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ORIGINAL PAPER

Concentration–response modeling of ECG data from early-phase clinical studies to assess QT prolongation risk of contezolid (MRX-I), an oxazolidinone antibacterial agent

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Abstract

The effects of contezolid (MRX-I, an oxazolidinone antibacterial agent) on cardiac repolarization were evaluated retrospectively using a population modeling approach in a Phase I study incorporating single ascending dose, multiple ascending dose, and food effect assessments. Linear mixed effect models were used to assess the relationships between MRX-I plasma concentrations and QT/QTc/ΔQTc (baseline-adjusted), in which different correction methods for heart rate have been included. The upper bound of the one-sided 95% confidence interval (CI) for predicted ΔΔQTc was < 10 ms (ms) at therapeutic doses of MRX-I. Model performance/suitability was determined using diagnostic evaluations, which indicated rationality of one-stage concentration-QT model, as well as C-QT model suggested by Garnett et al. The finding demonstrated that MRX-I may have no clinical effects on the QT interval. Concentration-QT model may be an alternative to conventional thorough QT studies.

Keywords MRX-I · QT interval · Population pharmacodynamics · C-QT model

Introduction

Contezolid (MRX-I), a new *ortho-fluoro* dihydropyridone oxazolidinone antibacterial agent, exhibits potent activity against resistant Gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), vancomycin-resistant *Enterococcus* (VRE), *Moraxella catarrhalis*, and certain anaerobic bacteria [1]. In preclinical studies, MRX-I displayed high antimicrobial activity in multiple animal models, generally similar to that for linezolid, coupled with markedly reduced potential for myelosuppression and monoamine oxidase inhibition (MAOI) [2]. Recently, a research reported that the in vitro activity of MRX-I was similar to that of linezolid against *Mycobacterium tuberculosis* [3].

Extensive in vitro and nonclinical testing of MRX-I support the supposition that MRX-I has a low risk of QT prolongation in humans. In the human ether-go-go-related gene (hERG) in vitro assay, MRX-I inhibited current with an IC_{50} of 160 μM, similar to the effect of linezolid (unpublished data). Moreover, a thorough QT study assessing the effect of linezolid on QTc interval suggested an absence of clinically significant QTc prolongation with 600 and 1200 mg of linezolid [4].

The effect of MRX-I in humans was first studied in a Phase I, single-center, three-periods, randomized study to assess the safety, tolerability, and pharmacokinetics (PK) of ascending single and multiple doses of MRX-I administrated orally to healthy adult subjects in China, including

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single ascending doses (dose range 200–1800 mg), food effect evaluation (dose range 300–900 mg), and a 15-day multiple dose tolerability assessment (600 mg twice daily (q12h) and 800 mg twice daily (q12h)) [1]. MRX-I was absorbed quickly and Tmax was about 2 h, and food had a significant effect on the absorption of MRX-I at higher doses. C_{max} and $AUC_{0-\infty}$ was more 2-fold higher those in subjects receiving high-fat diet than those under fasted condition. In MAD study, no drug accumulation was observed in either 600 mg or 800 mg cohort after 15 days of continuous twice daily administration of MRX-I [1]. Meanwhile, intensive assessment of electrocardiogram (ECG) monitoring was conducted along with evaluation of PK for MRX-I.

Non-antiarrhythmic drugs, including antibacterial agents, may delay cardiac repolarization, which manifests as prolongation of the QT interval. A delay in cardiac repolarization may result in cardiac arrhythmias, most severely torsade de points (TdP), leading to ventricular fibrillation and sudden death. In 2005, the International Conference on Harmonization (ICH) E14 guidance was implemented, requiring a thorough QT (TQT) study to be conducted during the development program in order to assess a new chemical entity's potential to prolong the QT interval [5]. Over last few years, many new drugs have been evaluated for their potential to prolong the QT interval in the drug development [6], and the statistical analysis has evolved. Due to high cost and low efficiency of TQT study, consideration has been given to replace thorough QT studies with data gathered from early phase clinical studies. Therefore, E14 guidance has been revised to strengthen the role of concentration–response modeling in QT assessment [7], which has been used to evaluate the risk of drugs in both general and special populations [8].

The purpose of this study is to explore the effect of MRX-I on QT intervals using linear mixed model with the data from the Phase I clinical trial. Three different population pharmacokinetic/pharmacodynamic (PK/PD) models were employed to describe the concentration–QT/QTc relationship of MRX-I. Therefore, a through QT (TQT) study has being conducted which could be a validity of the results of the C-QT models from Phase I study.

Methods

Study design

The Phase I study of MRX-I, which was conducted in Huashan Hospital, Fudan University, incorporated single ascending dose period, food effect period, and multiple ascending dose period, which have been described in detail elsewhere [1]. The study was approved by the Ethics Committee of Huashan Hospital, Fudan University and all participants provided written informed consent before participating in this study. The single ascending dose (SAD) period of the study was randomized, double-blinded, and placebo-controlled, consisting of 6 cohorts with 8 subjects per cohort (6 active, 2 placebo). Subjects were randomized to single oral doses of 50, 100, 200, 400, 800, 1200, 1600, and 1800 mg MRX-I or placebo following fasting for at least 10 h, and subjects were given the meal 4 h after the administration. Food effect period include 2 arms. Arm 1 was randomized, open-label, 3-period with three doses in a Latin square (3×3) design, and included 12 subjects. Each subject was administrated 300, 600, and 900 mg MRX-I under fasting conditions at each period, respectively. The washout was 7 days between every period. Another 12 subjects in arm 2 which is a randomized, single-dose, two-crossover study received 900 mg MRX-I under fasting condition and were fed a high-fat diet, with a washout of 7 days between the two dosing. Multiple ascending dose (MAD) period of the study was randomized, double-blinded, and placebo-controlled, incorporating 2 arms with 16 subjects per arm (12 active, 4 placebo) with the 1st and 15th day with dosing once while dosing twice daily in days 2–14 with 600 and 800 mg of MRX-I. In the two arms of the MAD period, a standard regular breakfast was given to the subjects after treatment was administered. All the groups were balanced by gender.

