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Statistical power analysis of cardiovascular safety pharmacology studies in conscious rats

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ABSTRACT

Cardiovascular (CV) toxicity and related attrition are a major challenge for novel therapeutic entities and identifying CV liability early is critical for effective derisking. CV safety pharmacology studies in rats are a valuable tool for early investigation of CV risk. Thorough understanding of data analysis techniques and statistical power of these studies is currently lacking and is imperative for enabling sound decision-making.

Methods: Data from 24 crossover and 12 parallel design CV telemetry rat studies were used for statistical power calculations. Average values of telemetry parameters (heart rate, blood pressure, body temperature, and activity) were logged every 60 s (from 1 h predose to 24 h post-dose) and reduced to 15 min mean values. These data were subsequently binned into super intervals for statistical analysis. A repeated measure analysis of variance was used for statistical analysis of crossover studies and a repeated measure analysis of covariance was used for parallel studies. Statistical power analysis was performed to generate power curves and establish relationships between detectable CV (blood pressure and heart rate) changes and statistical power. Additionally, data from a crossover CV study with phentolamine at 4, 20 and 100 mg/kg are reported as a representative example of data analysis methods.

Results: Phentolamine produced a CV profile characteristic of alpha adrenergic receptor antagonism, evidenced by a dose-dependent decrease in blood pressure and reflex tachycardia. Detectable blood pressure changes at 80% statistical power for crossover studies ($n = 8$) were 4–5 mm Hg. For parallel studies ($n = 8$), detectable changes at 80% power were 6–7 mm Hg. Detectable heart rate changes for both study designs were 20–22 bpm.

Discussion: Based on our results, the conscious rat CV model is a sensitive tool to detect and mitigate CV risk in early safety studies. Furthermore, these results will enable informed selection of appropriate models and study design for early stage CV studies.

1. Introduction

Rigorous safety assessment is a crucial part of the drug development process and safety pharmacology studies are a key component of these assessments. Safety pharmacology is a sub-discipline of pharmacology, related to toxicology, which focuses on studying direct and/or indirect unwanted pharmacodynamic effects on vital physiological functions (Bass et al., 2011). Nonclinical safety pharmacology studies provide key decision making data that enable progression of novel therapeutic entities from preclinical to clinical testing. The core battery of safety pharmacology studies is designed to investigate effects on cardiovascular, central nervous and respiratory systems. Cardiovascular safety, in particular, is a major concern for any new pharmaceutical under development. Cardiovascular related toxicity is responsible for >20% attrition during clinical testing (Laverty et al., 2011). Additionally, this attrition is more prevalent during or after Phase II as compared with Phase I clinical studies (Laverty et al., 2011). Attrition of drug candidates during late clinical development delays availability to patients and adds significant cost. Furthermore, small drug-induced changes in cardiovascular parameters such as blood pressure represent a safety concern, particularly in vulnerable patient populations such as the elderly and patients with other cardiovascular risk factors and/or co-morbidities (Sager et al., 2013).Thus, identifying cardiovascular risk early and with high confidence is critical. To achieve this, it is imperative that preclinical safety studies be carefully designed to detect meaningful changes.

Cardiovascular safety pharmacology studies primarily assess effects on hemodynamics, electrocardiogram and cardiac function. Per the regulatory guidance ICH S7A, these cardiovascular data are preferably obtained from conscious, unrestrained ambulatory animals (S7A, 2001). Radio telemetry is the state-of-the-art method for collecting high quality cardiovascular data from ambulatory animals (Kramer & Kinter, 2003). Indeed, telemetry is widely utilized within the pharmaceutical

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industry to collect cardiovascular data from ambulatory animals. Definitive cardiovascular safety studies to enable first-in-human clinical trials are performed using large animals (dogs or nonhuman primates) and tend to be conducted at later stages (post-candidate selection) of preclinical development (Guth, 2007). However, use of smaller species (e.g. rats) to perform early assessments has several advantages. Due to lower body weights, cardiovascular safety assessments in rats are a bulk-sparing alternative for hazard identification early in the program life cycle. Such early evaluations provide valuable information to identify risks associated with the target mechanism or chemical structures of the molecules under assessment. Further, since rats are used for most in vivo efficacy models, a therapeutic window can be established within the same species. In a 2008 survey benchmarking industry best practices, Lindgren et al. reported that approximately 70% of the survey respondents utilized rodent cardiovascular studies as part of early frontloaded assays (Lindgren et al., 2008). The prominent use of rats in cardiovascular safety assessment is evidence of their utility within the field of safety pharmacology.

