

CHAPTER 1.

1.1 Q.T. Interval

The Q.T. interval on the electrocardiogram (ECG) represents the duration from the onset of the QRS complex to the completion of the T wave. It encompasses the time required for the ventricular tissue to repolarize after depolarization (Thomas et al. 1996). The current guideline considers a corrected Q.T. (QTc) interval > 470 (milliseconds) ms on repeated ECGs as a diagnostic cut off value for QTc lengthening for men and > 480 ms for women (Rautaharju et al., 2009).

1.1.1 Correction Method for the Q.T. Interval

The Q.T. interval is affected by changes in heart rate, and so formulas must be used to correct the Q.T. interval for heart rate. The patient's heart rate and Q.T. interval are used to calculate a corrected Q.T. (QTc) interval value (Rabkin and Cheng, 2015; Rabkin et al., 2017). Several formulas have been proposed for Q.T. interval corrections (QTc) for changes in heart rate (Bazett, 1920; Fridericia, 1920; Sagie et al., 1992; Rautaharju et al., 2004; Rabkin and Cheng, 2015). There is not a "gold standard" among the formulas. However, the most commonly studied and applied QTc formulas are the Bazett, Fridericia, Framingham, and Rautaharju (Indik et al., 2006; Vandenberg et al., 2016; Rabkin et al., 2017). The Bazett formula is the most extensively used correction method in clinical practice, and the United States Food and Drug Administration (FDA) has recommended the Fridericia formula for clinical trials on drug safety (Phan et al., 2015).

In 1920, Henry Cuthbert Bazett proposed the Bazett formula (Eq. 2). The formula performs best with a heart rate range of 60–100 beats/min (Bazett, 1920). However, the formula is criticized because of its inaccuracy as it was only derived from 39 subjects and is supposedly found to "overcorrect" the measured Q.T. interval at heart rates > 100 bpm and "under correct" at heart rates < 60 bpm. (Sagie et al., 1992). Despite the critics, the Bazett formula remains a frequently used correction approach (Manion et al.; Ahnve, 1985; Sage et al., 1992). It is still widely used because

the thresholds for torsades de pointes risk have been determined using Bazett's formula (> 500 ms for marked increase risk of torsades; > 470 (men) and > 480 (women) for QTc prolongation. (Rautaharju et al., 2009).

$$QT_{cB} = QT\sqrt{RR} \quad (1)$$

The Framingham formula (QTcFra) was proposed by Sagie et al. in 1992 and was supported by a study of around 5000 patients from the Framingham population in the USA (Sagie et al., 1992). This linear equation (Eq. 3) gave a more uniform QTc over a broader range of heart rates and is considered a better and preferred formulas then Bazett's (Vandenberk et al., 2016). However, the Framingham formula is inconvenient for use at the bedside (Luo et al., 2004).

$$QT_{cFra} = QT + 0.154(1 - RR) \quad (2)$$

The Fridericia formula was derived by Louis Sigurd Fridericia in 1920 from observations of 50 healthy subjects aged 3 to 81 years. Fridericia found that taking the cube root of the R.R. interval gave the best adjustment formula in comparison to Framingham. The formula (Eq. **Error! Reference source not found.**) provides more accurate QTc values in subjects with tachycardia but has the same limitations as Bazett's formula for slow heart rates (Rautaharju and Zhang, 2002). Bazett's and Fridericia's formulas are logarithmic corrections (Fridericia, 1920).

$$QT_{cFri} = \frac{QT}{\sqrt[3]{RR}} \quad (3)$$

1.2 Electrophysiology of Ventricular Repolarization

The Q.T. interval measured on the ECG indicates the total duration of the ventricular electrical systole and diastole. The Q.T. interval includes the ventricular depolarization time (indicated by the QRS duration) and the repolarization time (indicated by the T wave) (**Figure 1. illustrations of Ventricular Action Potential Corresponded to Electrocardiogram (ECG)**

) (Locati et al., 2017). The transition of ventricular depolarization into ventricular repolarization conforms to the J-point on a standard ECG (Gussak et al., 1995). To appreciate the role of the Q.T. interval in evaluating ventricular repolarization, it is of utmost importance to understand the role of the cardiac action potential and the major ion channels involved in the process.

