced pluripotent stem cells, a transgenic LQT2 rabbit model, and patients with LQT2.

METHODS: Heart rate–corrected field potential duration, a surrogate for QTc, was measured in human induced pluripotent stem cells from 2 patients with LQT2 (KCNH2-p.A561V, KCNH2-p.R366X) before and after mexiletine using a multiwell multi-electrode array system. Action potential duration at 90% repolarization (APD_{90}) was evaluated in cardiomyocytes isolated from transgenic LQT2 rabbits (KCNH2-p.G628S) at baseline and after mexiletine application. Mexiletine was given to 96 patients with LQT2. Patients were defined as responders in the presence of a QTc shortening ≥40 ms. Antiarrhythmic efficacy of mexiletine was evaluated by a Poisson regression model.

RESULTS: After acute treatment with mexiletine, human induced pluripotent stem cells from both patients with LQT2 showed a significant shortening of heart rate–corrected field potential duration compared with dimethyl sulfoxide control. In cardiomyocytes isolated from LQT2 rabbits, acute mexiletine significantly shortened APD_{90} by 113 ms, indicating a strong mexiletine-mediated shortening across different LQT2 model systems. Mexiletine was given to 96 patients with LQT2 either chronically (n=60) or after the acute oral drug test (n=36): 65% of the patients taking mexiletine only chronically and 75% of the patients who performed the acute oral test were responders. There was a significant correlation between basal QTc and ΔQTc during the test (r= −0.8; P<0.001). The oral drug test correctly predicted long-term effect in 93% of the patients. Mexiletine reduced the mean yearly event rate from 0.10 (95% CI, 0.07–0.14) to 0.04 (95% CI, 0.02–0.08), with an incidence rate ratio of 0.40 (95% CI, 0.16–0.84), reflecting a 60% reduction in the event rate (P=0.01).

CONCLUSIONS: Mexiletine significantly shortens cardiac repolarization in LQT2 human induced pluripotent stem cells, in the LQT2 rabbit model, and in the majority of patients with LQT2. Furthermore, mexiletine showed antiarrhythmic efficacy. Mexiletine should therefore be considered a valid therapeutic option to be added to conventional therapies in higher-risk patients with LQT2.

Key Words: arrhythmias, cardiac ■ death, sudden, cardiac ■ genetics ■ long QT syndrome ■ mexiletine ■ pluripotent stem cells ■ precision medicine

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Clinical Perspective

What Is New?

- Mexiletine shortened heart rate–corrected field potential duration, a surrogate for QTc, in human induced pluripotent stem cell–derived cardiomyocytes from patients with long QT syndrome type 2 (LQT2) and significantly shortened action potential duration at 90% repolarization in cardiomyocytes isolated from LQT2 rabbits.
- Mexiletine was effective in shortening QTc by at least 40 ms in more than two thirds of patients with LQT2, and the oral drug test correctly predicted long-term effect in 92% of cases.
- Mexiletine showed antiarrhythmic efficacy in patients with LQT2, significantly reducing the mean yearly event rate.

What Are the Clinical Implications?

- Mexiletine should be considered a valid therapeutic option to be added to conventional therapies in higher-risk patients with LQT2.
- The greatest antiarrhythmic protection is observed in patients with a QTc on mexiletine <500 ms; therefore, when managing high-risk patients with LQT2, it is important to evaluate not only the degree of QTc shortening produced by mexiletine but also its actual final QTc value.

Nonstandard Abbreviations and Acronyms

AP	action potential
APD_{90}	action potential duration at 90% repolarization
DMSO	dimethyl sulfoxide
FPD	field potential duration
hiPSC	human induced pluripotent stem cell
hiPSC-CM	human induced pluripotent stem cell cardiomyocyte
LCSD	left cardiac sympathetic denervation
LQT2	long QT syndrome type 2
LQT3	long QT syndrome type 3
LQTS	long QT syndrome
PI3K	phosphatidyl inositol 3 kinase
PtPAmpl	peak-to-peak amplitude
SGK1	serum- and glucocorticoid-regulated kinase 1

The paradigm change brought about by the identification in 1995 and 1996 of the 3 main long QT syndrome (LQTS)–associated genes[1–3] included the search for new and gene-specific management strategies and therapies. The main effect on management followed the identification of gene-specific triggers for lethal arrhythmias[4] and resulted in highly specific recommendations,[4,5] whereas the main effect on therapy followed the realization that the arrhythmogenic mechanism in patients with long QT syndrome type 3 (LQT3) was the augmented inward late sodium current caused by gain-of-function variants in the sodium channel gene *SCN5A*.[6] The first suggestion to use the time-honored sodium channel blocker mexiletine was advanced in 1995 by showing a QTc shortening of 90 ms in 6 patients with LQT3 during an acute oral drug test.[7] After the initial suggestion to target a 50-ms shortening before going to chronic therapy,[8] a consensus was reached in 2013 guidelines[9] to use, in patients with LQT3, a 40-ms QTc shortening as an indication to move to chronic use. Today, for patients with LQT3 "with prolonged QT interval," mexiletine is a class I treatment recommendation.[10] Mexiletine was introduced 50 years ago as an antiarrhythmic drug,[11,12] and it was expected that, together with the QTc shortening, this would contribute to reducing arrhythmias in patients with LQT3, as indeed has been observed.[13] Thus, the original 1995 proposal to use mexiletine for patients with LQT3 with a prolonged QTc[7] has been validated over time, and mexiletine is now an integral part of the therapy for these patients.

Despite considerable progress, the management of LQTS remains complex because patients with LQT3 represent a small percentage (≈10%), whereas the larger group of patients with long QT syndrome type 2 (LQT2), involving 35% to 40% of patients with LQTS, is not fully protected by standard therapy, as indicated by all comparisons with patients with LQTS type 1.[14,15] For this reason, when we serendipitously observed QTc shortening in patients with LQT2, we examined our database, and then reported that 8 patients had shortened QTc with mexiletine.[16] Partly because of the very small number of patients, our preliminary report had relatively little effect on current management of patients with LQT2 seen outside LQTS referral centers.

It was on this background that we decided to tackle this important unresolved issue and designed a thorough study to assess whether mexiletine could be effective in the treatment of patients with not only LQT3 but also those with potassium channel–mediated LQT2. We evaluated its efficacy translationally, using cardiomyocytes derived from patient-specific human induced pluripotent stem cells (hiPSC-CMs), a transgenic LQT2 rabbit model, and a now expanded cohort of patients with LQT2. Our main clinical questions concerned the effect of mexiletine in patients with QT prolongation either at baseline or just on a Holter recording, the value of acute oral testing in predicting efficacy during chronic treatment, the relationship between QTc shortening and baseline values, and the association with LQT2-triggered arrhythmic events.

METHODS

Detailed Methods are available in the Supplemental Material. Data are available upon reasonable request.

HiPSC-Derived Cardiomyocytes

Patients with LQT2 with KCNH2-p.A561V or KCNH2-p.R366X variants signed appropriate informed consent forms, and the study was approved by the institutional review board of the IRCCS Istituto Auxologico Italiano (approval Auxologico 2019-04-16-04). The hiPSC line with the *KCNH2*-p.A561V variant was provided by Dr Joseph C. Wu from the Stanford Cardiovascular Institute (SCVI498).