Statistical power is the probability that an experiment will successfully reject the null hypothesis when the alternative hypothesis is true, and is an important factor to consider when selecting a preclinical model and study design. From a safety pharmacology perspective, this is the ability of a model to correctly detect a cardiovascular effect when one truly exists. Main factors that influence statistical power are magnitude of effect, sample size, level of significance (i.e. p-value) and variance across sample population. Therefore, imbalance of these factors may result in an inappropriately (i.e. under or over) powered study. For a high quality study, statistical power of 80%–90% is desired (Ludbrook, 2001). Underpowered studies generally result from insufficient sample size or suboptimal study design and will likely miss a true treatment related effect. Statistical power calculations demonstrate the sensitivity of an experimental model and provide a rationale for sample size selection (Valentin, Bass, Atrakchi, Olejniczak, & Kannosuke, 2005). Therefore, it is not surprising that assessment of power is a key recommendation for best practices in conducting nonclinical cardiovascular studies (Leishman et al., 2012). Statistical power for cardiovascular safety studies in dogs has been previously reported (Chiang, Smith, Main, & Sarazan, 2004; Sivarajah et al., 2010). However, despite wide utility of rodent models within industry as well as academia, similar analysis for rat cardiovascular studies is lacking. To address this gap, we utilized a historical dataset of rat cardiovascular studies conducted internally at Pfizer to perform statistical power calculations. The results of these calculations are reported here and demonstrate the sensitivity of these studies. We also report data from a positive control compound (phentolamine) as an example of current data analysis methods used within Pfizer.

2. Methods

2.1. Animals

All experiments involving animals were conducted as per the guidelines and study protocols reviewed and approved by Pfizer Institutional Animal Care and Use Committee. Conscious, male Wistar Han rats (Charles River, Raleigh, NC US) weighing 300–600 g were used and had ad libitum access to food and water. The rats were implanted with radio telemetry transmitters (TL11M2-C50-PXT/PT or HD-S10/11, Data Science International, St. Paul, MN) for transmission of blood pressure (BP), body temperature and relative physical activity data using the PONEMAH P3 Data Acquisition System (Gould Instruments, Inc. Valley View, OH, Versions 4.9–5.2). Data were continuously acquired from ~1 h predose to ~24 h post-dose.

2.2. Study design

Studies were conducted using either a crossover or parallel dosing design. For crossover studies with three dose levels plus vehicle, a Williams square design (n = 8 rats/group) divided over 4 treatment periods was used (2 treatment periods were used for single dose level studies). On each treatment day, rats received a single dose of either vehicle or test article, with a wash out period of at least 7 half-lives between dose administrations. By the end of the study, each rat had received vehicle as well as all the doses of test article. Additional satellite groups of rats (n = 3/dose group) were also dosed with test article and blood samples were collected at serial time points to characterize the pharmacokinetic profile. No telemetry data were acquired from satellite groups. Phentolamine treatment is described as a representative example of a crossover study with dose levels of 4, 20 and 100 mg/kg or vehicle (deionized water). Results from this study are reported as mean changes from vehicle treatment.

For parallel design studies, rats were divided into separate vehicle and test article treated groups. The groups were balanced for blood pressure and heart rate values, based on a short (4 h) telemetry data collection. Prior to any treatment, approximately 24 h of baseline cardiovascular data was collected for all study animals. On the day of treatment, each rat received either vehicle or test article. Satellite groups (n = 3/group) were included in each study to determine the pharmacokinetic profile as described above.

2.3. Cardiovascular data analysis

Average values for the following parameters were logged every 60 s and reduced to 15 min mean values: heart rate (HR), systolic (SBP), diastolic (DBP) and mean (MBP) blood pressure, body temperature, and activity. Data from each animal were subsequently binned into super intervals of 0–2, 2–4, 4–8, 8–12, 12–16, 16–20, and 20–24 h post-dose (hpd) and statistical analysis was performed on each time bin as follows:

For a cross-over design, each response was analyzed using repeated-measure analysis of variance (RM-ANOVA) with a Fisher's LSD post-hoc test, accounting for variation due to animal, period, time bin, and investigating differences due to treatment. Specifically, the model was:

$$\text{Parameter} = \text{Animal} + \text{Period} + \text{Treatment} + \text{Time} + \text{Treatment} \times \text{Time}.$$

For a parallel design, each response was analyzed separately for each time period using repeated measure analysis of covariance (ANCOVA) with a Fisher's LSD post-hoc test, accounting for variation due to time bin and baseline measurements, and investigating differences due to treatment. Specifically, the model was:

$$\text{Parameter} = \text{Predose} + \text{Treatment} + \text{Time} + \text{Treatment} \times \text{Time}.$$

Additionally, a double delta analysis was also performed for parallel design studies. RM-ANOVA was applied to the change from the baseline. In this case, rather than being used a covariate, the baseline was subtracted from the post-dose measurement. Specifically, the model was:

$$\text{Change from the baseline} = \text{Treatment} + \text{Time} + \text{Treatment} \times \text{Time}.$$

Fitted means for each dose group were calculated using the parameter estimates from the ANOVA or ANCOVA model. Confidence intervals for the treatment comparisons were calculated which indicated the likely range of values of the true treatment difference. If zero was not included within the 95% confidence interval, this indicated statistical significance at the 5% level (i.e., $p < 0.05$).

2.4. Analysis of statistical power

Statistical power was calculated for crossover and parallel studies using the entire dataset (all 24 hpd data). Additionally, power was