



Development of QT prolongation model in guinea pig with hERG assay-*in vivo* PK-*in vivo* QT effect to guide decision making in early drug discovery

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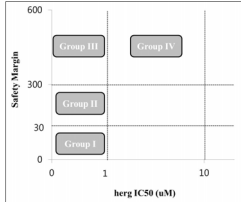
Introduction

hERG assay is a one of traditional approaches for evaluating cardiac safety of drug in early drug discover¹. But this assay has major limitation that it does not consider PK properties of drug candidates, leading to a weak correlation between *in vitro* (hERG IC₅₀) and *in vivo* (ΔQTcF) tests. For accurate prediction of QT prolongation in early drug development process, much improved evaluation method is required. In this study, we built the model for predicting QT prolongation considering hERG assay and PK parameters as well as *in-vivo* QT prolongation in guinea pig

Method

Selection of drugs for developing QT prolongation model

- For building QTc prolongation prediction model, drugs were selected from each of the four groups that were classified based on safety margin (IC₅₀/C_{max}: 0~30 / 30 ~ 300 / 300 ~ 600) and hERG IC₅₀ (~1 / 1 ~ 10 μM) values.
- 13 drugs were selected and were classified as high- (Group 1), moderate- (Group 2), low- (Group 3) and no- (Group 4) risk groups.
- Test dose of each drug was converted to animal dose from human maximum daily dose.



Drug	hERG IC ₅₀ (μM)	Group	Test dose (mg/kg)
Dofetilide	0.06	1	0.05
Droperidol	0.091	1	2.5
Astemizole	0.1	1	0.5
Halofantrin	0.197	1	25
Ketanserin	0.38	1	3
Clomipramine	0.083	2	5
Domperidone	0.16	2	1.5
Solifenacin	0.27	2	3
Sunitinib	0.27	2	15
Ziprasidone	0.165	3	10
Loratadine	0.173	3	3
Perphenazine	1.003	4	1.5
Olanzapine	6	4	1

Pharmacokinetics study design

- Animals : SPF Hartley guinea pig (male, 6 weeks) were housed under temperature/humidity-controlled conditions (22±3 °C, 50±10%) with a 12hour light-dark cycle. (n=5 for each drug group).
- Jugular vein was catheterized for I.V. injection and blood collection. And intraperitoneal injection of zoletil + rompun (1.13 mg/kg) was given to anesthetizing guinea pig.
- Blood collection
 - Sampling time: 0.083, 0.17, 0.25, 0.33, 0.42, 0.5, 1, 2, 4, 6 hours after dosing.
 - 300 μL blood was collected from jugular vein at each time points.
 - After collecting the blood, each blood samples were centrifuged at 15,000 rpm, 4 °C for 10min. Plasma was separated and stored at -70 °C until analysis.
- Analysis of samples was done with API 2000 (Applied Biosystems, USA) and Analyst software ver.1.5.1.
- Pharmacokinetic parameters(AUC_{last}, AUC_{inf}, C_{max}) were estimated using Phoenix ver. 6.4.0 (Pharsight, USA).

Pharmacodynamics study design

- For measuring ECG of guinea pig², lead 2 Hugo Sachs Elektronik ECG (Hugstetten-March, Germany) was used. ECG were measured after administration of drug only after heart rate was stabilized.
- ECG were measured at 5, 10, 15, 20, 25, 30 minute after dosing
- QT interval of guinea pig was corrected by RR interval as below:

Bazett eq.(QTcB)	Fridericia eq. (QTcF)	Van de Water eq. (QTcV)
$\frac{QT}{\sqrt{RR}}$	$\frac{QT}{\sqrt[3]{RR}}$	$QT - 0.087 \times (RR - 1000)$

As a representative *in vivo* PD parameter, maximum changes in QTc interval from baseline (ΔQTc interval) were used.

Simple regression analysis

- Linear regression analysis was performed using R ver.3.2.2 and Rstudio ver.0.99.
 - Linear regression between hERG IC₅₀ and ΔQTc interval was assessed and AUC_{last/inf} and C_{max} of each drug were tested as covariate candidates.
- R² values calculated from each regression analysis were compared.

Multiple nonlinear regression analysis

- Multiple nonlinear regression between ΔQTc interval, hERG IC₅₀ and AUC_{last/inf} or C_{max} was performed using SigmaPlot ver.10.0 (Systat Software, USA).
- Plane equation and Paraboloid equation were tested and r² values calculated from each regression analysis were compared.

Plane equation	$Z = z_0 + a^*x + b^*y$
Paraboloid equation	$Z = z_0 + a^*x + b^*y + c^*x^2 + d^*y^2$

Nonlinear mixed effect model for prediction of QT prolongation

- Nonlinear mixed effect regression model
 - For identifying relationship between hERG IC₅₀, ΔQTc interval and PK parameters, nonlinear mixed effect regression model was developed. ΔQTc interval, AUC_{last/inf} and C_{max} of each drug were natural log transformed.
 - Exponential model was used for explaining inter-individual variability (IIV) and additive or proportional error model was tested as a residual variability model. And first order conditional estimation with interaction method was used for estimating parameters.
 - Model equations for prediction of QT prolongation is given below

$$TV\alpha = \theta_1 * (1 + \theta_1 * \ln C_{max}) * (1 + \theta_2 * \ln AUC_{inf}) * e^{\eta}$$

- Base: Base ΔQTc interval when hERG IC₅₀ converge to zero.
- α : Decreasing rate constant of ΔQTc interval from base following hERG IC₅₀.