Human Induced Pluripotent Stem Cell Cardiomyocytes

HiPSCs derived from the 2 patients with LQT2 with either the KCNH2-p.A561V or KCNH2-p.R366X variant were cultured and differentiated to hiPSC-CMs with a modified version of a previously published Wnt/β-catenin signaling modulation protocol.[17,18]

The hiPSC-CMs carrying the KCNH2-p.R366X variant were generated from one of our patients, who was also enrolled in the clinical part of the study. The hiPSC line carrying the KCNH2-p.A561V variant was acquired within a research collaboration with the Stanford Cardiovascular Institute.

Electrophysiological experiments were conducted at 37°C on monolayers of hiPSC-CMs using a 24-well multiwell multielectrode array system (Multichannel Systems) as previously described.[19,20] Mexiletine HCl (Tocris no. 2596) was prepared from a 100-mM stock solution in dimethyl sulfoxide (DMSO) and used in cell culture medium at a final concentration of 10 μM. The time intervals considered for the analysis were baseline (before the addition of either DMSO or mexiletine) and 4, 24, and 48 hours after addition of either mexiletine or DMSO. Field potential duration (FPD), RR interval, and peak-to-peak amplitude (PtPAmpl) were calculated on field potentials from spontaneously beating monolayers of hiPSC-CMs.[20] The Bazett formula[21] was used to correct raw FPD values for the RR interval to obtain the beating rate–corrected FPD. Extensive details on hiPSC maintenance, cardiac differentiation protocol, multielectrode array procedures, data collection, and analysis are provided in the Supplemental Material.

LQT2 Rabbit Model

All animal experiments were performed in compliance with EU legislation (directive 2010/63/EU) and the Swiss Animal Welfare Ordinance after approval by the Cantonal Veterinary Office and the Animal Welfare Officer (Kanton Bern; approval number BE132-20).

Cardiomyocyte Isolation

Adult New Zealand transgenic LQT2 (KCNH2-p.G628S) rabbits of both sexes[22] were anesthetized with an intramuscular injection of ketamine S (12.5 mg/kg) and xylazine (3.75 mg/kg). After euthanasia with an intravenous injection of pentobarbital, hearts were excised rapidly, cannulated by the aorta, and mounted on a Langendorff perfusion system, at which time a standard enzymatic collagenase digestion was used to isolate ventricular cardiomyocytes.[23]

Patch Clamp Measurements

Action potential (AP) recordings in isolated left ventricular rabbit cardiomyocytes were performed with the perforated patch method (by using amphotericin 0.44 mM) using an Axopatch 200B amplifier and pClamp 11.1/Clampfit for data acquisition and data analysis. Mexiletine HCl (Tocris no. 2596) was solubilized in water as a stock solution of 100 mM.

In single rabbit cardiomyocytes, APs were measured at 37 °C using a modified Tyrode solution. Mexiletine was added in the external solution to a final concentration of 10 μM. APs were elicited at 1 Hz by 3-ms, ≈1.5× threshold current pulses through the patch pipette. Resting membrane potential, AP amplitude, maximal AP upstroke velocity (dV/dt max), and AP duration at 90% repolarization (APD_{90}) were analyzed. Recordings were started once APs were stabilized and the recordings were kept for 1 minute (baseline) and for ≈2 to 3 minutes for mexiletine bath application and temporal control measurements. Data from 5 consecutive APs measured before (baseline) and after mexiletine application (at minutes 2 to 3) were averaged and potentials were corrected for the calculated liquid junction potential (15 mV). Temporal control experiments solely with Tyrode application were performed to check the effect of temporal changes on the AP measures (temporal control group).

Late I_{Na} was measured at 37 °C as ranolazine-sensitive current (using 100 μM ranolazine to block late I_{Na}). To this end, from a holding potential of −100 mV, a 300-ms depolarizing step to −20 mV was applied in isolated LQT2 and wild-type rabbit cardiomyocytes.

Statistical Analysis (Experimental Part)

For multielectrode array experiments, data were collected from 3 independent differentiations for each line; the sample sizes and *P* values are reported in the figure legends. Comparison of mexiletine with DMSO at all time points was calculated with 2-way ANOVA followed by Šídák test to correct for multiple comparisons.

Baseline and mexiletine or baseline and temporal control measurements were performed in the same cardiomyocyte. Paired Student *t* test (for baseline versus mexiletine/temporal control and for wild-type versus LQT2 late I_{Na} density), unpaired Student *t* test (for ΔAPD shortening), and Wilcoxon signed rank test were used when appropriate. All statistical analyses were performed with GraphPad Prism, and statistical significance was defined as $P<0.05$.

Patients With LQT2

Patients with LQTS with a pathogenic or likely pathogenic *KCNH2* variant evaluated and treated at IRCCS Istituto Auxologico Italiano (Milan, Italy), the Windland Smith Rice Genetic Heart Rhythm Clinic at the Mayo Clinic (Rochester, MN), or Istituto Cardiocentro Ticino (Lugano, Switzerland) were analyzed retrospectively. Patients with LQT2 who received mexiletine for clinical indication (ie, QTc ≥470 ms in basal condition or QTc ≥500 ms during Holter recording), either by acute oral challenge or chronic administration, were included in the study. Twelve patients had been included in our previous publication[16]; we updated their clinical information with follow-up data. The study was approved by the local institutional review boards (IRCCS Istituto Auxologico Italiano, approval 2021-05-18-06;

the Mayo Clinic, approval 16-008436; Istituto Cardiocentro Ticino, approval 2019-00754/CE 3476), and patients gave informed consent.

The acute oral drug test was performed by giving 6 to 8 mg/kg of oral mexiletine[8,24] and recording the ECG before administration and then every 15 minutes in the first hour and every 10 minutes in the second hour. Usually, the peak of QTc shortening was observed between 70 and 90 minutes after oral administration. The shortest QTc obtained was compared with the basal QTc measured just before mexiletine administration. Given the known variability of basal QTc, there could be a difference between the basal QTc used to give the indication to perform the oral mexiletine test and the actual basal QTc measured on the day of the test. To evaluate the response to mexiletine during the acute loading test, the QTc just before mexiletine assumption was considered. The effect of mexiletine in chronic treatment was evaluated by reviewing the ECG and calculating the QTc before and after treatment initiation as well as at last follow-up. We evaluated the basal ECG and, whenever available, the ECG 12-lead Holter recording. The first follow-up ranges available were from 6 months to 1 year after initiation of mexiletine for those performing an oral acute test; for those patients taking mexiletine as chronic therapy, the first ECG was requested after 1 month in IRCCS Istituto Auxologico Italiano and Istituto Cardiocentro Ticino; the time was more variable at the Mayo Clinic.

