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Original article

Sensitivity of pharmacokinetic–pharmacodynamic analysis for detecting small magnitudes of QTc prolongation in preclinical safety testing

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ABSTRACT

Introduction: Preclinical concentration–effect (pharmacokinetic–pharmacodynamic, PKPD) modeling has successfully quantified QT effects of several drugs known for significant QT prolongation. This study investigated its sensitivity for detecting small magnitudes of QT-prolongation in a typical preclinical cardiovascular (CV) safety study in the conscious telemetered dog (crossover study in 4–8 animals receiving a vehicle and three dose levels). Results were compared with conventional statistical analysis (analysis of covariance, ANCOVA). **Methods:** A PKPD model predicting individual QTc was first developed from vehicle arms of 28 typical CV studies and one positive control study (sotalol). The model quantified between-animal, inter-occasion and within-animal variability and described QTc over 24 h as a function of circadian variation and drug concentration. This "true" model was used to repeatedly ($n = 500$) simulate studies with typical drug-induced QTc prolongation (ΔQTc) of 1 to 12 ms at high-dose peak concentrations. Simulated studies were re-analyzed by both PKPD analysis (with varying complexity) and ANCOVA. Sensitivity (power) was calculated as the percentage of studies in which a significant ($\alpha = 0.05$) drug effect was found. One simulation scenario did not include a concentration–effect relationship and served to investigate false-positive rates. Exposure–effect relationships were derived from both PKPD analysis (linear concentration–effect) and ANCOVA (linear trend test for dose) and compared. **Results:** PKPD analysis/ANCOVA had a sensitivity of 80% to detect the effects of 7/13 ms ($n = 4$), 5/10 ms ($n = 6$) and 4.5/8 ms ($n = 8$), respectively. The false-positive rate was much higher using ANCOVA (40%) compared to PKPD analysis (1%). Typical drug effects were more precisely predicted using estimated concentration–effect slopes ($\pm$1.5–2.8 ms) than dose–effect slopes ($\pm$3.3–3.7 ms). **Discussion:** Preclinical PKPD analysis can increase the confidence in the quantification of small QTc effects and potentially allow reducing the number of animals while maintaining the required study sensitivity. This underscores the value of PKPD modeling in preclinical safety testing.

1. Introduction

Cardiovascular safety (CV) studies in the conscious telemetered dog are part of standard preclinical safety pharmacology testing (ICH S7A Guideline, 2001). One particular objective is to evaluate the potential of a drug to delay ventricular repolarization (QT interval) and to establish respective dose–effect or concentration–effect relationships (ICH S7B Guideline, 2005). Since the QT interval is highly variable between and within individuals, high sensitivity is required to detect meaningful changes. In fact, a heart-rate corrected QT (QTc) prolongation as small as 2–8 ms in the conscious dog may correlate with a relevant 5–10 ms QTc prolongation in humans (Chain, Dubois, Danhof, Sturkenboom, & Della Pasqua, 2013; Parkinson et al., 2013a). Methods

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to ensure best sensitivity of CV studies have thus been proposed (Leishman et al., 2012), which address the study design, conduction, and analysis.

Concerning the data analysis, drug effects are typically assessed by different models of analysis of (co)variance (ANCOVA) (Aylott, Bate, Collins, Jarvis, & Saul, 2010). Such models compare the mean QTc under different dose levels versus the mean QTc after vehicle administration at pre-defined time points. Through linear regression, a dose–effect relationship can be established in terms of a dose–effect slope (e.g. QTc increase in [ms] by a dose increase of 1 mg/kg). It has been shown that such statistical models allow detecting a 5–10% effect (12–25 ms) in 80% of studies (Aylott et al., 2010; Chiang et al., 2007; Ewart et al., 2013) and 2% (4 ms) (Sivarajah et al., 2010), respectively, when using 4 animals in a Latin-square design.

A possibly more sensitive, informative and efficient approach is pharmacokinetic–pharmacodynamic (PKPD) analysis, which takes the drug's pharmacokinetics (predicted course of plasma drug concentration over time) and circadian effects into account. A drug effect on the QT interval can here be detected in terms of a significant concentration–effect relationship, for example expressed as concentration–effect slope (e.g. QTc increase in [ms] by a concentration increase of 1 ng/mL). This type of analysis improves the temporal understanding of drug effects and facilitates the in vitro–in vivo-human translation of drug effects (Chain et al., 2013; Jonker et al., 2005; Parkinson et al., 2013b; Rohatagi & Carrothers, 2009; Watson et al., 2011) and has been successfully applied in the preclinical setting to characterize the effect of drugs known for significant QT prolongation of 30–60 ms, such as sotalol (Chain et al., 2013), dofetilide (Jonker et al., 2005; Ollerstam et al., 2006) and moxifloxacin (Ollerstam et al., 2007; Watson et al., 2011). Knowledge about the usefulness of PKPD modeling to detect small effects in the preclinical setting is however limited.