Figure 1. illustrations of Ventricular Action Potential Corresponded to Electrocardiogram (ECG)

All mammalian cardiac cells have channels embedded in the plasma membrane to allow for the inward and outward movement of ions in the cells. These cardiac ion channels are essential in shaping the action potential. The channels may be open, closed, or inactivated, depending on changes in cardiac membrane potential. Depolarization occurs as a result of the coordination of action potentials in the ventricles and atria, whereas repolarization occurs as a result of the recovery and return of membrane potential to the baseline resting phase (Lester, Pagliarunga et al. 2019). The essential ions responsible for the membrane potential are Na^+ , K^+ , Ca^{2+} , and Cl^- . The intracellular concentration of K^+ is higher, and the concentration of Na^+ , Ca^{2+} , and Cl^- are higher outside the cell than inside (Klabunde 2017). In a normal cardiac cycle, there is an inward flow of positive ions (mainly sodium and calcium currents) activating depolarization and outward movement of positive ions (mainly potassium currents) activating repolarization. There are five distinct phases (0 through 4) to the cardiac action potential, as described below, the start and end being the baseline resting phase (4) (Locati, Bagliani et al. 2017).

- Phase 0 (Rapid depolarization): A sudden influx of Na^+ ions (I_{Na}) through Na^+ channels produces an abrupt spike of the action potential.
- Phase 1 (Notch or rapid repolarization): A partial repolarization following rapid depolarization due to closure of Na^+ channels and the temporary outward movement of K^+ (I_{to}) via the K^+ channels.
- Phase 2 (Plateau): The cardiac muscle fiber is in the depolarized state. There is a balance of Ca^{2+} (I_{Ca}) inward via the opening of L-type Ca^{2+} channels and outward of potassium through closure of rectifier K^+ channels.

- Phase 3 (Repolarization): Cardiac repolarization primarily occurs due to the K^+ efflux through delayed rectifier current (I_{Kr} and I_{Ks}). I_{Kr} is the current primarily responsible for ventricular repolarization.
- Phase 4 (Diastolic): The resting membrane potential or polarized state is maintained by the sodium-potassium ATPase pump, Na^+/Ca^{2+} exchanger, and the inward rectifier K^+ current (I_{K1}).

Any changes in phase 3 occurring in the absence of dispersion of depolarization are considered a primary reason for Q.T. prolongation, which indicates a lengthening of the refractory period of cardiac tissue. Q.T. prolongation may be associated with blockade or modification of K^+ efflux through delayed rectifier currents (I_{Kr} and I_{Ks}) (Roden 2008). A fundamental assumption in the interpretation of cardiac electrophysiology states that a Q.T. prolongation is secondary to an increased action potential duration in at least a few regions of the ventricle. This increase in action potential duration, in turn, indicates a decrease in outward current or an increase in inward current (Keating and Sanguinetti 2001).

1.3 Drug-Induced QTc Prolongation and TdP

Prolongation of the Q.T./QTc can point to a potentially deadly ventricular arrhythmia known as Torsade de pointes (TdP) which was first described by the French physician Francois Dessertenne in 1966 (Dessertenne 1966). Torsade de pointes is an unusual polymorphic ventricular tachycardia characterized by a continuously changing amplitude and the twisting appearance of the QRS complexes around the isoelectric line on an ECG, that first appear positive and then negative (Drew, Ackerman Michael et al. 2010). The mechanism of the relationship between the duration of the Q.T. interval and the risk of TdP remains inconclusive. However, in general a QTc of higher than 500 ms or an increase in the QTc interval greater than 60 ms is usually considered to confer a high risk of TdP in an individual patient (Heist and Ruskin 2005, Barnes and Hollands 2010).