Akin to the previously used threshold for patients with LQT3, patients were defined as responders in the presence of a QTc shortening of at least 40 ms.[9]

Clinical and demographic characteristics; disease history, presentation, and outcomes; family history; past and current treatments; and genetic data were extracted from the medical records. LQTS-related outcomes were evaluated for all patients before diagnosis, after establishment of standard therapy, and after having added mexiletine. An LQT2-associated breakthrough cardiac event was defined as an LQTS-attributable syncopal event, seizure, sudden cardiac arrest, or appropriate ventricular fibrillation–terminating implantable cardioverter defibrillator (ICD) shock.

Statistical Analysis (Clinical Part)

Statistical analysis was performed using GraphPad Prism (version 10.0.1). Continuous variables were expressed as mean±SD or as median and interquartile range (25th to 75th percentile) and compared using Student *t* test or Mann-Whitney *U* test on the basis of the normality and homoscedasticity assumptions. Categorical data were expressed as number and percentage and compared by Fisher exact tests or with McNemar test for pre–post comparison. Spearman rank correlation analysis was used to evaluate the possible relationship between baseline QTc before mexiletine and change in QTc (ΔQTc). To evaluate the difference in QTc in each patient and the number of arrhythmic events before and after mexiletine, the Wilcoxon matched-pairs test was used. Kaplan-Meier analysis with a log-rank test for comparison was used to compare probability of cardiac event–free survival during the follow-up period with mexiletine. The end point was the time from initiation of chronic mexiletine to any first cardiac event. To measure the effect of mexiletine on event counts over time, the comparison was made by a Poisson regression model and the incidence rate ratio, with its 95% CI, was reported. For each analysis, $P<0.05$ was considered statistically significant.

RESULTS

Mexiletine in HiPSC-Derived Cardiomyocytes

After acute treatment with mexiletine, hiPSC-CMs from both lines showed a significant increase in the normalized RR interval at all the time points considered (KCNH2-p.A561V: 4 hours, +23%; 24 hours, +19%; 48 hours, +17% versus DMSO; KCNH2-p.R366X: 4 hours, +53%; 24 hours, +34%; 48 hours, +57% versus DMSO). Normalized FPD was not altered (Figure 1A.A through 1A.C; Figure 1B.A through 1B.C). In KCNH2-p.A561V hiPSC-CMs, mexiletine significantly shortened the normalized beating rate–corrected FPD at all time points considered (4 hours, −13%; 24 hours, −8%; 48 hours, −6% versus DMSO; Figure 1A.D). In KCNH2-p.R366X hiPSC-CMs, mexiletine shortened the normalized beating rate–corrected FPD from 24 hours onwards (24 hours, −13%; 48 hours, −16% versus DMSO); a trend toward shortening also emerged at 4 hours (4 hours, −8% versus DMSO, not significant; Figure 1B.D).

Treatment with mexiletine progressively decreased the PtPAmpl from both lines over time, becoming statistically significant at 4 and 24 hours (KCNH2-p.A561V hiPSC-CMs: 4 hours, −57%; 24 hours, −49%, respectively, versus DMSO; KCNH2-p.R366X hiPSC-CMs: 4 hours, −50%; 24 hours, −29%; Figure 1A.E and 1B.E).

Mexiletine in LQT2 Rabbit Model

Acute bath application of mexiletine significantly shortened the APD_{90} (1 Hz: baseline 509±35 ms versus mexiletine 397±26 ms, at 2- to 3-minute perfusion; ΔAPD shortening 113 ms; Figure 2A through 2D). None of the other AP measures investigated, such as AP amplitude, resting membrane potential, or maximal AP upstroke velocity (dV/dt max), were altered by acute mexiletine application (Figure 2C).

To exclude a rundown of APD_{90} as a cause of the acute mexiletine-induced APD shortening, we performed the same experiment with a temporal control (Tyrode bath application only, for a similar duration). No significant temporal change was observed in APD_{90} (Figure S1A through S1C). No other AP measures examined changed over time (Figure S1C).

These observations and the significantly larger ΔAPD_{90} shortening with acute mexiletine over time than in the temporal control (Figure 2E) indicate that the APD_{90} shortening during acute mexiletine application is due to the late I_{Na}-blocking effect of the compound itself and not attributable to any rundown-induced APD shortening over time.

We further verified that late I_{Na} is indeed enhanced in LQT2 cardiomyocytes, and could confirm an enhancement of late I_{Na} in LQT2 to 182% of the current density in healthy wild-type cardiomyocytes (Figure S2).

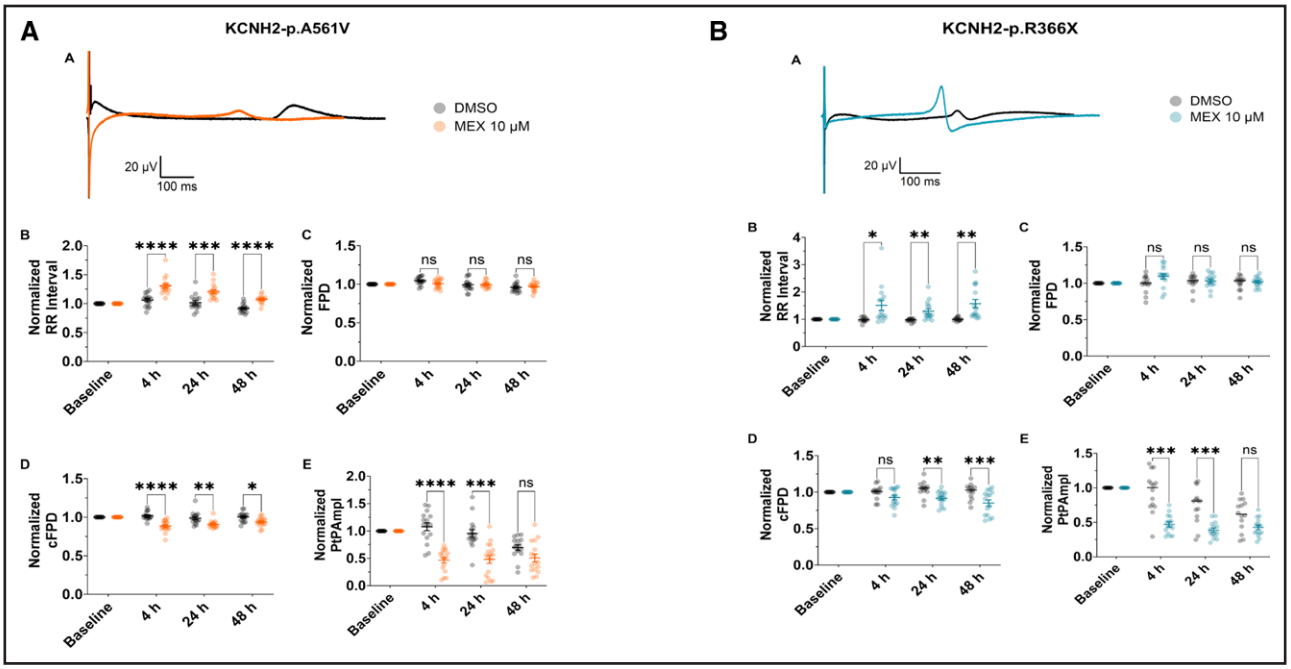

Figure 1. Effect of mexiletine on human induced pluripotent stem cell–derived cardiomyocytes from patients with long QT syndrome type 2.

