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Demonstration of Clinically Relevant Detection Sensitivity with ICH E14/S7B Q&A: Ascending Dose Cardiovascular Telemetry Study in Dogs

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Introduction

As part of the AMED-funded research initiative in Japan, a positive-control study was conducted in accordance with the *Best Practice* recommendations described in the ICH E14/S7B Q&A (2022)[1] and the methodologies reported by Rossman et al.[2, 3] to evaluate the assay sensitivity of the test system. A previously conducted Latin-square crossover study demonstrated sufficient assay sensitivity to support a potential waiver of the clinical thorough QT (TQT) study.

Latin' Square Crossover Design		
Method: Beagle dog (n=4), Moxifloxacin (0 [vehicle], 3, 10, 30 mg/kg), single oral gavage (5 mL/kg) with 1-week intervals PK: 1, 2, 4, 6, 8, 24 and 48 hours postdose (HPD) in the PK study T30: 30-minute average at PK points SI: 0.5-4, 6-10, 10-16, 16-22, 22-25 and 46-48 HPD average		
Result	T30	SI
LSD, median (min-max), msec	9.6 (2.7 – 13.2)	3.8 (3.1 – 9.5)
RMSE, median (min – max), msec	5.5 (1.6 – 7.7)	2.2 (1.8 – 5.5)
ΔΔQTc +10 msec conc., mean in vivo/human ratio	1.9	1.9

Adopted from data presented at the 17th Annual Meeting of Japanese Safety Pharmacology Society

In the present study, we evaluated the assay sensitivity of an ascending-dose design, which is useful when the dose–toxicity relationship has not been fully characterized. In addition, to assess the potential impact of blood sampling on the sensitivity for detecting QT prolongation, we compared cardiovascular (CV) telemetry study designs with and without integrated pharmacokinetic (PK) sampling. We also investigated whether the QTc averaging interval at each assessment time point influenced assay sensitivity.

Methods

A CV telemetry study in beagle dogs was conducted using moxifloxacin with an ascending-dose design (N=4; vehicle, 3, 10, 30 mg/kg), with PK study after CV measurement. Root mean square error (RMSE) and Fisher's least significant difference (LSD) were calculated using 30-minute average QTc values (T30) and super-interval averaged QTc values (SI) by ANOVA.[2] The concentrations producing a ΔΔQTc prolongation of 10 msec were estimated using a linear regression model by concentration-QTc (C-QTc) analysis. RMSE/LSD and C-QTc were reviewed alongside crossover design data; no direct statistical comparison was performed because different study designs and analytical methods were used.
In addition, CV measurements were conducted during the PK study and RMSE/LSD were compared between with (w/PK) and without (w/o PK) serial PK sampling.

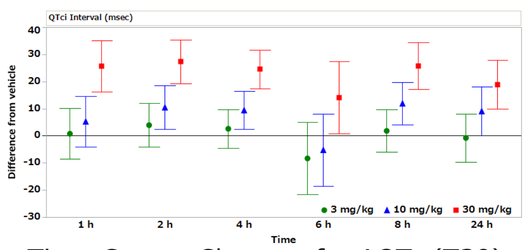
	CV without serial PK (w/o PK)	CV with concurrent serial PK (w/PK)
Animals	Beagle dog (male, n=4)	
Test Article, Doses, Dose regimen	Moxifloxacin: 0 (vehicle), 3, 10, 30 mg/kg single oral gavage ascending dose study design (5 mL/kg) with 1-week intervals	
PK sampling	4.5 HPD	1, 2, 4, 8 and 24 HPD
T30 analysis point	1, 2, 4, 6, 8 and 24 HPD	0.75, 1.75, 3.75, 6, 7.75 and 23.75 HPD
SI analysis point	0.5-4, 6-10, 10-16, 16-22 and 22-24 HPD	0.5-4, 6-10, 10-16, 16-22 and 22-24 HPD
QTc formula	log (QTc) = log (QT) – β [(log (RR) – log (RR ref)]	
Statistical Model	ANOVA, ΔQTc = (Animal) + (Treatment)	
Sensitivity (LSD)	$LSD=[t_{1-\alpha,\ 3(n-2)}]\sqrt{2/n}\cdot RMSE$	
C-QTc analysis, Data set	Linear regression model based on ΔΔQTc from the w/o PK study at T30 and SI analysis points and plasma concentrations obtained from the w/PK study	Not conducted

Vehicle: 0.5 w/v% methylcellulose solution. Statistical analysis was performed using JMP (ver. 19.1).
PK sampling, and T30 and SI analysis were conducted at each time-point in w/o PK and w/PK studies.