1.3.1 Risk Factors for Drug Induced QTc Interval Prolongation

Major factors prolonging the prevalence of QTc interval prolongation and TdP include Q.T. prolonging drug therapy, electrolyte abnormalities (e.g. a decrease in serum potassium and elevated in serum magnesium and calcium), and genetic polymorphisms that increase TdP risk. Other evident risk factors include a QTc interval >500 ms, a ≥ 60 ms increase in QTc interval from the pretreatment value, female sex (females have a two-fold increased risk compared to males), advanced age, heart failure with reduced ejection fraction, acute myocardial infarction, bradycardia, use of diuretics, inadequate dose adjustment of renally eliminated drugs in patients with kidney disease, elevated plasma concentrations of QTc interval prolonging drugs caused by drug interactions, and rapid intravenous administration of high risk drugs (Tisdale et al., 2016). Also, the multiplicity of risk factors further raises a patient's risk of TdP, and if not properly managed, TdP can progress to ventricular fibrillation (V.F.) or sudden cardiac death (SCD) (Li, Esterly et al. 2010).

Abnormal Q.T./QTc prolongation is of two categories, the congenital (cLQTS) and the acquired (aLQTS). The congenital form was first described in 1975 and is a relatively uncommon but crucial clinical disorder. Congenital Long-QT syndrome is subdivided based on the underlying gene in which causative mutations occur. The most common forms are LQT1, LQT2, and LQT3, respectively. The clinical manifestations of LQT syndrome involve syncopal episodes, which can, albeit uncommonly, include sudden death due to cardiac arrest. It has been demonstrated that QTc-prolonging medications increase the risk of SCD in LQT patients two to eight times higher (Straus et al., 2005; Straus et al., 2006).

1.3.2 Drug-Induced QTc Interval Lengthening in Patients with Heart Failure

Patients with heart failure (H.F.) are at a threefold increased risk of developing TdP (Lo and Hsia 2008). As heart failure progresses there are alterations in the expression and function of several ion channels, including downregulation of KCNH2 (Beuckelmann, Nabauer et al. 1993, Nabauer, Beuckelmann et al. 1993, Tomaselli and Marbán 1999). The KCNH2 gene, also referred to as the human Ether-a-go-go Related Gene (hERG), encodes a potassium channel responsible for the rapid

component of the delayed rectifier outward K^+ current (I_{Kr}) in the heart (Sanguinetti, Jiang et al. 1995). I_{Kr} is crucial for ventricular repolarization, and inhibition of this current prolongs the Q.T. interval on a surface ECG and can influence the development of TdP (Vandenberg, Perry et al. 2012). Even alterations in KCNH2 function that do not present as Q.T. interval prolongation can increase susceptibility to arrhythmias by increasing the dispersion of repolarization or by the depletion of the repolarization reserve (Wu, Rajamani et al. 2009, Yasuda, Yasuda et al. 2015). The term repolarization reserve refers to the redundancy of outward currents that drive repolarization. In addition to I_{Kr} , the slow-component of the delayed rectifier current (I_{Ks}), contributes to ventricular repolarization, particularly during rapid heart rates or when I_{Kr} is compromised. Consequently, the decrease of a single outward potassium current, such as I_{Kr} , may not lead to noticeable changes in QT-interval.

1.4 miRNA Biogenesis and Function

Over the last decade, microRNAs (miRNAs) have been identified to regulate genes important for proper cardiac function. miRNAs are short, 22 base-long segments of non-coding RNA that are involved in the regulation of gene expression in a wide variety of cellular processes (Van Wynsberghe, Chan et al. 2011). The first miRNA (lin-4) was observed in *C. Elegans* in 1993 (Lee, Feinbaum et al. 1993). Since then, hundreds of miRNAs have been discovered in several species, particularly emphasizing their role in embryonic development and disease regulation (Pritchard, Cheng et al. 2012). It is not recognized that miRNAs are evolutionarily conserved among species, and account for up to 3% of the mammalian genome (Bartel 2009).