A, Effect of mexiletine on human induced pluripotent stem cell–derived cardiomyocytes (hiPSC-CMs) with the KCNH2-p.A561V variant. **A.A**, Representative corrected field potential (FP) traces after 4 hours of incubation with either dimethyl sulfoxide (DMSO) or 10 µM mexiletine. Dot plots comparing normalized RR intervals (**A.B**), field potential duration (FPD; **A.C**) beating rate–corrected FPD (cFPD; **A.D**), and peak-to-peak amplitude (PtPAmpl; **E**) after 4, 24, and 48 hours of incubation with either DMSO or 10 µM mexiletine (n=17 at baseline from 3 independent differentiations: **P*<0.05; ***P*<0.01; *** *P*<0.001; *****P*<0.0001). Normalized FPDs were corrected for the beating frequency using the Bazett formula to generate cFPDs. **B**, Effect of mexiletine on hiPSC-CMs with the KCNH2-p.R366X variant. **B.A**, Representative corrected FP traces after 4 hours of incubation with DMSO or 10 µM mexiletine. Dot plot comparing normalized RR interval (**B.B**), FPD (**B.C**), cFPD (**B.D**), and PtPAmpl (**B.E**) after 4, 24, and 48 hours of incubation with either DMSO or 10 µM mexiletine (n≥14 at baseline from 3 independent differentiations: **P*<0.05; ***P*<0.01; ****P*<0.001; *****P*<0.0001). Normalized FPDs were corrected for the beating frequency using the Bazett formula to generate cFPDs.

Mexiletine in Patients With LQT2

Mexiletine was used in the treatment of 96 patients with LQT2 either chronically (n=60) or through the acute oral drug test (n=36). Age at diagnosis was 16±14 years, basal QTc at the same time was 531±53 ms, and 30 patients (31%) were symptomatic before diagnosis, including 4 with previous sudden cardiac arrest. A family history of sudden cardiac death was present in 37 patients (39%) and for LQTS in 66 (69%); 59 (61%) were probands, and 53 (55%) were female (Table 1).

Indicative of a relatively higher-risk cohort of patients with LQT2, after diagnosis and before mexiletine, 94 patients (98%) were prescribed beta-blocker therapy, 30 (31%) underwent left cardiac sympathetic denervation (LCSD), and 28 (29%) received an ICD. Also, 24 (25%) had a cardiac event: 19 after initiation of antiadrenergic therapy (19 with beta-blockers, and 4 of them also with LCSD); 11 (46%) had only syncopal events, whereas 13 (54%) had a sudden cardiac arrest or an appropriate ICD shock.

The decision to add mexiletine was clinical and was made on the basis of QTc duration: QTc ≥470 ms in basal condition (n=83) or QTc <470 ms but exceeding 500 ms on a 12-lead 24-hour ECG Holter recording (n=13; Figure 3). Before starting mexiletine, the longest QTc at the Holter recording was 540±26 ms.

Mexiletine and QTc

In 36 patients, the acute oral drug test was performed with a loading dose of 6 to 8 mg/kg. Mean QTc at baseline was 518±53 ms and decreased to 446±37 after the acute mexiletine oral administration. The QTc shortening was significant (*P*<0.001) and 27 patients (75%) had a QTc shortening ≥40 ms (ie, were responders to therapy; Figure 4). As shown in Figure 3, 22 of 28 (79%) were responders in the group with a baseline QTc ≥470 ms and 5 of 8 (63%) among those with QTc <470 ms; however, the QTc reported in Figure 3 refers to the one identified during the visit in which the indication to perform the acute oral test was given, whereas the basal QTc considered to evaluate the response to acute mexiletine oral administration was the basal one measured on the day of the test, just before mexiletine administration, to use internal control analysis.

The mean basal QTc of the responders was 537±47 ms, and that of nonresponders was 463±19 ms on the day of the test (*P*<0.001). Mean basal heart rate was not

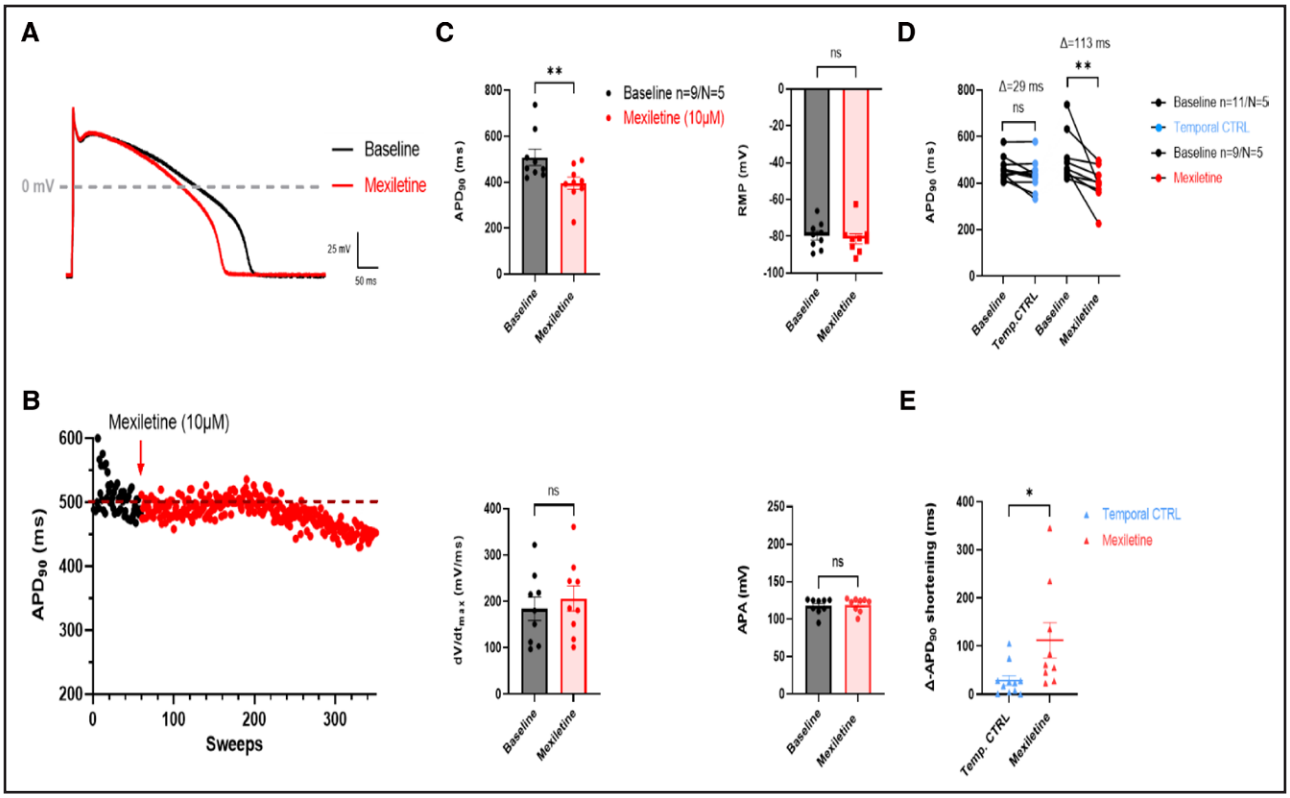

Figure 2. Effects of mexiletine on action potential characteristics in isolated ventricular long QT syndrome type 2 rabbit cardiomyocytes in perforated-patch configuration at 37 °C.

