Journal of Pharmacological and Toxicological Methods 74 (2015) 26–32

Contents lists available at ScienceDirect

Journal of Pharmacological and Toxicological Methods

journal homepage: www.elsevier.com/locate/jpharmtox

Original article

Long-term stability, reproducibility, and statistical sensitivity of a telemetry-instrumented dog model: A 27-month longitudinal assessment☆

Ryan M. Fryer [a,*], Khing Jow Ng [a], Liguo Chi [a], Xidong Jin [b], Glenn A. Reinhart [a]

[a] Department of Cardiometabolic Diseases Research, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield 06877, CT, USA
[b] Department of Biometrics and Data Management, Boehringer Ingelheim Pharmaceuticals Inc., Ridgefield 06877, CT, USA

ARTICLE INFO

Article history:
Received 16 January 2015
Received in revised form 9 April 2015
Accepted 28 April 2015
Available online 3 May 2015

Keywords:
Telemetry
Dog
Hemodynamics
dl-sotalol
Drug discovery
In vivo
Safety pharmacology
QT-interval
Heart rate
dP/dt

ABSTRACT

Introduction: ICH guidelines, as well as best-practice and ethical considerations, provide strong rationale for use of telemetry-instrumented dog colonies for cardiovascular safety assessment. However, few studies have investigated the long-term stability of cardiovascular function at baseline, reproducibility in response to pharmacologic challenge, and maintenance of statistical sensitivity to define the usable life of the colony. These questions were addressed in 3 identical studies spanning 27 months and were performed in the same colony of dogs.

Methods: Telemetry-instrumented dogs (n = 4) received a single dose of *dl*-sotalol (10 mg/kg, p.o.), a β_1 adrenergic and I_{Kr} blocker, or vehicle, in 3 separate studies spanning 27 months. Systemic hemodynamics, cardiovascular function, and ECG parameters were monitored for 18 h post-dose; plasma drug concentrations (Cp) were measured at 1, 3, 5, and 24 h post-dose.

Results: Baseline hemodynamic/ECG values were consistent across the 27-month study with the exception of modest age-dependent decreases in heart rate and the corresponding QT-interval. *dl*-Sotalol elicited highly reproducible effects in each study. Reductions in heart rate after *dl*-sotalol treatment ranged between −22 and −32 beats/min, and slight differences in magnitude could be ascribed to variability in *dl*-sotalol Cp (range = 3230–5087 ng/mL); *dl*-sotalol also reduced LV-dP/dt_{max} 13–22%. *dl*-Sotalol increased the slope of the PR–RR relationship suggesting inhibition of AV-conduction. Increases in the heart-rate corrected QT-interval were not significantly different across the 3 studies and results of a power analysis demonstrated that the detection limit for QTc values was not diminished throughout the 27 month period and across a range of power assumptions despite modest, age-dependent changes in heart rate.

Discussion: These results demonstrate the long-term stability of a telemetry dog colony as evidenced by a stability of baseline values, consistently reproducible response to pharmacologic challenge and no diminished statistical sensitivity over time.

1. Introduction

Regulatory guidelines, as well as best-practice and ethical considerations, provide strong rationale for the use of telemetry-instrumented conscious animals for cardiovascular safety assessment. Indeed, measurement of blood pressure in the conscious state, using radiotelemetry in freely moving animals, is considered the gold-standard technology and has been endorsed by the AHA council on high blood pressure research (Kurtz, Griffin, Bidani, Davisson, & Hall, 2005) and international regulatory agencies (ICH S7A) prior to FIH testing of novel therapeutics. While multi-site comparisons of *in vivo* safety pharmacology studies conducted in telemetry dog to support ICH S7A and S7B regulatory submissions have yielded similar results (Ewart et al., 2013; Sasaki, Shimizu, Suganami, & Yamamoto, 2005), few have investigated the long-term stability of hemodynamic and ECG endpoints at baseline, reproducibility in response to pharmacologic challenge, and maintenance of statistical sensitivity, over the usable-life of the colony. These questions were addressed in 3 identical studies spanning 27 months that were performed in the same colony of instrumented dogs.