miRNAs are transcribed by RNA polymerase II to generate a sizeable stem-loop structure called the primary miRNA (pri-miRNA) (Cai, Hagedorn et al. 2004). The stem-loop structure in the nucleus is bound by an RNase Drosha complex and its cofactor Dgcr8, which cleaves the structure to produce the hairpin loop-shaped precursor miRNA (pre-miRNA) (Denli, Tops et al. 2004). The pre-miRNA is then exported to the cytoplasm by the nuclear transport receptor Exportin5, and the nuclear G-protein Ran-GTP (Bohnsack, Czaplinski et al. 2004). In the cytoplasm, another RNase known as Dicer cleaves the loop off of the stem-loop structure, thus producing a duplex nucleotide containing miRNA. Finally, one strand from the duplex degenerates, and the other strand is assimilated into the RNA induced silencing complex (RISC) which includes Argonaute, the

catalytic unit of the complex (Schwarz, Hutvagner et al. 2003, Du and Zamore 2005). The mature RNA in the RISC complex finds the target mRNA by binding to the 2-8 nucleotide "seed region" at the 3' end of the target mRNA. This results in translational repression or mRNA degradation and hence silences the target gene (Pasquinelli 2012). The miRNA-RISC complex interaction with the 3'-untranslated regions (3' UTR) of the target gene to silence gene expression is well accepted. However, many studies have shown that a few miRNAs can also interact with the 5'-UTR (Gu, Xu et al. 2014, Broughton, Lovci et al. 2016), coding sequences (Brummer and Hausser 2014), and can even activate translation (Xiao, Li et al. 2017). This interaction is dependent on various factors, including subcellular localization, miRNA abundance, and the level of complementation of the miRNA-mRNA complex (O'Brien, Hayder et al. 2018).

The sequencing of the human genome in 2001 has paved a path for expanded identification of new miRNAs and their target genes. As more miRNAs have been identified, the research focus has shifted towards the functional characterization of miRNAs as they relate to human diseases and developmental anomalies. Many miRNAs have been found to play important roles in critical biological processes, including cell division and death, immunity, signal transduction, metabolism, and cell locomotion (Li and Kowdley 2012). Given this, the aberrant expression of several miRNAs associated with cancer, viral pathogenesis, and immune dysfunction was not surprising. Among the various conditions, cancer is the most prominent human disease that has a stable association with miRNA mis regulation. The earliest association was investigated in 2002, wherein the deletion of miR-15 and miR-16 was observed in 65% of patients with B-cell chronic lymphocytic leukemia (Calin, Dumitru et al. 2002). Widespread expression profiling studies further strengthened the association with miRNA dysregulation and increased susceptibility to various cancers, most notably, breast cancer and lung cancer. Moreover, miRNA expression patterns have been assessed to pinpoint the cancer progression stages (Acunzo, Romano et al. 2015). In addition to their substantial role in cancer development and progression, miRNAs have been implicated in degenerative diseases, especially neurodegenerative diseases like Parkinson's and Alzheimer's (Sharma and Lu 2018). Also, they have been implicated in a variety of other degenerative disorders of bone (van Wijnen, van de Peppel et al. 2013), retina (Loscher, Hokamp et al. 2008), muscle (Wang, Yang et al. 2018), and heart (Ultimo, Zauli et al. 2018).

1.4.1 microRNA & Regulation of Gene Expression

MicroRNAs regulate gene expression by direct base pairing to the 3'UTR region of target transcripts (Oliveto, Mancino et al. 2017). The miR-3'UTR complex disrupts translation by silencing the gene or promoting degradation of mRNA. The characteristics and function of miRNA and antisense miRNA (anti- miRNA) have been studied as potential therapeutic agents. Anti-miRNA consist of reverse complementary sequences that lower endogenous miRNA expression and enhance gene expression (Marquez and McCaffrey 2008). Miravirsen is the first anti-miR (inhibits miR-122) that has been FDA approved in patients with viral hepatitis C (Hu, Xu et al. 2012). The results from the clinical trials demonstrated that miravirsen is well-tolerated; hence the enthusiasm for developing other anti- miRNA -based drugs has increased.

The distribution of miRNA or anti-miRNA to cardiac muscle tissue remains a challenge due to several physiological and cellular obstacles experienced *en route* to target cells (Guzman-Villanueva, El-Sherbiny et al. 2012). Nevertheless, pathophysiological signal targeting mechanisms that enhance miRNA expression may be useful to normalize the expression of gene targets. However, the mechanisms of the physiologic and pathophysiologic regulation of miRNA expression is an underserved area of research. This provides an opportunity for the development of pharmacological inhibitors targeting the regulatory pathways of miRNA in diseases such as H.F. Furthermore, the circulation of miRNA in the blood makes them attractive as potential biomarkers for clinical diagnosis (Reid, Kirschner et al. 2011, Paraskevi, Theodoropoulos et al. 2012).