A, Representative action potential (AP) traces triggered at 1 Hz in long QT syndrome type 2 (LQT2) isolated rabbit cardiomyocytes at baseline (black line) and after mexiletine bath application (10 µmol/L; red line). **B**, Representative action potential duration at 90% repolarization (APD_{90}) trend over time in all individual APs recorded before (baseline; black) and after mexiletine application (red) from 1 cardiomyocyte. **C**, Average values for APD_{90}, resting membrane potential (RMP), dV/dt max, and AP amplitude (APA) at baseline and after mexiletine application (10 µmol/L) in LQT2 rabbits (n=5 rabbits; n=9 cells). Results are expressed as mean±SEM and every dot represents an individual value derived from 1 cardiomyocyte before (baseline) and after mexiletine application (n). **D**, APD_{90} comparison between the temporal control (CTRL) group and mexiletine group. **E**, ΔAPD_{90} shortening between the temporal CTRL group and mexiletine group. *P<0.05; **P<0.005. Paired Student and Wilcoxon signed rank tests were performed.

different between responders and nonresponders (48±5 versus 52±6 bpm; P=0.1). There was a significant correlation between basal QTc and the ΔQTc obtained during the mexiletine test (r=–0.8; P<0.001; Figure 5). Thus, longer the baseline QTc, the greater the probability of being responsive to mexiletine. Among patients with a baseline QTc ≥500 ms (n=22), 21 (95%) were responders and the mean QTc shortening was 104±51 ms, with 14 (64%) patients having a QTc shortening ≥100 ms (Figure 5).

In 60 patients, chronic therapy with mexiletine, at a dose of 9±4 mg/kg, was initiated without the acute oral drug test. Mean QTc at baseline was 528±50 ms and decreased to 473±35 ms on mexiletine (P<0.001). A QTc shortening by at least 40 ms was observed in 39 of the 60 patients (65%). Responders had a significantly longer QTc before mexiletine compared with nonresponders (543±54 versus 500±25 ms; P<0.001), with a significant correlation between basal QTc and ΔQTc (r=–0.8; P<0.001).

Of the 13 patients who had a basal QTc <470 ms but a QTc ≥500 ms during Holter recording, 8 were tested through an acute oral test, whereas 5 were directly prescribed chronic therapy (Figure 3). Among these, we have Holter ECG recordings before and after mexiletine in 11 patients prescribed chronic therapy: 7 responders and 4 nonresponders. In the 7 responders, maximum QTc during Holter recordings was 533±26 ms before and 487±29 ms after mexiletine (P<0.001).

Acute Oral Drug Test to Predict Long-Term Efficacy

Mexiletine was prescribed to 29 patients who had undergone the acute oral drug test, with follow-up data available in 28: 22 were responders and 6 were nonresponders (Figure S3). Most of the 22 (95%) remained responders during chronic administration, and 5 of the 6 nonresponders (83%) also did not respond during chronic administration. This indicates a correct prediction of the long-term effect in 93% of patients, and proves that the acute oral drug test is accurate in rapidly assessing the likelihood that the patient will respond to chronic mexiletine.

Table 1. Clinical Characteristics of the Study Population

Characteristics	Total LQT2 study population	Chronic treatment with mexiletine without acute test	Acute oral drug test
Patients	96	60	36
Female	53 (55)	40 (67)	13 (36)
Probands	59 (61)	30 (50)	29 (81)
Age at diagnosis, y	16±14	12±14	23±13
Symptoms before diagnosis	30 (31)	18 (30)	12 (33)
Syncope/TdP/VT	26 (27)	16 (27)	10 (28)
SCA/ICD shock/VF	4 (4)	2 (3)	2 (6)
Family history of LQTS	66 (69)	40 (67)	26 (72)
Family history of SCD	37 (39)	21 (35)	16 (44)
QTc at diagnosis, ms	531±53	535±54	523±52
Beta-blockers	94 (98)	58 (97)	36 (100)
LCSD	30 (31)	19 (32)	11 (31)
ICD	28 (29)	22 (37)	6 (17)

Values are n (%) or mean±SD. ICD indicates implantable cardioverter defibrillator; LCSD, left cardiac sympathetic denervation; LQT2, long QT syndrome type 2; LQTS, long QT syndrome; SCA, sudden cardiac arrest; SCD, sudden cardiac death; TdP, torsades de pointes; VF, ventricular fibrillation; and VT, ventricular tachycardia.

Outcome on Chronic Mexiletine Therapy

Among the 96 patients, 88 were followed on chronic mexiletine therapy at a dose of 8±4 mg/kg (28 after oral test and 60 started directly on chronic therapy). Overall, 61 (69%) were responders, with no significant difference in the dose of mexiletine assumed compared with nonresponders (8.3±4 versus 7.6±3 mg/kg; P=0.5).

During a median postdiagnosis follow-up of 54 months (interquartile range, 15–120), 23 patients (26%) had at least 1 cardiac event before taking mexiletine; 10 of these (43%) had only syncopal events, whereas 13 (57%) had a sudden cardiac arrest or an appropriate ICD shock. The total number of events before mexiletine was 65.

After taking mexiletine, during a median follow-up of 22 months (interquartile range, 11–48), 8 patients had a cardiac event, but 2 were not compliant; among the 6 taking mexiletine regularly, 2 had a syncope and 4 appropriate ICD shocks. Thus, mexiletine significantly reduced the number of symptomatic patients (P<0.001). The total number of LQT2-associated breakthrough cardiac events among patients regularly taking mexiletine was 7, indicating that the number of cardiac events had been significantly reduced (P<0.001). The 6 patients with cardiac events despite mexiletine (3 responders and 3 nonresponders) had a very severe form of the disease, their QTc at diagnosis was 564±77 ms, 4 of these 6 patients experienced symptoms after diagnosis and before prescription of mexiletine despite beta-blockers (all 4) and LCSD (in 2), and 5 had an ICD. On mexiletine, their QTc was 507±68 ms. In these patients, the mexiletine dose was 11±6 mg/kg compared with 8±3 mg/kg in those asymptomatic on mexiletine (not significant; P=0.1). Among the patients asymptomatic on mexiletine, 19 experienced cardiac events after diagnosis and before starting mexiletine, 14 of these despite taking beta-blockers, and their QTc on mexiletine was 469±26 ms, thus significantly lower compared with the QTc of the patients who were symptomatic despite mexiletine therapy (P=0.003). Survival curves clearly showed that having a QTc ≥500 ms on mexiletine significantly increased the risk of experiencing recurrences on therapy (P=0.006; Figure 6).