SAD/MAD study data

In the SAD period of the study (except for 50, 100, 1600 and 1800 mg groups), plasma PK samples were collected at 30 min before dosing, and at 0.5, 1, 2, 4, 6, 8, 12, 24, and 48 h post-dose, and digital ECGs were recorded at pre-dose (baseline), and at 1, 2, 6, 12, 24, and 48 h post-dose. In the effect of food part of the study, plasma PK samples were collected at 30 min before dosing, and at 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h post-dose, and digital ECGs were recorded at pre-dose (baseline), and at 1, 2, 6, 12, 24, and 48 h post-dose. In the MAD period of study, plasma PK samples were collected at 30 min before dosing, and at 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 16 h post-dose on Day 1 and at 30 min before dosing, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, and 48 h post-dose time-points on Day 15. On Days 2, 3, 4, 8, 12, 13, and 14, PK samples were collected at 30 min pre-dose, and at 1.5 h post-dose. ECGs were recorded at 30 min pre-dose (baseline), and at 1, 2, 4, 6, and 12 h post-dose on Days 1 and 15; ECGs also were collected at 30 min pre-dose, and at 1, 2, and 6 h post-dose for first dosing and 30 min pre-dose, and at 1 and 2 h post-dose of the second dosing on Day 2 to Day 14. On Day 15, ECGs were collected at 24, 36, and 48 h post-dose.

Subjects were to rest quietly in the supine position for a minimum of 5 min before each ECG acquisition. ECG at every timepoint was collected once with the electrodes placed in the same position. The measurement of ECG intervals was conducted according to semi-automated approaches by a trained cardiologist. To avoid the conflict between PK sample and ECG collections, ECGs collections were always 15 min before PK sampling when scheduled at the same timepoints. Plasma concentrations of MRX-I were determined by a validated LC/MS/MS assay [1].

Statistical analysis

Pharmacokinetic model

A two-compartment model with first-order elimination and absorption input from the central compartment with a lag time was employed using WinNonlin (version 6.3, Pharsight Corporation, Mountain View, CA, USA). Weighting factor of 1/C were investigated for the model fitting. There were 1922 concentrations from 72 subjects included in the model. 149 (7.8%) concentrations of the 1922 concentrations were below the limit of quantification (BLQ, 0.0025 mg/L), and all the BLQ values occurred at the last timepoint. BLQ data were discarded in the model. No outlying data were found. Note that, as PK samples of 50, 100, 1600 and 1800 mg groups in SAD period has not been collected, so these groups were not included in the PK model.

Heart rate-corrected QT and changes in adjusted QTc

Changes in heart rate have significant influence on drug's effect on repolarization (i.e., QT interval) and correction methods with different characteristics are often applied [7]. In this study, three different heart rate correction methods were employed to develop concentration-QT modeling [9]. The most common correction formulae QTcB and QTcF were calculated as follows:

$$\text{Bazett correction}, QTcB = QT/RR^{1/2}$$

$$\text{Fridericia correction}, QTcF = QT/RR^{1/3}$$

$$\text{Study-specific correction}, QTcP = QT/RR^{\beta}$$

The calculations of study-specific correction (QTcP) was illustrated clearly by Xu et al. [9]. Study-specific correction, $QTcP = QT/RR^{\beta}$, where β is the slope for the regression of logarithm (log) of QT on log(RR) for an individual subject with drug-free data in the study population, which indicates that all the subjects have the same power term for the QT correction.

One-stage concentration-QT interval model

The one-stage concentration-QT interval model, as described by Xu et al. [9], which indicated the raw QT interval, was directly estimated using the predicted MRX-I concentration and RR. The relationship between MRX-I concentration and QT interval was evaluated by a linear mixed effects model. The model was described as follows:

$$QT = (\text{Intercept} + \text{Slope} \times \text{Conc} + \varepsilon) \times (RR/1000)^{\beta} \quad (1)$$

In the equation, Intercept and Slope were regarded as the intercept and slope of the linear mixed effects model, respectively. Conc represented predicted concentration of MRX-I from population pharmacokinetic (PPK) model. RR represented RR interval in the ECG. The correction factor β was used to adjust the relationship between QT and RR interval. Lastly, ε was the residual error, is assumed to have normal distribution.

Two-stage concentration-QTc interval model

Two-stage concentration-QT interval model, as described by Xu et al. [9], which showed that the dependent variable of C-QT model, QTc and ΔQTc, was calculated by different heart rate correction methods. The models were displayed using Eqs. 2 and 3, and the meaning of Intercept, Slope, Conc, and ε were similar to one-stage concentration-QT interval model.

$$QTc = (\text{Intercept} + \text{Slope} \times \text{Conc} + \varepsilon) \quad (2)$$

$$\Delta QTc = (\text{Intercept} + \text{Slope} \times \text{Conc} + \varepsilon) \quad (3)$$

Another linear mixed effect C-QTc model, suggested by Garnett et al. [10], incorporates ΔQTc as dependent variable, intercept, slope, influence of baseline on intercept, treatment (active = 1 or placebo = 0) and nominal time from first dose as fixed effect parameters, along with the subject is included as an additive random effect on both intercept and slope terms. The linear mixed effect formulae have been described previously in detail [10]. In brief, Eq. 4 summarized the model.

$$QTc = \left(\theta_0 + \eta_{0,i}\right) + \theta_1 TRT_j + \left(\theta_2 + \eta_{2,i}\right) C_{ijk} + \theta_3 TIME_j + \theta_4 \left(QTc_{i,j=0} - \overline{QTc_0}\right) \quad (4)$$

Model development

Inter-individual variability (IIV) of one-stage and two-stage models were evaluated using exponential error model, and IIV was assumed to be independent and followed normal distribution with mean 0 and variance ω^2. Additive error model was used to describe the variability

between the observation and individual prediction of QT/QTc interval. The residual error was assumed to be normally distributed with 0 mean and variance σ^2.