Final Model evaluation

- For evaluating the final QT prolongation prediction model visual predictive check was used.

Results

Pharmacokinetic & Pharmacodynamic study results

- From PK study (n=5, each drug), PK parameters (AUC_{last}, AUC_{inf}, C_{max}) of each drug were estimated by noncompartmental analysis.
- From PD study (n=5, each drug), maximum ΔQTc interval (QTcB, QTcF, QTcV) of each drug were calculated.

Simple regression analysis

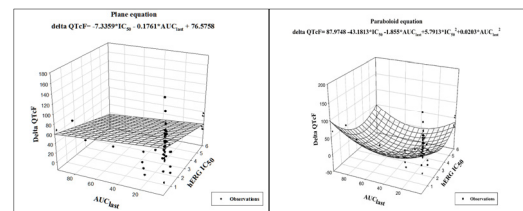
- 4 models were tested in R and the second model (Y~X1*x2) was selected because of r² value (0.2209) and significance of variable.

Model No.	Model	Variable	R _{adj} ²	Significant variable
1	Y~x1+covar	X1: IC ₅₀ , Covar: AUC _{inf}	0.05398	X1
2	Y~x1*x2	X1: IC ₅₀ , X2: AUC _{inf}	0.2209	All variables
3	Y~X1*X2*X3	X1: IC ₅₀ , X2: AUC _{inf} , X3: C _{max}	0.2129	X1, X2, X1:X2
4	Y~X1*X2*X3	X1: IC ₅₀ , X2: AUC _{last} , X3: C _{max}	0.1993	X1, X2, X1:X2

Multiple nonlinear regression analysis

- R² values of plane equation and paraboloid equation were 0.1421 and 0.2504, respectively.

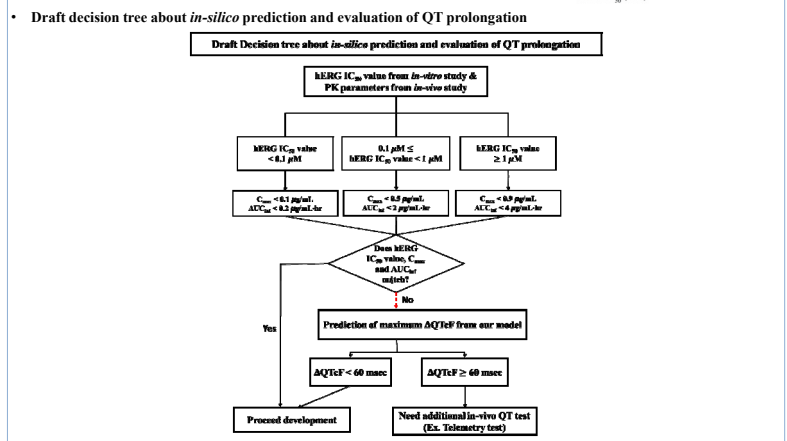
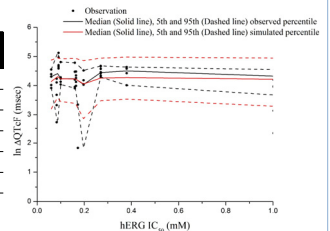
Plane equation	$\Delta QTcF = 76.5758 - 7.3359 * IC_{50} - 0.1761 * AUC_{last}$	R ² = 0.1421
Paraboloid equation	$\Delta QTcF = 87.9748 - 43.1813 * IC_{50} - 1.855 * AUC_{last} + 5.7913 * IC_{50}^2 + 0.0203 * AUC_{last}^2$	R ² = 0.2504



Nonlinear mixed effect model for prediction of QT prolongation

- Model for identifying nonlinear relationship between hERG IC₅₀ and ΔQTc interval was developed using NONMEM and In AUC_{inf}, C_{max} were selected as covariate of alpha parameter
- Estimated values from final model and visual predictive check (n=1,000) are shown below

Parameter	Estimated value (%RSE)	IIV CV% (%RSE)
Base (msec)	74.4 (7.7 %)	28.5 % (26.0 %)
α (1/μM)	23.3 (35 %)	59.2 % (36.6 %)
θ ₁ (ln C _{max})	-0.706 (19.5 %)	-
θ ₂ (ln AUC _{inf})	-0.531 (62.9 %)	-
Residual variability		
Additive error (msec)	1.31 (15.3 %)	



Discussion

- With the results from PK and PD study, simple regression and multiple nonlinear regression analysis were successfully developed.
- Equations from these regression analysis are simple but show low r² values, and cannot explain high variance of PK and PD parameters.
- Regression model of hERG IC₅₀ and ΔQTc interval, considering PK parameters as covariate was successfully developed using NONMEM.
- Natural log transformed AUC_{inf} and C_{max} were found to be significant covariate for α.
- Our final model can estimate the ΔQTc interval considering hERG IC₅₀ value as well as PK parameters of given drugs.
- Our study had limited number of drugs in each of the classified risk groups, so we plan to update our model with PK and PD data from more drugs.
- Using final model, we suggest draft decision tree for predicting and evaluating of QT prolongation using *in-silico* method. This draft version of the decision tree will be updated with future studies.

Reference

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Acknowledgement

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2014R1A1A1006006).