If we consider only the 78 patients on optimal antiadrenergic therapy before mexiletine, 63 (81%) were taking only beta-blockers, whereas 15 (19%) also underwent LCSD. In this subgroup, the mean yearly event rate was lower in the postmexiletine period (0.04 [95% CI, 0.02–0.08]) compared with the premexiletine period (0.10 [95% CI, 0.07–0.14]), with an incidence rate ratio of 0.40 (95% CI, 0.16–0.84), which would correspond to a 60% reduction in event rate (P=0.01; Table 2).

Side Effects of Mexiletine

In some patients, mexiletine may cause minor side effects. The oral drug test was generally well tolerated, with 7 patients (22%) reporting minor symptoms such as heartburn, nausea, vertigo, and epigastric pain. During chronic assumption of mexiletine, only 8 patients (9%) reported heartburn or nausea. In 4 cases, symptoms resolved simply by favoring assumption after meals and having the higher dose in the evening; in 4 cases, therapy was suspended.

DISCUSSION

The current data provide solid evidence that mexiletine significantly shortens cardiac repolarization in hiPSC-CMs from patients with LQT2, in the LQT2 rabbit model, and in the majority of patients with LQT2. Furthermore, mexiletine prevented arrhythmia recurrences in most patients.

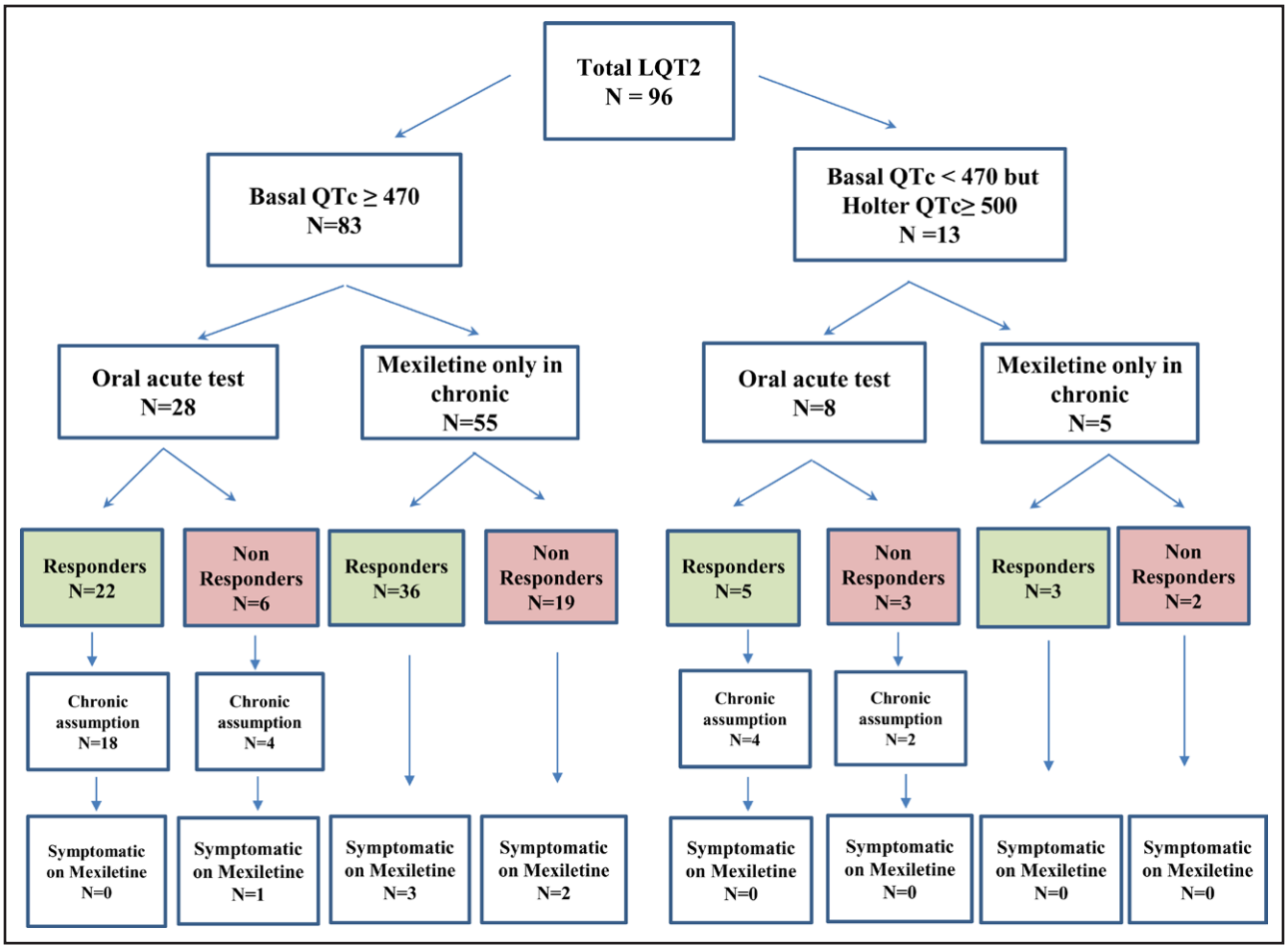

Figure 3. Flowchart showing criteria of enrollment of patients with long QT syndrome type 2.
Among the 96 patients with long QT syndrome type 2 (LQT2) analyzed in the study, 83 had a basal QTc ≥470 ms and 13 had a basal QTc <470 ms but QTc values during Holter recordings ≥500 ms. The QTc shown in this figure refers to the QTc observed at the last visit before prescription of mexiletine in either the chronic or acute oral test. The bottom part of the figure shows how many patients were responders (green) or nonresponders (pink) to mexiletine given either the chronic or acute oral test. Patients were defined as responders if mexiletine caused a QTc shortening of at least 40 ms. In this figure, patients are divided according to the QTc observed at the last visit before prescription of mexiletine, but for those patients performing the acute oral mexiletine test, the QTc reduction was evaluated considering the basal QTc present on the day of the test, which could be different from the basal QTc present at the last visit. In the bottom part of the figure, numbers of patients with cardiac events on chronic mexiletine therapy are indicated in the different subgroups.

Response to mexiletine is largely dependent on the basal QTc, with the major benefit obtained in those with a basal QTc >500 ms. Furthermore, the QTc on mexiletine is the major determinant of its therapeutic efficacy; indeed, patients with a QTc on mexiletine <500 ms are those at lowest risk for cardiac events. These findings should affect the management of patients with LQT2 assessed as being at higher risk.