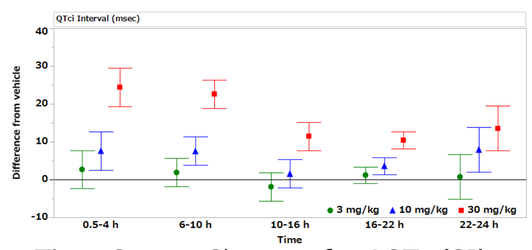
Results

Ascending dose study design w/o PK

Dose-Response Analysis by Time

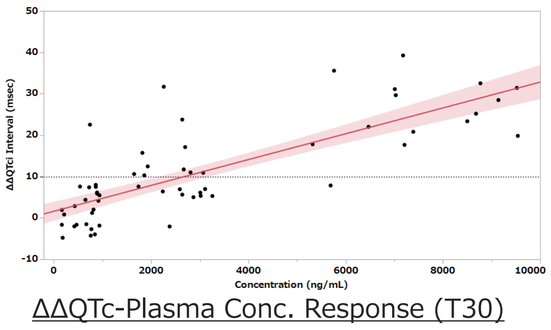


Time-Course Changes for ΔQTc (T30)

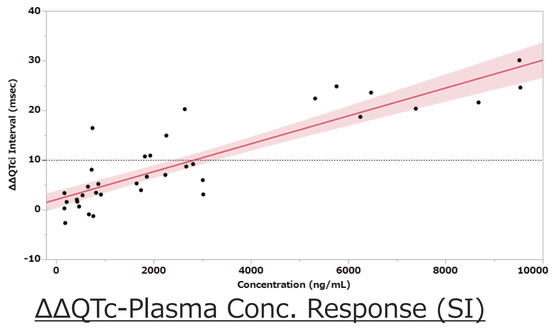


Time-Course Changes for ΔQTc (SI)

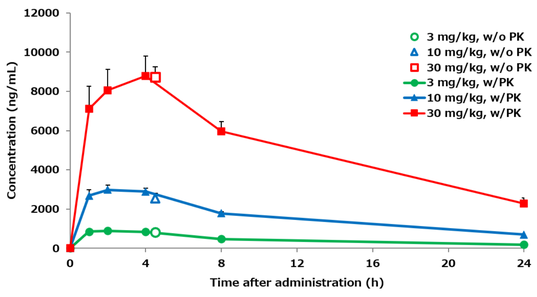
C-QTc analysis



ΔΔQTc-Plasma Conc. Response (T30)



ΔΔQTc-Plasma Conc. Response (SI)

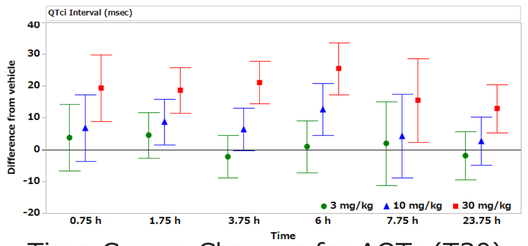


Plasma Conc.-Time Profile of Moxifloxacin

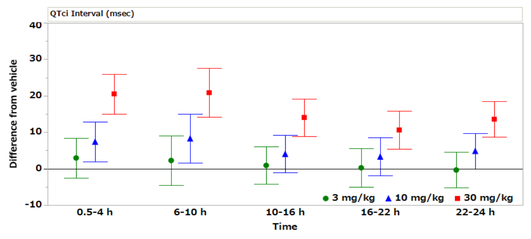
ΔQTc (difference from vehicle) and ΔΔQTc changed over time in parallel with plasma concentrations in both the dose-response and C-QTc analyses, increasing in a dose- and concentration-dependent manner across the evaluated dose range.
Additionally, ΔQTc values for T30 exhibited greater variability than those for SI.