Model verification was performed by examination of the goodness-of-fit plots [11], and model evaluation was based on objective function value (OFV), bootstrap methods and visual predictive check (VPC). In the bootstrap procedure, 1000 resampled bootstrap datasets were generated. Parameter estimation based on bootstrap procedure was performed. The median and 95% prediction interval for each final parameter estimate were calculated. The VPC produced 1000 Monte-Carlo-simulated data for each observed QT interval by using the population mean values and a covariance matrix based on the one-stage final model. Then raw data points were plotted, along with the median and 95% prediction interval based on the simulations from the C-QT model. As female may have longer QT interval than male, sex was used to evaluate the influence of covariates on C-QT model parameters. An α value of 0.05 was used for the determination of the statistical significance of covariate effects in the model.

All modeling work were conducted by NONMEM 7.3 (ICON company, USA), and all runs were performed using the first-order conditional estimation (FOCE) with interaction method. Figures were generated using R 3.5.0 and GraphPad Prism 7. The magnitude of change (mean and 95% confidence interval) in QTc for the observed geometric mean of C_{max} (maximum observed plasma concentration) in 900 mg high-fat food cohort was calculated.

Results

Pharmacokinetic model

A two-compartment model with first-order elimination and absorption with lag time described time profiles of MRX-I.

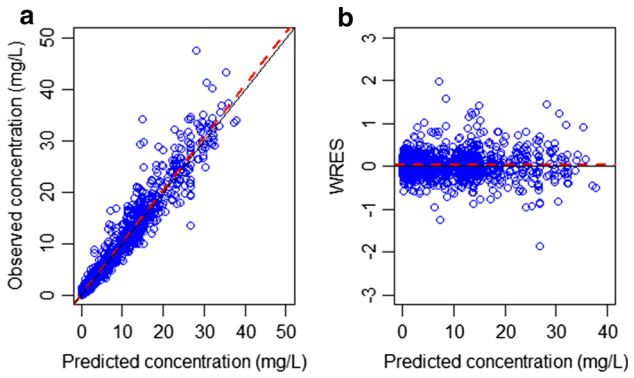

Fig. 1 Diagnostic plots of pharmacokinetic model. **a** Predicted concentration versus observed concentration. **b** Weighted residuals versus time. The red line represents the regression line (Color figure online)

Figure 1 was the diagnostic plots of PK model for MRX-I, indicating the favorable fit of the model. MRX-I concentrations at corresponding timepoints of ECG measurements were simulated using the two-compartment PK model.

QT evaluation and categorical analysis

As the QT interval has an inverse relationship to heart rate, it is necessary to appreciate the effect of the drug on HR [10]. Firstly, we evaluated the time course of mean ΔHR by treatment (Fig. 2a). However, there is no consensus on the specific threshold effect on HR that can influence QT/QTc assessment, though mean increases or decreases > 10 bpm have been problematic [10]. The mean changes of HR did not exceed 10 bpm in our study.

Figure 2d demonstrates the mean ΔQTcF data over time on Day 1 for the MAD 800 mg cohort, the SAD 800 mg cohort, and the food effect 900 mg cohort, along with that of placebo in each cohort except for the 900 mg cohort. Compared with the placebo group, mean ΔQTcF on MRX-I treatment for the 800 mg cohort including the MAD and SAD subjects was mostly negative. Figure 2e shows the mean ΔQTcF data over time on Day 15 for the MAD 800 mg cohort and that of placebo in the MAD study. There was no statistical significance difference of ΔQTc values between the MRX-I and placebo group ($P > 0.05$), even ΔQTcF values of MRX-I treatment were higher than that of placebo group at most of the timepoints. The mean change of QTcF at 2 h was 2.99 ms for the 900 mg cohort. Because food increased the exposure of MRX-I, 900 mg dosing with the high-fat diet was deemed a supratherapeutic dose compared with the therapeutic dose of 600 mg or 800 mg. Meanwhile, plasma concentrations of MRX-I for different cohorts over time were displayed in Fig. 2b, c. Actually, no time delay between MRX-I concentration and ΔQTcF concordance has been observed.

Scatter plots of raw QT vs. RR interval clearly demonstrated an increase in QT with increasing RR interval (Fig. 3a), indicating the need to correct QT for heart rate. It is a subject of controversy about the best correction approach, so we evaluate the four correction formulae with QTcF (Fig. 3b), QTcB (Fig. 3c), and QTcP (Fig. 3d). Scatter plots of QTc vs. RR suggest that Fridericia's, Study-specific, and Individual correction adequately corrected the data for heart rate. QTcB, however, showed slightly overcorrected QT values at lower RR interval and slightly undercorrected QT values at higher RR interval, indicating that Bazett's formula did not adequately correct for heart rate, which is consistent with previous reports [12]. The methods were assessed to determine if QTc is independent of HR, as depicted in Fig. 3.

Categorical analysis of QT and QTc (QTcF, QTcB, QTcP) prolongation was demonstrated in Table 1. There were no subjects on MRX-I or placebo with the QT/QTc interval