Effect of Mexiletine in Experimental LQT2 Models

Several experimental studies conducted in animal models, tissues, or hiPSC-CMs suggest that late I_{Na} blockade might be a potential repolarization-normalizing and antiarrhythmic target in LQT2. Indeed, Na^+-channel blockers such as mexiletine can shorten APD and reduce proarrhythmic APD dispersion in drug-induced LQT2 canine wedge preparations,[25,26] and the Na^+-channel blocker ranolazine could shorten APD and prevent proarrhythmic early afterdepolarizations in an LQT2 hiPSC-CM model.[27] We provided evidence that the relatively specific late I_{Na} inhibitor GS967 could suppress polymorphic ventricular tachycardia formation in transgenic LQT2 rabbit hearts by reducing Ca^{2+}-mediated early afterdepolarizations.[28] These data suggest that late I_{Na} can be enhanced in the context of prolonged cardiac APs due to a delayed inactivation of I_{Na} also in the K^+ channelopathy LQT2. As further support for this hypothesis, it has been demonstrated in the context of drug-induced LQTS that I_{Kr} blockade and resulting APD prolongation may enhance late I_{Na} by PI3K (phosphatidyl inositol 3 kinase)–dependent mechanisms.[29,30] A recent review article postulated that mexiletine might

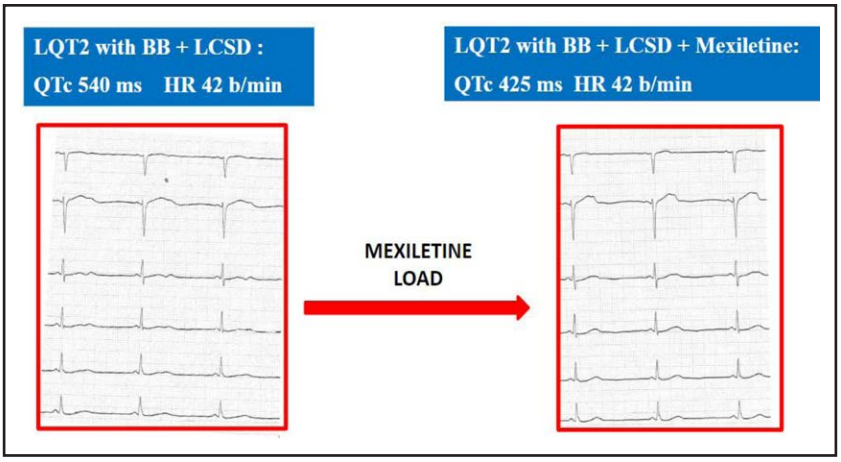

Figure 4. ECGs showing a long QT syndrome type 2 responder at the acute oral mexiletine test.
The ECG on the left is the basal ECG performed just before assumption of oral mexiletine load in a patient with long QT syndrome type 2 (LQT2) already on full dose beta-blockers (BB) and who performed left cardiac sympathetic denervation (LCSD). The QTc at a heart rate (HR) of 42 beats per minute (b/min) is 540 ms and notched/diphasic T waves are evident in all leads presented. The ECG on the right shows the effect of mexiletine obtained after the oral administration of 8 mg/kg of mexiletine. The QTc after 80 minutes from oral assumption normalized (QTc 425 ms at the same heart rate of 42 bpm) and the T-wave morphology improved substantially.

shorten QT/APD irrespective of the cause of the QT/APD prolongation.[31]

In line with these observations, we have previously shown in transgenic LQT2 rabbit models and several LQT2 hiPSC lines with different pathogenic *KCNH2* variants that the modulation of other pathways involved in late I_{Na} enhancement, such as inhibition of SGK1 (serum- and glucocorticoid-regulated kinase 1), can similarly shorten APD in LQT2 by 20% to 30% irrespective of the disease-causing variant.[18,32] To further enhance this compelling evidence of a potential therapeutic role of late I_{Na} blockade in LQT2, irrespective of the underlying sequence variation, it has been proved in transgenic LQT2 rabbits that late I_{Na} is indeed detectable, albeit small, in LQT2 cardiomyocytes,[28] which we could confirm by demonstrating an enhancement of late I_{Na} in LQT2 to 182% of the current density in healthy wild-type cardiomyocytes. Clinical data and in vitro studies in other LQTS subtypes suggest that the APD prolongation per se may be the strongest contributor to late I_{Na} enhancement, as suggested by the effect of mexiletine in one patient with Timothy syndrome[33] and in some patients with calmodulin-related LQTS.[34,35]

Here, we demonstrated in both a transgenic rabbit model of LQT2 and in different LQT2 hiPSC-CM models that the beneficial effect of mexiletine can already be appreciated acutely due to its direct interaction with the late I_{Na} current, in line with what we observed in patients. This is consistent with previous studies in wedge preparations, demonstrating that mexiletine reduced dispersion of repolarization by shortening APD more prominently in cardiomyocytes with longer APD.[25,26] By using patient-specific hiPSC-CMs from a patient with LQT2, who also performed the acute oral mexiletine test, we could show

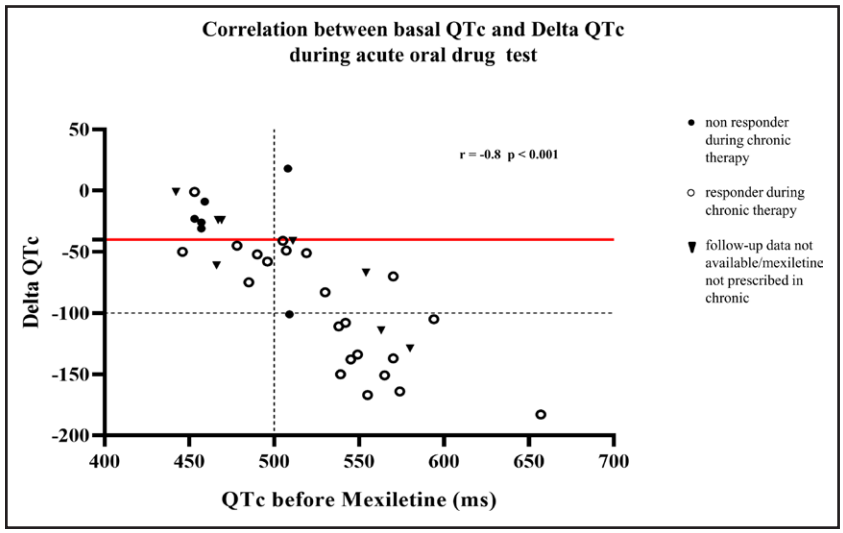

Figure 5. Correlation between basal QTc and ΔQTc obtained during acute oral mexiletine test.
Indicated on the *x* axis is the QTc measured on the day of the oral acute mexiletine test, just before oral administration of the drug. Indicated on the *y* axis is the maximum ΔQTc shortening observed in the 2 hours after mexiletine administration. The red line shows the QTc shortening of 40 ms that is considered the cut-off value to consider a patient a responder to therapy. Patients are indicated with a black dot if nonresponsive to chronic treatment, with a white dot if responsive to chronic treatment, or with a black triangle if mexiletine was not prescribed as chronic treatment or follow-up data are unavailable. R indicates Spearman rank correlation analysis coefficient.