Abbreviations: dP/dt_{max}, left ventricular contractility; Cp, plasma concentration; C_{max}, maximal plasma drug concentration; T_{max}, time associated with C_{max}; CV, cardiovascular; ICH, international committee on harmonization; ECG, electrocardiogram; ms, milliseconds; FIH, first in human clinical trial; QTi, QT-interval; LO, lead optimization; QTcV, QTi corrected for changes in heart rate with Van de Water or Fridericia (QTcF) formulae.

☆ Limitations of the present study, and other observations not directly related to the stability and sensitivity of the model, are noted in the supplemental text.

* Corresponding author at: Boehringer Ingelheim Pharmaceuticals, Inc., 900 Ridgebury Road, Ridgefield CT 06877-0368, USA. Tel.: +1 203 778 7928; fax: +1 203 837 5928.
E-mail address: ryan.fryer@boehringer-ingelheim.com (R.M. Fryer).

http://dx.doi.org/10.1016/j.vascn.2015.04.007
1056-8719/

In a 2004 assessment of the underlying cause of attrition in drug development from 1991–2000, while lack of efficacy accounted for approximately 27% of failures in 2000, this represented a slight decrease from 1991 (30%). However while toxicology and clinical safety accounted for a similar percentage of failures, unlike efficacy values, failures due to toxicology and clinical safety tended to rise during the 9-year period (from a combined value of approximately 21% to 33%) (Kola & Landis, 2004) demonstrating the need for development of *in vivo* models with enhanced clinical translation. Toward this end, others have suggested an expanded application of predictive technologies to guide pre-clinical safety decisions including a narrowed focus on problems that contribute most to adverse drug reactions whereby cardiac (and hepatic) toxicity contributes disproportionately to drug withdrawals (Stevens & Baker, 2009). Prior to FIH studies CV safety studies are largely performed in conscious dogs or non-human primates (Authier, Tanguay, Gauvin, Fruscia, & Troncy, 2007) where there is a broad literature base surrounding their predictivity for acute CV effects in humans. However, while ethical and cost considerations suggest that telemetry-instrumented animals can be used repeatedly over multiple studies, the stability of the model with regards to hemodynamic and ECG parameters at baseline, reproducibility of the response to pharmacologic challenge, and sustained statistical sensitivity has not been determined. Maintenance of statistical power is particularly important, for example, for detecting changes in QTc since in the clinical setting, a 5-ms change is considered detectable (Authier et al., 2007; U.S.Department of Health and Human Services, 2005) and sufficient to impact the label of marketed products (*e.g.* New cardiac warnings for Kaletra, 2009).

Thus, we evaluated the effect of an industry-standard and established positive control for CV safety studies, *dl*-sotalol (a β_1 adrenergic, and I_{Kr} channel, blocker) on hemodynamics, CV function, and the electrocardiogram (ECG) in the same colony of dogs in three identical studies separated, in total, by 27 months. We report a stable and highly consistent response to pharmacological challenge and demonstrate the consistency in the statistical sensitivity to detect effects across a number of CV endpoints.

2. Methods

All experiments and procedures were performed under protocols approved by the Boehringer-Ingelheim Institutional Animal Care and Use Committee and according to the United States Animal Welfare Act.

Male inbred beagle dogs (n = 4, 9–13 kg; Marshall Farms) instrumented with telemetry implants (T27F-9, Konigsberg) were evaluated in three identical studies over a period encompassing 27 months and spaced 13–14 months apart; the same four dogs were used throughout the study. For simplicity, the three study groups are referred to as Month 1 (M1), Month 14 (M14), and Month 27 (M27) representing their temporal separation.