The primary objective of this work was thus to evaluate the sensitivity of PKPD (concentration–effect) analysis to detect small magnitudes of drug-induced QTc prolongation (ΔQTc), and to compare it with traditional ANCOVA (dose–effect) analysis. Additionally, we assessed the false positive rate (1-specificity), i.e. the percentage of studies in which a significant drug effect is found when in reality there is no drug effect.

Secondary objectives were to assess the confidence in predicted exposure–effect relationships in terms of accuracy and precision of predicted typical maximal ΔQTc, to evaluate the influence of different study designs and conditions on predictions (number of animals, density of ECG sampling, PK half-life of the drug), and to explore the performance of alternative, simpler PKPD models for data analysis (typical/median population predictions, instead of individual concentration predictions for every ECG measurement, non-compartmental PK analysis (concentration interpolation), ignorance of circadian variation).

2. Methods

Briefly, a simulation study was performed in the following steps (Fig. 1a): First, a mathematical–statistical model describing circadian QT variation, HR dependency, between-subject, inter-occasion and residual variability (baseline model) was developed from 28 experimental vehicle-arm study data sets (Table 1). Second, a realistic PKPD model ("true" PKPD model) was developed from a positive control drug study (sotalol, showing maximal ΔQTc of ≈50 ms at high concentrations, Table 1) and the baseline model. The "true" PKPD model was then used to simulate repeatedly CV studies with decreasing dose to mimic a hypothetical drug with a small effect on the QT interval, that is maximal ΔQTc of 1–12 ms. Simulated studies were reanalyzed by both PKPD analysis and ANCOVA. Sensitivity was calculated as the % of studies in which a significant drug–effect was detected.

Non-linear mixed-effect PKPD modeling and simulations were performed using the NONMEM software (version 7.3.0; Icon Development Solutions, Ellicott City, MD, USA) and Pearl-speaks-NONMEM scripts (PsN version 3.7.6, http://psn.sourceforge.net). Parameters were estimated using the first-order conditional estimation (FOCE) method (baseline PD model) and FOCE with interaction (PKPD model), respectively. ANCOVA, statistics and figures were created using R (version 2.10.1; R Development Core Team, Vienna, Austria, http://www.r-project.org).

2.1. Data

All experiments were performed in accordance with "the Provision of the European Convention" on the protection of vertebrate animals and "Appendices A and B", made at Strasbourg on March 18, 1986 (Belgian Act of October 18, 1991), and were approved by appropriate ethics committees.

The characteristics of the vehicle data and sotalol study data are summarized in Table 1. For ECG, the results of the automatically calculated parameters (PQ, QRS and QT intervals, based on average of 10 consecutive beats before and as close as possible to the selected time points) were checked visually, adjusted if necessary and validated, using a semi-automatic program especially developed for this purpose.

2.2. Model development

Model building was guided by graphical data examination, goodness-of-fit plots (observations versus predictions, residual plots, parameter distributions, individual fits), physiological plausibility and relevance, standard errors, and the likelihood ratio test ($\alpha = 0.01$). The latter criterion corresponds to a decrease of the objective function value (OFV, equivalent to −2 log-likelihood) by 6.6 and 9.2 for one and two additional parameters respectively. The adequacy of predicted QT variability from the final baseline model was evaluated by a visual predictive check (VPC), using 1000 Monte Carlo simulations.

2.2.1. Baseline model: dynamic QTc variability in the absence of a drug

To describe the individual QT–HR or QT–RR relationship, asymptotic relationships with RR interval were tested (in particular exponential (Carmeliet, 1995) and power/log-linear (Chain et al., 2013) relationships), and a linear relationship with HR (Aylott et al., 2010; Ollerstam et al., 2006). HR was tested as hemodynamic and ECG derived measurement via RR-interval (HR_{ECG}). One to several cosine functions with different periods (6 to 24 h) were considered to describe dynamic variation, i.e. diurnal variation over clock time (Piotrovsky, 2005). In addition, a possible influence of i.v. versus s.c/oral drug administration, and slinged versus freely moving conditions on the parameters characterizing the dynamic and hear-rate dependent QT interval changes were evaluated.

Between-subject and inter-occasion variability (BSV and IOV, respectively) was evaluated for all parameters, considering normal and log-normal distributions. Residual variability was assumed to follow a normal distribution (additive error model).

2.2.2. PKPD model

The PKPD model was built in a step-wise procedure: first the PK model was built, and then PK parameters and baseline model parameters were fixed to their final estimates for characterizing the concentration–effect relationship. Simultaneous modeling was tested but resulted in difficulties to estimate reliable PK model parameters.

According to the literature (Chain et al., 2013) a two-compartment model was used to describe sotalol drug concentrations over time. A sigmoidal E_{max} model was used to describe the concentration–effect relationship. The presence of a delayed drug effect was assessed visually (hysteresis in individual drug effect over concentration plots), and modeled using an effect-compartment.

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