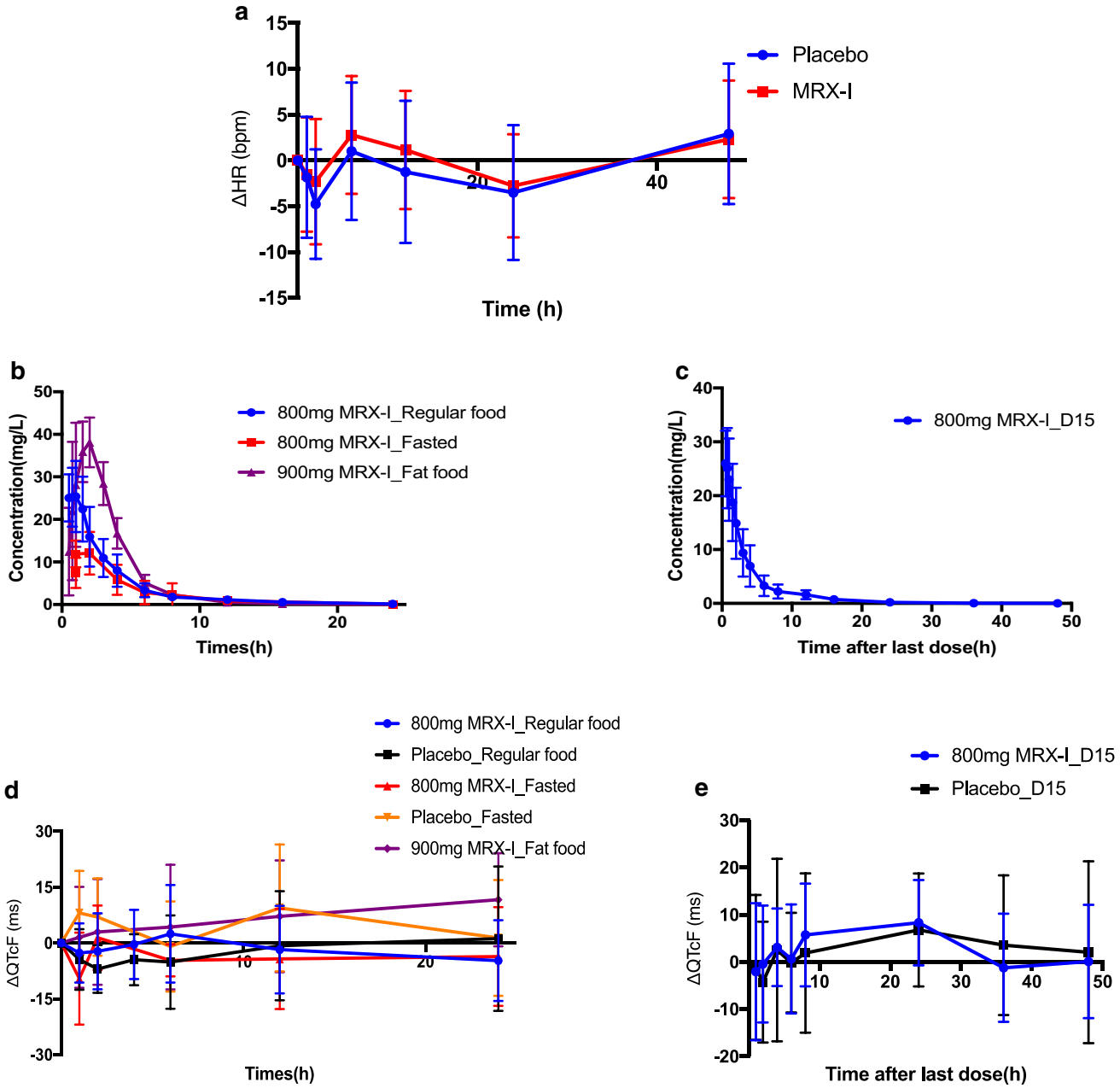

Fig. 2 Evaluation of change of heart rate, MRX-I concentration and ΔQTcF over time. **a** Time course of the mean change from baseline in heart rate. MRX-I treatment includes groups of SAD and food effect part; **b**, **c** time course of mean and SD of MRX-I plasma concentration in three different cohorts, on Day 1 and Day 15; **d**, **e** time course of mean and SD of ΔQTcF by treatment in 3 different cohorts, on Day 1 and Day 15. The blue line in **b**, **d** indicates the 800-mg dose in MAD cohort on Day 1, the red line in **b**, **d** indicates 800-mg dose in SAD study, the purple line in **b**, **d** indicates 900-mg dose in effect of food study. The blue line in **c**, **e** shows 800-mg dose in MAD cohort on Day 15 (Color figure online)

exceeding 480 ms. 7 (0.23%) and 4 (0.13%) observations on MRX-I had QT and QTcB values > 450 to ≤ 480 ms, respectively. No changes from baseline > 60 ms were observed for MRX-I for QTcF, QTcB or QTcP corrections methods. Only one subject had an increase in QTcF, QTcB, and QTcP exceeding 60 ms in the placebo group. The proportions of observations with changes from baseline > 30 ms on MRX-I and placebo for QT/QTc interval were similar.

One-stage concentration QT interval model

We also assessed the predicted concentration-QT plot incorporating a trend line in the process of modeling assumption (Supplemental Fig. s1). The weak correlation ($P > 0.05$) between QT and predicted plasma MRX-I concentration suggested that MRX-I may not exert a clinical effect on QT interval. According to the guidance of