1.4.2 miRNAs in Cardiovascular Function and Anomalies

miRNAs are expressed in cardiovascular tissue and have been identified as regulators of cardiovascular function. In addition to serving an essential role in cardiac development, they play a role in the pathogenesis of various cardiovascular diseases, including cardiac arrhythmias, myocardial infarction, atherosclerosis, and coronary artery disease (Romaine, Tomaszewski et al. 2015). The cardiac-specific knockout of the endonuclease Dicer, which effectively disrupts all miRNAs, results in severe cardiac developmental anomalies, pericardial edema, and death (da

Costa Martins, Bourajjaj et al. 2008). A global genomic study on healthy human heart muscle in 2008 revealed a handful of miRNAs including miR-1, miR-16, miR-27b, miR-30d, miR-126, miR-133, miR-143, miR-208 and let-7 which were found abundantly in cardiac tissue compared to other tissues. Taken together, this suggests that miRNAs have a definite role in cardiac muscle function and maintenance (Landgraf, Rusu et al. 2007, Thum, Catalucci et al. 2008). Current research has revealed more specific functions for these miRNAs, along with the identification of newly associated miRNAs in cardiac disease. For example, miR-1 has been identified as an indicator of an increased risk for heart failure development (Sygitowicz, Tomaniak et al. 2015). Additionally, miR-126 is upregulated in response to vascular injury of the heart and is crucial for cell proliferation and maintenance (Chistiakov, Orekhov, et al. 2016). Any loss of its expression has been associated with atrial fibrillation and heart failure due to loss of its protective function against cardiac hypoxia (Wei, Han et al. 2015).

1.4.3 Dysregulations of miRNA in Patients with Heart Failure

There is increasing evidence suggesting that miRNA dysregulation is associated with heart failure and comorbid pathological conditions. Both miR-1 and miR-210 regulation are positively correlated with New York Heart Association (NYHA) functional classifications (Endo, Weng et al. 2013, Sygitowicz, Tomaniak, et al. 2015, Zhou, Jin, et al. 2018). In addition, decreased expression of the miRNAs let-7i, miR-18b, miR-18a, miR-223, miR-301a, miR-652, and miR-423 are correlated with increased risk of mortality in H.F. patients (Ovchinnikova, Schmitter, et al. 2016). Contrarily, increased levels of miR-1254 and miR-1306 are correlated with hospitalization and death, and increased levels of miR-208b and miR-499 are strongly correlated with the development of H.F. and death (Bayes-Genis, Lanfear, et al. 2018).

The screening and examination of miRNA expression profiles in healthy and failing human hearts has been accomplished with deep sequencing and bioinformatics. Around 250 miRNAs were differentially expressed in failing human hearts compared to that of controls; high expression of miRNAs was observed in patients with refractory end-stage heart failure. (Leptidis, El Azzouzi, et al. 2013).

1.4.4 miR-362-3p Targets KCNH2

The mechanism for downregulation of KCNH2 during heart failure and the potential role of miRs regulation is unknown. Our laboratory have correlated five miRs with KCNH2 mRNA down-regulation and increased in patients with a single nucleotide polymorphism (SNP) linked to Q.T. interval lengthening (Shao M., et al 2013). Through *in vitro* and *ex vivo* examination, miR-362-3p has been identified as binding to the 3'UTR region of KCNH2, resulting in decreased KCNH2-mRNA and protein expression leading to lower I_{Kr} density.(Assiri A., et al. [2019](#))

miR-362-3p is endogenously expressed in the heart, and further deep sequencing showed that it is upregulated during cardiac hypertrophy (Leptidis, El Azzouzi et al. 2013). It has also been suggested that miRNAs may be promising biomarkers in the diagnosis H.F. both in patients with reduced ejection fraction (HFrEF) and with preserved ejection fraction (HFpEF) (Schulte, Westermann, et al. 2015). Interestingly, our laboratory has demonstrated that miR-362-3p is upregulated in cardiac tissue during HFrEF(Mourad NA., et al 2015). The ventricular tissue of patients HFrEF demonstrated a 60% increase in miR-362-3p coupled with a 40% reduction in KCNH2 expression. These data support that increased expression of miR-362-3p downregulates KCNH2 to increase the susceptibility to arrhythmias during HFrEF. Overall there is clear evidence to advocate the crucial role of miRNAs in heart failure pathogenesis and their use as a potential biomarker in heart failure therapy.