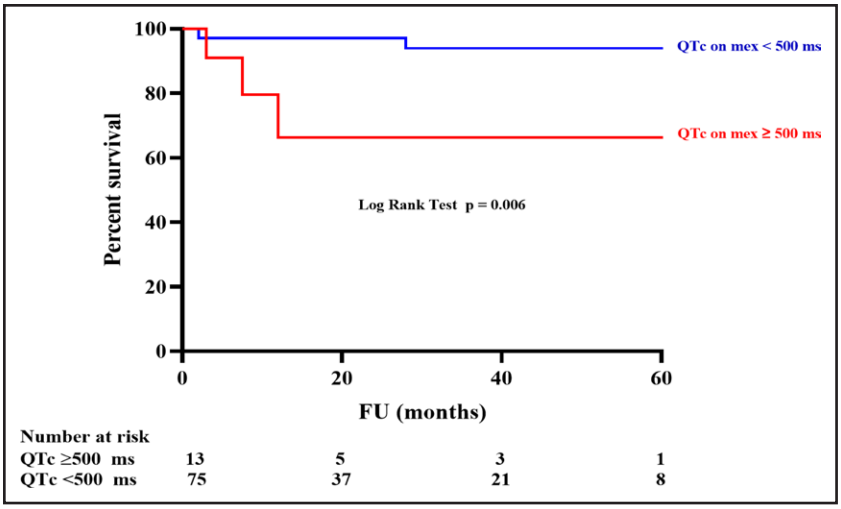

Figure 6. Kaplan-Meier curves.
Survival curves comparing cardiac events on mexiletine in patients with a QTc on mexiletine ≥500 ms and <500 ms. Having a QTc ≥500 ms is associated with a higher risk of long QT syndrome type 2–associated recurrences (*P*=0.006). FU indicates follow-up time in months.

a similar response in vitro and in vivo, further supporting the predictive role of in vitro data in patient-specific hiPSC-CMs for preclinical drug screening.

Therapeutic Efficacy of Mexiletine in Patients With LQT2

The groundbreaking 1995 proposal to implement the first gene-specific therapy by administering mexiletine to patients with LQT3[7] is enriched by our evidence that this old sodium channel blocker can be useful also for the majority of patients with LQT2.

During the past 30 years, we witnessed enormous advancement in understanding of LQTS, its genetic basis, and the effect of genetics in risk stratification and therapeutic management.[36] However, particularly in the genetic subtypes 2 and 3, some patients are not fully protected by available therapies.[4,14,15,37] For patients with LQT2 stemming from a pathogenic loss-of-function variant in *KCNH2*, the risk of major arrhythmic events is greater in female patients after puberty and whenever QTc is >500 ms,[38] and the overall protective effect of beta-blockers is lower than for patients with LQTS type 1.[4,14,15] LCSD, despite adding further protection, is not always sufficient in LQT2,[37] and patients with a QTc persisting >500 ms despite antiadrenergic interventions remain at higher risk for arrhythmias.[39,40]

Mexiletine is a sodium channel blocker with a proven efficacy in patients with LQT3 with pathogenic gain-of-function variants in the sodium channel gene *SCN5A* in both reducing the QTc[7] and in preventing arrhythmic risk,[13] and, indeed, is the only gene-specific therapy with a class I indication in European guidelines for the prevention of sudden cardiac death.[10]

In 2019, we described 8 patients with LQT2 who showed a significant QT shortening on mexiletine,[16] but such an anecdotal observation required a larger study to evaluate whether mexiletine could indeed become a treatment consideration for patients with LQT2 with evidence that would merit a future guideline indication. We therefore evaluated 96 patients with a pathogenic variant in *KCNH2* and an overall higher risk phenotype (mean QTc 531 ms, 31% symptomatic for cardiac events, 39% with family history of sudden cardiac death), and showed that mexiletine is effective in reducing the QTc by at least 40 ms, the previously designated cut-off to indicate clinical efficacy,[9] in most of the patients. The response to mexiletine was influenced by the basal QTc, supporting the concept that APD itself may enhance late I_{Na}.[25,26] Indeed, a significant correlation between ΔQTc and basal QTc was observed both in patients performing an oral mexiletine test and in patients taking chronic mexiletine treatment directly. A total of 95% of the patients with a basal QTc ≥500 ms undergoing an oral mexiletine test were responders, with a mean QTc reduction of 104 ms. These results may suggest that the acute oral test could be skipped in patients with very prolonged QTc in basal condition and chronic administration could be started immediately.

Another clinically relevant observation from the current study is that the oral mexiletine test correctly predicts the response to chronic administration. Indeed, most of the responders (95%) remain responders with chronic administration of the drug, and only 1 of 6 nonresponders to the acute test had a sustained

Table 2. Effect of Mexiletine on Morbidity and Event Rate

Mexiletine timing	No.	Symptomatic, n (%)	Person-years	CEs, n	Mean yearly event rate (95% CI)	IRR (95% CI)	*P* value
Before mexiletine	78	15 (19)	403	41	0.10 (0.07–0.14)	1	0.01
On mexiletine	78	6 (8)	172	7	0.04 (0.02–0.08)	0.40 (0.16–0.84)	

CE indicates cardiac event; and IRR, incidence rate ratio.

attenuation of their QTc during chronic administration. These few patients who took mexiletine chronically despite being nonresponders during the acute mexiletine test were patients who did not have a very prolonged basal QTc but had considerable nocturnal QT prolongation, which led us to assess in chronic therapy whether mexiletine could prevent such QT prolongation. In the only patient who was defined a responder only during chronic administration, we observed that the positive effect of mexiletine was mainly evident at nighttime.

Because the degree of QT prolongation is an important contributor to the arrhythmic risk in LQTS,[5] it would be logical to expect that mexiletine-mediated QTc attenuation could also reduce the arrhythmic risk, as previously reported for LQT3.[13] Furthermore, as additional antiarrhythmic mechanisms, mexiletine also decreases dispersion of ventricular repolarization and reverses use-dependent QT prolongation.[31,41] In a recently published case reporting a 28-year-old woman with LQT2 with recurrent torsades de pointes and ventricular fibrillation despite beta-blocker therapy, mexiletine not only considerably shortened her QTc, but also completely suppressed arrhythmias.[42] Also, in a cohort of 12 patients with torsades de pointes caused by acquired LQTS, mexiletine not only significantly shortened the QTc, but also completely suppressed arrhythmic episodes after failure of conventional treatments.[43] Our data are promising in this regard, showing a significant reduction with mexiletine of the number of symptomatic patients and of the number of LQT2-triggered recurrences. Indeed, out of 23 patients symptomatic after diagnosis, only 6 had recurrence of symptoms on mexiletine, with 3 of these being nonresponders. Furthermore, even correcting for time of observation, the mean yearly event rate was significantly reduced from 0.10 to 0.04. The risk of cardiac events despite mexiletine treatment can be predicted; as seen for LCSD,[39,40] greater protection is observed in patients with a QTc <500 ms on therapy. Therefore, when managing higher-risk patients with LQT2, there should be careful consideration not only for the degree of QTc shortening produced by mexiletine, but also the actual final value of QTc.

Conclusions

Mexiletine shortens cardiac repolarization in LQT2 hiPSC-CMs, in the LQT2 rabbit model, and in the majority of patients with LQT2, particularly those with a pretreatment QTc >500 ms. Furthermore, in these patients, mexiletine has substantial antiarrhythmic efficacy. For higher-risk patients with LQT2, mexiletine should be added to conventional therapies.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Methods

Figures S1–S3

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