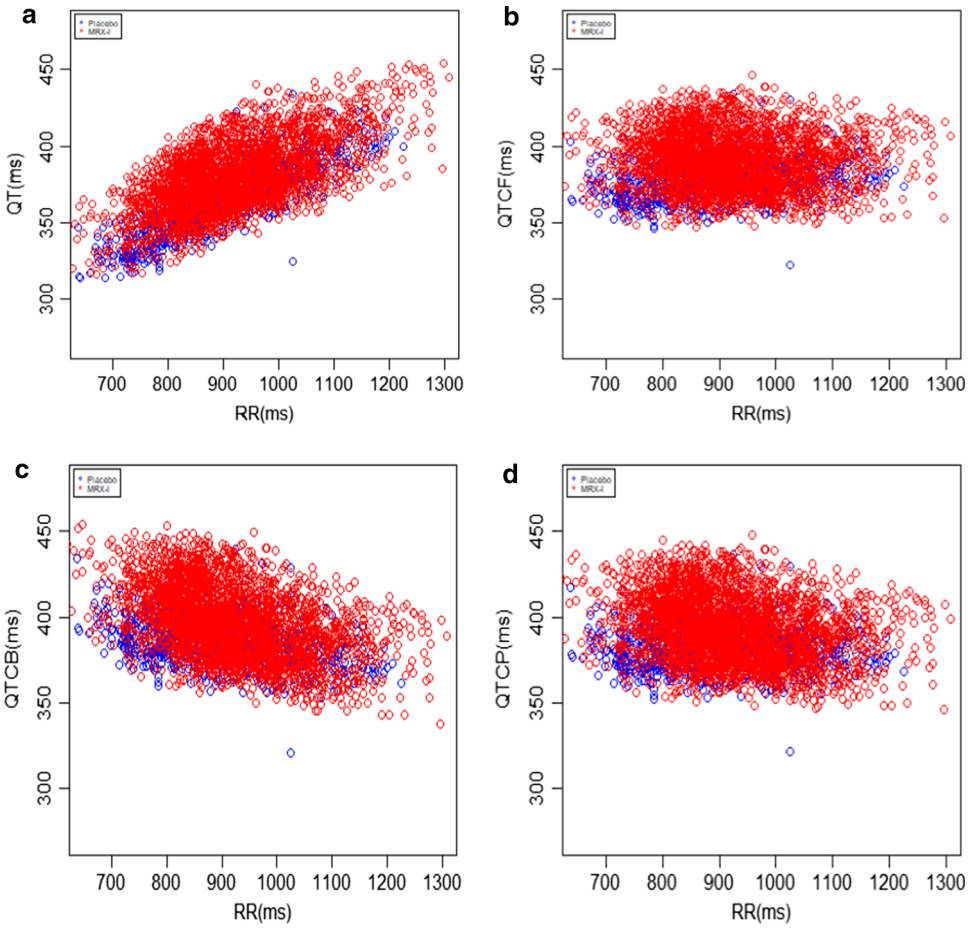

Fig. 3 Evaluation the relationship between the QT/QTc interval and RR interval. **a–e** The scatterplot of QT, QTcF, QTcB, and QTcP and RR interval in MRX-I treatment and placebo group, respectively. The red dots represent the MRX-I group and the blue dots show the placebo group (Color figure online)

Table 1 Number of observation (ratio of observation) with categorical analysis of QT, QTcF, QTcB, QTcP prolongation in Phase I clinical trial

	QT (%)	QTcF (%)	QTcB (%)	QTcP (%)
Number of observation about absolute QT or QTc prolongation				
Placebo				
> 450 ms	0 (0)	0 (0)	0 (0)	0 (0)
> 480 ms	0 (0)	0 (0)	0 (0)	0 (0)
> 500 ms	0 (0)	0 (0)	0 (0)	0 (0)
MRX-I				
> 450 ms	7 (0.23)	0 (0)	4 (0.13)	0 (0)
> 480 ms	0 (0)	0 (0)	0 (0)	0 (0)
> 500 ms	0 (0)	0 (0)	0 (0)	0 (0)
Number of observation about absolute QT or QTc prolongation change from baseline				
Placebo				
> 30 ms	222 (23.85)	22 (2.36)	23 (2.47)	18 (1.93)
> 60 ms	9 (0.97)	1 (0.11)	1 (0.11)	1 (0.11)
MRX-I				
> 30 ms	544 (17.57)	79 (2.55)	121 (3.91)	72 (2.32)
> 60 ms	21 (0.68)	0 (0)	0 (0)	0 (0)

ICH E14 (R3) [7], a linear mixed effects model was used in a one-stage concentration QT interval model to describe the concentration–QT relationship. MRX-I concentrations matched to the corresponding timepoints of ECG records were predicted well using individual PK parameter estimates, as indicated by the good match between observed and model-predicted concentrations (Fig. 1). The effect of sex on the intercept and slope of QT interval versus plasma concentration of MRX-I was evaluated in the model development. In final model, sex was regarded as a significant covariate on intercept (Eq. 7), where $\theta_{\text{intercept}}$ (382 ms) was the population parameter of intercept, and θ_{sex} (0.044) is the parameter of sex effects on intercept (sex = 0 for males, and sex = 1 for females). Hence intercept for females was longer (16.8 ms) than for males.

$$\text{Intercept} = \theta \times (1 + \theta_{sex} \times \text{Sex}) \quad (7)$$

The diagnostic plots (Fig. 4) suggested favorable fit of the C-QT final model. Observed QT interval and the population-predicted QT interval were distributed evenly around identity line as well as the observed interval versus the individual-predicted QT interval (Fig. 4a, b). The changes of conditional weighted residual value (CWRES) over population-predicted QT interval and concentrations were distributed evenly around 0 and most points were located between − 3.5 and + 3.5 (Fig. 4c, d), illustrating the adequate model fit of the final model.

The results of parameter estimates and their inter-individual variation (IIV) and results of the bootstrap of final one-stage C-QT interval model were summarized in Table 2. The estimated correction factor β was 0.368. The estimated slope was 0.0812 per mg/L. The QT effect (ΔΔQTc) were predicted to be 2.08 and 3.39 ms at the geometric mean C_{max} (25.6 mg/L) of clinically therapeutic dose (800 mg q12h under regular food condition) and at maximum observed geometric mean C_{max} (41.7 mg/L) of 900 mg administration under high-fat diet condition, respectively. Furthermore, both one-sided upper limit of 95% CIs for therapeutic and supratherapeutic doses were less than 10 ms. Figure 5 shows the relationship between population predicted values of QTc interval and concentration for female and male subjects, which also illustrates the higher QTc interval in female than male subjects.

Figure 6 showed the results of visual predictive check (VPC) for the one-stage C-QT interval final model. Most of the open circles representing the observed QT interval were within the confidence interval (CI). The red solid and dashed lines representing the median and the 95% CI of observed QT intervals were within the 1000 times simulation results, indicating the adequate predictive power of the final model.