1.5 Population Pharmacokinetics Pharmacodynamic Model Concept

Population pharmacokinetic (P.K.) and pharmacodynamics (P.D.) modeling techniques assist in the identification of inter-and intra-individual vulnerability that affect drug safety and efficacy (Sun, Fadiran et al. 1999). The P.K. modeling concept was first introduced by Sheiner et al. (1972) and describes the relationship between drug concentration(s) and Time. Initially, P.K. modeling dealt with sparse P.K. concentration data collected during therapeutic drug monitoring (Sheiner and Beal, 1980), but later expanded to include a PK-dependent model. The term population pharmacokinetics is defined as the study of the Time that described the drug concentration and to correlate the drug exposure variability between individual representative. (Aarons 1991). According to Mould et al. (Mould and Upton 2012), population pharmacokinetics

is "the study of pharmacokinetics at the population level, in which data obtained from all individuals in a population are evaluated simultaneously using a non-linear mixed-effects model."

Traditional P.K. studies are designed with an exhaustive sampling schedule and collect multiple samples at fixed intervals. The population P.K. method, on the other hand, is designed to include sparse sampling schedules but in a larger number of patients. These patients are usually given similar or different doses of drugs, and blood samples from these patients are taken from different time intervals (Charles 2014). Pharmacokinetic modeling is an important part of the drug development and is an extensive investment for new therapeutic agents. As such, P.K. assessment involve a complex process requiring the rigorous procedures necessary to provide orderly data, appropriate computing platforms, adequate resources and effective delivery (Mould and Upton 2012).

1.5.1 Non-linear Mixed-effects Modeling Concept

The term population P.K. usually refers to 'mixed-effects models' (parameterization), which is a combination of fixed and random effects Parameters that do not differ across individuals are known as the model's fixed effects (structural). In contrast, in the random effects or variance model are parameters vary across individuals and include parameters such as inter-subject variability, which may account for the remaining unexplained variation after fitting the model to the data (Mould and Upton 2012, Charles 2014).

1.5.2 Aspects of PK/PD Modeling

There are four key sequential processes generally required to develop population pharmacokinetic modeling aspects; (1) dataset, (2) structural analysis model, (3) inter-subject and intra-subject variability (statistical) model, and (4) covariate models. The structural models generally describe the typical drug concentration versus time relationship of the population. They can include the systemic model (e.g. description of kinetics following intravenous dosing) and the absorption model (e.g. description of drug uptake in the blood for extravascular dosing). The inter-subject and intra-subject variability model detail the random variability in the population (e.g. residual,

occasional and inter-subject). Covariate models detail the variability predicted by covariates of the individual study subjects. Non-linear mixed-effect modeling combines the models, applying an estimation method for the determination of structural analysis model, inter-subject and intra-subject variability model, and covariate model parameters that describes the data (Bonate 2011).

1.5.3 Statistical Modeling

The development of an appropriate inter-subject and intra-subject variability model is essential for proper evaluation of PK/PD data, as well as for simulation. In population P.K. models, three inter-subject and intra-subject variability (statistical) models are used (Lala and Gobburu 2012). The between-subject variability (BSV) or interindividual variability model indicates a random unexplained variance between subjects, while the between occasion variability (BOV) model describes individual variance between different occasions. Within subject variance (WSV) or residual variability model describe unknown or unexplained variability despite controlling for known sources of variability. The WSV, also known as intra-individual variability, may represent model misspecification and error in the measurement of the assay. The parameters obtained from these inter-subject and intra-subject variability models quantify random, unknown variations. (Mould and Upton 2012, Burger, Fiebig, et al. 2014).