All dogs received *dl*-sotalol (10 mg/kg, p.o.) or an empty capsule as vehicle whereby each dog was administered both treatments at M1, M14, and M27 in a randomized fashion. At each test phase (M1 in January of Year 1, M14 in March of Year 2, or M27 in April of Year 3) n = 2 dogs received the empty capsule and the remaining n = 2 dogs received sotalol; one week later the dosing was repeated with the opposite treatment. Blood samples were collected *via* a cephalic vein in each study at baseline, 1, 3, 5 and 24 h post-treatment for the determination of *dl*-sotalol Cp by mass spectrometry. The CV and ECG data were recorded continuously over a 19-hour period (from 1-hr baseline to 18 h post-treatment using 10-min averaged values) using a data acquisition/analysis system (TD-14 Base Station from RMISS, CA-recorder/VRR software from DISS) while dogs were conscious, freely moving, and single-housed. CV endpoints collected included systolic, diastolic, and mean arterial pressure, heart rate, left ventricular pressure, dP/dt_{max}, and left ventricular end-diastolic pressure (LVEDP; results not shown). Calculation of ECG endpoints was automated using CA-recorder software; endpoints included the PR-, QRS-, QT-, and RR-intervals. QT-interval values were corrected for changes in heart rate as described by Fridericia (Fridericia, 1920) and Van de Water (Van de Water, Verheyen, Xhonneux, & Reneman, 1989). Of note, the dogs included in this study were also used repeatedly for the CV safety evaluation of other drugs (~10 compounds), spanning a wide range of mechanisms, during the 27-month timespan of the study.

2.1. Statistical analysis

At baseline, defined as the mean of values 1 h prior to oral dosing, a paired two-tail *t* test was used to compare the vehicle and *dl*-sotalol treatment groups for each parameter; paired tests were used since each animal received each treatment. Within a treatment group (vehicle or *dl*-sotalol) values were also compared at Month 1, 14, and 27 (M1 *vs.* M14, M1 *vs.* M27, and M14 *vs.* M27) using a paired two-tail *t* test. For comparison of CV functional values from study-to-study during pharmacological challenge with *dl*-sotalol, values were compared on Month 1, 14, and 27 (M1 *vs.* M14, M1 *vs.* M27, and M14 *vs.* M27) using a paired two-tail *t* test at 2, 4, and 6 h post-dose. Regression analyses were performed using GraphPad Prism software.

2.2. Power analysis

For each study (M1, M14, and M27) and each animal, mean and standard deviation values from 0–18 h post-dose were calculated to enable a power analysis for endpoints mechanistically-impacted by I_{Kr} or β-blockade by *dl*-sotalol (heart rate, LV dP/dt_{max}, QTi, PRi) as well as mean arterial blood pressure. Subsequently, the analysis was performed (NQuery; paired *t* test, vehicle *vs.* treated) to calculate the estimated detectable change in selected endpoints under a series of statistical power assumptions (*e.g.* power values from = 70–95%).

3. Results

The pharmacokinetic profile of *dl*-sotalol (10 mg/kg; p.o.) was comparable across the three studies; mean *dl*-sotalol Cp at 1, 3, 5, and 24 h post-dose during each study as well as in the 4 individual dogs based on dose, independent of study, at the same timepoints are shown (Fig. 1). The observed T_{max} occurred at 2.0, 1.5, and 2.5 h post-dose at Month 1, Month 14, and Month 27, respectively; corresponding C_{max} values were 4643, 5087, and 3230 ng/mL.

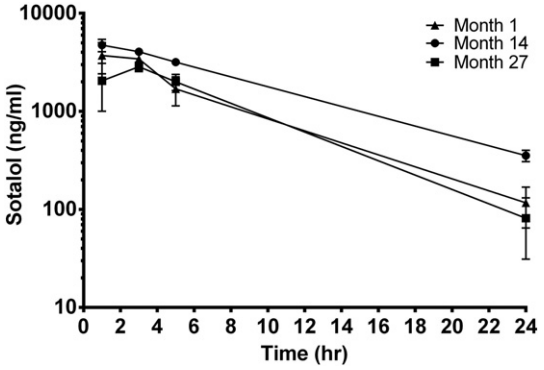

Fig. 1. Sotalol Plasma Concentrations. Plasma concentrations (mean ± SEM) of *dl*-sotalol over three studies, spanning 27 months, in telemetry-instrumented dogs (n = 4/timepoint) as measured at 1, 3, 5, and 24 h post-dose.