Two-stage concentration QT interval model

Then we constructed two-stage concentration-QT interval models by using QTc and ΔQTc as dependent variables. The parameter and standard error estimates from the two-stage concentration-QT interval models are displayed in Table 3. The point estimates and corresponding upper limit of the one-sided 95% CIs for different correction methods

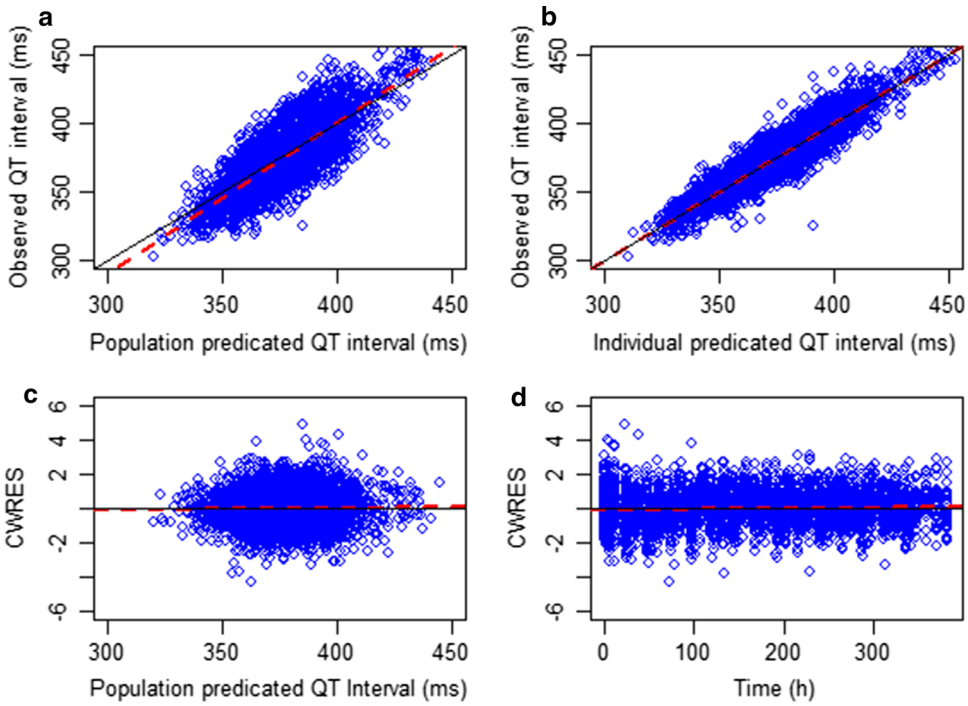

Fig. 4 Diagnostic plot of the one stage concentration-QT interval final model. **a** Population predicted QT interval versus observed QT interval. **b** Individual predicted QT interval versus observed QT interval. **c** Conditional weighted residuals versus population prediction. **d** Conditional weighted residuals versus predicted concentrations. The red line represents the regression line (Color figure online)

Table 2 Parameter estimates, relative standard error, and bootstrap confidence intervals of the pharmacodynamics final model

	Final model		Shrinkage (%)	Bootstrap result		Bias (%)
	Estimation (RSE %)	95% CI[a]		Median	95% CI	
Intercept (ms)	382 (0.4)	379.12–384.88		382.022	379.26–385.02	− 0.0128
Slope (ms*L/mg)	0.0812 (37.8)	0.021–0.141		0.0821	0.0004–0.1508	0.00126
β	0.368 (2.8)	0.348–0.388		0.368	0.348–0.388	0.0003
Sex	0.044 (14.3)	0.032–0.056		0.0445	0.0317–0.0572	0.0005
Interindividual variability						
Intercept (%)	3.2 (13.3)		4.1	3.1	2.7–3.5	− 0.00002
Slope (%)	103.9 (54.5)		61.8	99.3	52.0–182.3	0.1405
β (%)	16.8 (26.7)		38.5	16.4	12.1–21.2	− 0.00054
Residual variability						
Additive error (ms)	77.2 (6.1)		2.3	77.36	68.1–85.9	0.0807

95% CI confidence interval

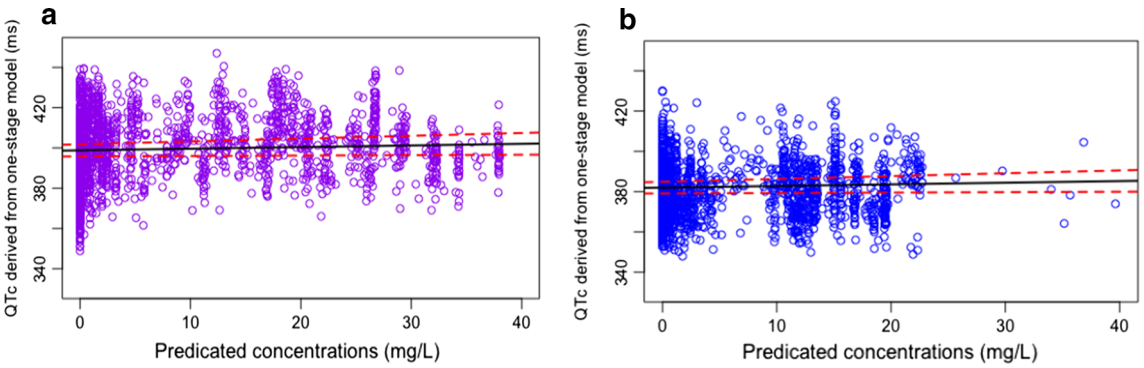

Fig. 5 Population predicted values of QTc interval vs predicted concentrations of MRX-I. **a** For female subjects. **b** For male subjects. The solid black line and dashed red lines represent the model-predicted mean QTc intervals with 95% CI (Color figure online)