Between-Subject Variability (BSV)

It is unlikely that a population model parameter will be consistent across all individuals. Therefore, model parameters are assumed to have variability among all individuals. However, this assumption cannot always be accurately determined due to incorrect or insufficient data collection to estimate the variability of a particular parameter in a population. Depending on the sampling a model may need to be simple and consider a particular parameter to be a fixed effect (Lacey, Keene et al. 1997, Limpert, Stahel et al. 2001, Mould and Upton 2013). Consequently, it is commonly necessary to have a model with some fixed parameters across all individuals and some parameters with variability across individuals. In some instances, the choice of modeling the variability often depends on the type of data provided that the variability between individualTime be estimated (Mould and Upton 2013).

Between-Occasion Variability (BOV)

Individual P.K. parameters can vary with Time, and the source of the variability is determined by the occasion. Initially, between-occasion variability (BOV) was defined as an residual unexplained variability component (RUV) (Karlsson and Sheiner 1993) and was later cited as a component of BSV. The BOV should be evaluated whenever appropriate. As reported by Karlsson and Sheiner (1993), bias was observed to exist in both variance and structural parameters when BOV was completely ignored. However, the degree of bias depended on the design of the study, the magnitude of the BOV, and the inter-individual variability. Failure to consider BOV may result in an increased chance of statistically significant bias in the period of Time in parameter estimates. In particular, the omission of BOV could lead to a misapprehension of therapeutic drug monitoring. For the determination of BOV, intensive serial sample of the P.K. data on each subject must always be collected on each occasion.

Within-Subject Variability

Within-subject variability (WSV), or RUV, arises from a variety of sources, such as assay variability, incorrect sample time collection, dosing errors, and model misspecification. The WSV model selection usually depends on the type of data evaluated and is similar to the BSV model (Mould and Upton, 2013). The model that determines the within-subject variability is known as the residual variance model. The more heterogeneous and substantial the residual variance, the higher the need for it to be included in the overall model. WSV depends on specific covariates, such as changes in the assay between studies, variations in the execution of studies, or the involvement of different patient populations requiring different WSV models (Mould, Holford, et al. 2002).

1.5.4 Covariate Model Analysis

One of the critical terms in population P.K. study is "covariate," which relates to any variable that is limited to an individual. Covariates are categorized into intrinsic factors such as those that are genetically determined or inherited, or age, height, weight, and race; and extrinsic factors that are influenced by the external environment such as drug dose, degree of medication adherence, smoking habits, and presence of adjuvant medications (Holford, 2013). Covariates can be

classified as continuous (e.g., age), dichotomous (e.g., sex), or polychotomous/categorical (e.g., race). Generally, intrinsic covariates do not differ over a short duration, whereas extrinsic covariates can differ during the course of a study. (Bonate 2011). The covariate modeling method can be univariate or multivariate, and various methods for covariate selection have been described, including the classical stepwise covariate modeling method.

1.6 Pharmacodynamic (P.D.) Modeling Concept

Pharmacodynamics (P.D.) is the study of the effect of drugs on the body. In other words, P.D. represents a drug effect at the physiological, biological, and molecular levels (Benet and Zia-Amirhosseini 1995, Campbell and Cohall 2017). The recognition of P.D. science began in the mid-1960s with the work of Dr. Gerhard Levy, who first described an association between drug concentrations and reversible drug reactions using mathematical equations (Levy 1964, Levy 1966). Since then, P.D. modeling has emerged as a significant field to mathematically outline drug effects (Mager, Wyska, et al. 2003). P.D. modeling is crucial for regulatory review of new therapeutics (Huang, Abernethy, et al. 2013).

Population P.D. models evaluate the physiological and biochemical effects of drugs on the body at a population level using non-linear mixed-effect modeling approaches. The evolution of the population P.D. model approach is similar to P.K. modeling and addresses the same significant aspects (data, structural analysis model, inter-subject and intra-subject variability model, and covariate model) of P.K. modeling. (Upton and Mould 2014). Different types of P.D. models range from linear models to more complex models. The relationship between plasma concentrations and P.D. response of a drug whether beneficial or adverse is evaluated using a static function such as a E_{max} model (Holford, 2011). Nevertheless, some drugs require Time for their response due to an equilibration delay between the plasma compartment and the site of action. This delay is seemingly not related to plasma concentrations but is associated with effect-compartment ('link') model (Hull et al., 1978; Stanski and Maitre, 1990).