Ascending dose study design w/PK

Dose-Response Analysis by Time



Time-Course Changes for ΔQTc (T30)



Time-Course Changes for ΔQTc (SI)

Sensitivity of Ascending Dose Study Design w/PK

Comparison of LSD/RMSE by T30 and SI

Dose escalation design w/PK	T30	SI
LSD, median (min-max), msec	7.9 (6.7 – 13.1)	5.2 (4.9 – 6.7)
RMSE, median (min – max), msec	4.9 (4.2 – 8.2)	3.3 (3.1 – 4.2)

Ascending-dose study design with concurrent PK sampling demonstrated sufficient assay sensitivity to detect a 10-msec prolongation of QTc (LSD<10 msec), comparable to that of the design without serial PK sampling. Appropriate optimization of the analysis interval can minimize the impact of PK sampling on QTc detection sensitivity.

Sensitivity of Ascending Dose Study Design w/o PK

Comparison of LSD/RMSE by time bin

	T15	T30	T60	T120	SI
LSD, msec median (min-max)	9.2 (8.7-15.4)	8.3 (7.1–13.2)	8.5 (6.8-11.0)	5.9 (4.2-7.3)	3.8 (2.2–5.9)
RMSE, msec median (min-max)	5.8 (5.4-9.6)	5.3 (4.4–8.3)	5.3 (4.2-6.9)	3.7 (2.6-4.5)	2.3 (1.4–3.7)

T15, T30, T60: 15-, 30- and 60-min average at 1, 2, 4, 6, 8, 24 HPD. T120: 120-min average at 2, 4, 6, 8, 24 HPD.
SI: 0.5-4, 6-10, 10-16, 16-22 and 22-24 HPD

Ascending-dose study design demonstrated sufficient assay sensitivity to detect a 10-msec QTc prolongation (LSD<10 msec), comparable to that of the Latin's square crossover design, and the analysis based on SI showed greater sensitivity than that based on T30. Comparison of T15, T30, T60, T120 and SI analyses showed that wider averaging intervals reduced variance and improved assay sensitivity. Particularly, SI with the analysis interval optimized based on the plasma concentration and pharmacodynamic effect profile provides the highest sensitivity.

Comparison of C-QTc analysis between T30 and SI

	T30	SI
Slope (p-value), msec per ng/mL	0.00312 (p<0.001)	0.00280 (p<0.001)
Intercept, msec	1.64	2.04
ΔΔQTc +10 msec conc., total, mean (90%CI), ng/mL	2682 (2158-3183)	2837 (2386-3298)
ΔΔQTc +10 msec conc., free, mean (90%CI), ng/mL	1904 (1532-2260)	2014 (1694-2342)
in vivo/human* ΔΔQTc +10 msec conc. ratio, mean	1.7	1.8

＊ Critical concentration (ng/mL, free) that gives a 10-msec increase in humans, Mean (90%CI): 1120 (955, 1313)

The linear regression model-predicted concentrations producing a 10-msec ΔΔQTc prolongation were within 2-fold of the clinical concentration across all study designs and analysis methods (crossover and dose escalation; T30 and SI).

Conclusion

- **An ascending-dose study design demonstrated sufficient assay sensitivity** to detect a 10-msec QTc prolongation, comparable to that achieved with the crossover design, thereby supporting a waiver of the thorough QT (TQT) study requirement.
- The sensitivity of the cardiovascular (CV) telemetry study was influenced by the averaging interval applied at each assessment time point. **A "super-interval," optimized based on the PK/PD profiles and encompassing a broader analysis window, provided the greatest assay sensitivity.**
- **The impact of blood sampling on assay sensitivity was minimal**, and the sensitivity of this PK-integrated study was comparable to that of the stand-alone CV study.
- Therefore, an ascending-dose design incorporating PK assessments may be particularly valuable for candidate compounds whose dose–toxicity relationship has not been fully characterized, as well as during early-stage drug discovery.

Reference

1) ICH E14/S7B Q&As Training Materials, 2022.
2) *Eric I. Rossman*, *et al*. J Pharmacol Toxicol Methods 123 (2023) 107270
3) *Li, D*., and *Nichols, J*., Statistical Best Practices to Support the New ICH S7B Q&A, Safety Pharmacology Society Webinar, 2022.

Conflicts of interest (COI): None

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