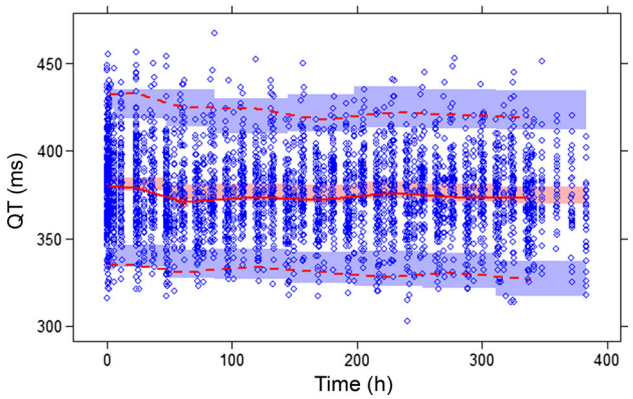

Fig. 6 The visual predictive checks for the pharmacodynamics final model. Open circles represent observed QT, and the solid and dashed lines represent median and the 95% CI of observation. The middle red shadow areas represent the 95% CI of median for the results of 1000 times simulation of the model, and the blue shadow areas represent the 95% CI of the 2.5th and 97.5th percentiles of the results of 1000 times simulation of the pharmacodynamics model (Color figure online)

were calculated at the geometric mean C_{max} of therapeutic dose and supratherapeutic dose, respectively. For the two-stage models with heart rate correction and sex as covariates on intercept of the models, the slight positive relationships between the concentration of MRX-I and QTcF, QTcB and QTcP were observed, with the estimated slopes ranged from 0.0395 to 0.124 ms per 1 mg/L. And the corresponding upper bound of the one-sided 95% CIs for QTcF, QTcB and QTcP at the geometric mean C_{max} of MRX-I for supratherapeutic dose were 3.33, 7.85, and 5.47 ms, respectively. For the two-stage models with heart-rate and baseline corrections, the estimated slopes of ΔQTcF, ΔQTcB, and ΔQTcP were ranged from 0.135 to 0.26 ms per 1 mg/L. The one-sided upper limit of 95% CIs in ΔQTcF model was 8.67 ms at geometric mean C_{max} of supratherapeutic dose. While, the highest corresponding one-sided upper limit of 95% CIs at the geometric mean C_{max} of supratherapeutic does exceeded 10 ms for ΔQTcB model, and the one-sided upper limit of 95% CIs was slightly greater than 10 ms for ΔQTcP model (Table 3).

Table 3 Parameter estimates, objective function value, standard error and one-side upper limit of 95% confidence interval at maximum MRX-I plasma concentration in different pharmacodynamic models

Method	OFV	Intercept (SE, ms)	Slope (SE, ms)	Therapeutic dose		Supratherapeutic dose	
				Point estimate (ms)[c]	Upper bound of 95% CI[d]	Point estimate (ms)[c]	Upper bound of 95% CI[d]
One-stage model [1]							
QT_Base model	21,510	390 (1.44)	0.0813 (0.0319)	2.08	3.42	3.39	5.58
QT_Final model	21,467	382 (1.47)	0.0812 (0.0307)	2.08	3.37	3.39	5.49
Two-stage model with heart rate correction [2]							
QTcF_Final model	21,911	382 (1.51)	0.0395 (0.0245)	1.01	2.04	1.65	3.33
QTcB_Final model	22,547	382 (1.45)	0.124 (0.0391)	3.17	4.82	5.17	7.85
QTcP_Final model	21,910	382 (1.42)	0.0791 (0.0316)	2.02	3.36	3.30	5.47
Two-stage model with heart rate and baseline correction [3]							
ΔQTcF_Base model	22,580	− 0.891 (0.255)	0.135 (0.0443)	3.46	5.32	5.63	8.67
ΔQTcB_Base model	23,125	− 0.964 (0.282)	0.26 (0.0482)	6.66	8.69	10.84	14.15
ΔQTcP_Base model	22,477	− 0.889 (0.898)	0.19 (0.042)	4.86	6.63	7.92	10.80
Two-stage adapted model with heart rate and baseline correction [4]							
ΔQTcF_adapted model	21,733	− 2.05 (2.19)	0.054 (0.0344)	2.30	2.35	3.17	3.23
ΔQTcB_adapted model	22,383	− 3.29 (1.94)	0.146 (0.0433)	5.80	5.87	8.15	8.22
ΔQTcP_adapted model	21,738	− 2.54 (2.03)	0.101 (0.0367)	3.92	3.98	5.54	5.60

TRT treatment effect, *DF* degrees of freedom, *OFV* objective function value, *SE* standard error

[c]The QT prolongation of MRX-I estimate at the geometric mean C_{max} of MRX-I, which is calculated as $\theta_{Slope} \times C_{max}$ in model [1], [2] and [3], while calculated as $\theta_{Slope} \times C_{max} + \theta_{TRT}$ in model [4]. θ refers to the estimate of the parameters

[d]Upper bound of 95% CI was calculated as $(\theta_{Slope} + t(0.95,DF) \times SE_{Slope}) \times Cmax$ in model [1], [2] and [3], while calculated as $(\theta_{Slope} \times C_{max} + \theta_{TRT}) + t(0.95,DF) \times SE(Slope, TRT)$ in model [4]

However, the one-sided upper limit of 95% CIs at therapeutic dose did not exceed 10 ms for the three ΔQTc models.

The linear mixed effect model, considering influence of baseline on intercept, treatment, and nominal time from first dose, developed ΔQTcF, ΔQTcB, and ΔQTcP as dependent variables. The estimated slopes of two-stage adapted model with heart rate and baseline correction were consistent with the values of two-stage model with heart rate correction. The corresponding one-side upper limit of 95% CIs, calculated based on the formulae suggested by Garnett [10], did not exceed 10 ms at therapeutic and supratheraputic doses.

Discussion

In order to evaluate the effect of MRX-I on the QT interval, a concentration-QT linear mixed effect model was developed by analyzing the data from an early Phase I study of MRX-I in healthy volunteers. The finding of the modeling and simulation analysis showed that MRX-I at therapeutic concentrations may not have a clinically meaningful effect on the QT interval. The International Conference on Harmonization (ICH) E14 guidance described design elements and statistical endpoints for a thorough QT trial (TQT) [5]. A TQT study is resource intensive, time-consuming, and increases the risk of exposing subjects to a drug that causes QT prolongation with a positive control, and thus replacing a TQT study with Phase I SAD and SAD data has been explored over the last few years [13]. In terms of using Phase I SAD/MAD data as an alternative to a TQT study, a key focus is that cohorts are too small for analysis by timepoint. With the development of models and simulations, many researchers have developed concentration-QT effect models to evaluate the relationship between concentrations and QTc intervals, and the results have been approved by FDA. The hallmark of using C-QT models to evaluate the QT effect in a standard first-in-human (FIH)

study was the collaboration between the Cardiac Safety Research Consortium (CSRC) and Consortium of Innovation and Quality in Pharmaceutical Development (IQ-CSRC) and FDA, which conducted a study evaluating the ability of C-QT modeling to detect QT prolongation of five QT-positive and one QT-negative drugs in early-phase clinical trials [14]. Due to success of the study [14] and experience from C-QT modeling of TQT studies, the E14 document [7] was subsequently revised in December 2015 to describe the approach of using C-QT modeling applied to data from small studies to replace the TQT study.

Because of the spare PK samples on Day 2 to Day 14 in MAD cohort, along with slight discrepancy between ECG and PK collection timepoints, a two-compartment PK model was employed to predict the concentrations at time-matched ECG data. Before constructing the C-QT model, basic exploratory plots evaluating model independence have been made using simple graphics as provided in Fig. 2. Firstly, Fig. 2a supported the assumption that MRX-I at the therapeutic and supratherapeutic doses did not have a clinically relevant effect on heart rate. Meanwhile, QTc interval was independent of heart rate using QT correction methods including QTcF and QTcP as demonstrated in Fig. 3. Lastly, implicit in Fig. 2b–d was that no time delay between MRX-I concentration and QTc concordance was found in the study.

QT interval can vary widely depending on the heart rate correction method used. The different correction factor may produce different conclusions. In our study, the MRX-I concentration-QT/QTc model was well described by the one- and two-stage linear models by population modeling approach. In the one-stage C-QT modeling, the RR interval correction factor β is 0.368, which is between 0.33 and 0.5, as well as the Study-specific correction of QTcP (correction factor = 0.41) in two-stage modeling. In most of concentration-QT/QTc/ΔQTc models, the upper limits of the one-sided 95% CIs of ΔΔQTc at geometric mean C_{max} of supratherapeutic dose did not exceed 10 ms (Table 3). The slope of Bazett's correction were greater than those derived using the subject- and individual-specific method. Of note, one-sided upper limit of 95% CIs in ΔQTcB model with two-stage correction was greater than 10 ms (14.15 ms). But the scatterplots of measured QTc/ΔQTc intervals versus the corresponding predicted values demonstrated inadequate fit of two-stage models (Supplemental Figs. s2–4), which indicated that other factors needed to be added in the model. Another linear mixed model incorporating the effect of treatment, QTc baseline changes and time on intercept suggested by Garnett et al. [10] showed the good fit between predicted and measured ΔQTc intervals (Supplemental Figs. s5–7). Furthermore, the one-sided upper limit of 95% CIs in the three ΔQTc models were less than 10 ms at either therapeutic or supratherapeutic doses. Moreover, all models predicting one-sided upper limit of 95% CIs at geometric mean C_{max} of therapeutic dose did not exceed 10 ms. All of the results indicated that MRX-I has no clinical effects on QT prolongation (placebo corrected and baseline corrected) at therapeutic dose. Additionally, diagnostic plots of C-QT final model, as presented in Fig. 4, suggested that goodness-of-fit was acceptable for one-stage C-QT model. The Bootstrap and VPC approach also proved the reliability of the one-stage final model. Similarly, diagnostic plots of C-QT model suggested by Garnett et al. [10] also demonstrated a good fit. These two models indicated a negative effect of MRX-I on QT interval at therapeutic and supratherapeutic doses.

The wide dose range was one of the advantages of collecting intensive ECG data in a Phase I study, which provides enough concentration range for C-QT modeling development [10, 13]. In Phase II and Phase III studies of MRX-I, 800 mg twice daily (q12h) was deemed the therapeutic dose in the clinical development program. The maximum geometric mean C_{max} value for MRX-I achieved in the food effect portion of the study with 900 mg given with a high-fat diet was 1.63–fold greater than the expected steady-state C_{max} values at the therapeutic dose given with regular food. The FDA's Interdisciplinary Review Team has recommended that exposures should be at least twice the highest clinically relevant exposure to obviate the need for a positive control [10]. Based on one-stage model, predicted QTc prolongation was to be 4.16 ms at twice the observed stead-state C_{max} of the recommended therapeutic dose (geometric mean, 25.6 mg/L) of MRX-I, and the upper bound of the one-sided 95% CI was 6.74 ms, which was well below 10 ms.

There are limitations to detect the prolongation of QT/QTc interval as the risk of TdP [15]. On one hand, not every patient that develops QT interval prolongation, even beyond 500 ms, experiences TdP. On the other hand, the changes of QT were so sensitive that it was difficult to measure the QT precisely. Many factors, such as inter-individual variability in performing measurements, diurnal variability, and heart rate were found to influence QT intervals [15]. Hence, ECG quality plays a vital role in QTc interval assessment in Phase I studies. In our research, even only a single ECG measurement was collected at each timepoint, a trained cardiologist was responsible to evaluate the quality of ECG. Diurnal variability is characteristic for QT/QTc [16], and baseline and placebo evaluations were included in our study to correct the influence of diurnal variability.

Another risk factor related to QTc interval prolongation is sex [15]. Generally, females have inherently longer QT intervals than adult males, and drug-induced inhibition of

potassium current (I_{kr}) is more likely to happen in females [15]. According to the result of model, sex has been considered as a factor to affect intercept of the C-QT model. We did not find any statistically significance of food on the intercept or slope (data not presented).

Conclusions

In summary, the results of C-QT model in Phase I has suggested that the relationship between ΔΔQTc and plasma concentrations of MRX-I may not be clinically relevant at the therapeutic and supratherapeutic dose. This is the first research to evaluate the effect of MRX-I on the QT interval. Additionally, our research provides a population modeling method to analyze the concentration–QT relationship using the data from Phase I study, especially for there were time discrepancy between the PK samples and ECG data.

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Compliance with ethical standards

Conflicting of interest The authors declare no conflicts of interest.

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