Pharmacodynamic models can also be classified according to the time dependency, known as time-invariant and time-variant models. Time-invariant models are so named because the P.D. parameters remain constant over Time. This model is sufficient for most drugs because their

changes are directly related solely to their concentration at the effect site. However, with some drugs, the P.D. parameters can change as a result of tolerance (i.e. a decrease in the response) (Meibohm & Derendorf, 2002) or sensitization (decrease in the number of receptors, or in the receptor affinity) in the patient's system (Ploeger, van der Graaf, & Danhof, 2009).

1.6.1 The Indirect Pharmacodynamic Response Model

P.D. modeling can use a mechanism-based approach to offer insight into the mechanistic process behind drug effects. These mechanistic response models are commonly sub-classified as indirect pharmacodynamic models, in which the effect of a drug indirectly related to secondary or a more intermediate response steps (Agoram, Martin, & van der Graaf, 2007; Danhof, de Lange, Pasqua, Ploeger, & Voskuyl, 2008). This classification can be dependent upon the pathophysiology of the disease state and requires an understanding and consideration of the mechanisms. Indirect response models are used to study the P.D. of a wide variety of drugs with an indirect mechanism of action. The first formal proposal of indirect response models was presented as four basic models by Dayneka and colleagues (Dayneka, Garg, et al. 1993). These basic models include inhibition of the production variable (k_{in}) (Model 1), inhibition of the dissipation variable (k_{out}) (Model 2), stimulation of the production variable (k_{in}) (Model 3), and stimulation of the dissipation variable (k_{out}) (Model 4), as presented in **Figure 2. Structure of four basic indirect response models**

Model 1 inhibition of the production variable (k_{in}), Model 2 inhibition of the dissipation variable (k_{out}), Model 3 stimulation of the production variable (k_{in}), and Model 4 stimulation of the dissipation (variable (k_{out})).

(Jusko and Ko 1994, Sharma and Jusko 1998)

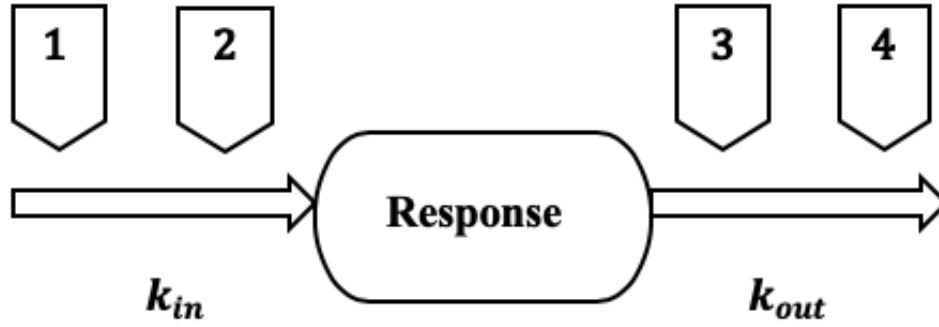


Figure 2. Structure of four basic indirect response models

Model 1 inhibition of the production variable (k_{in}), Model 2 inhibition of the dissipation variable (k_{out}), Model 3 stimulation of the production variable (k_{in}), and Model 4 stimulation of the dissipation (variable (k_{out})).

Model 1 (Inhibition- k_{in})

Model 1 outlines the drug response formed as a consequence of inhibiting the factors regulating the output of the response variable k_{in} . The I_{max} and IC_{50} inhibition mechanism are known to operate on a k_{in} as shown in Eq. **Error! Reference source not found.:**

$$\frac{dR}{dt} = k_{in} \left(1 - \frac{I_{max} \times C_p}{IC_{50} + C_p} \right) - k_{out} \times R \quad (3)$$

(k_{in} is the zero order rate constant. K_{out} is the first order rate constant. I_{max} is the maximum fractional ability of the drug to influence the inhibition processes. IC_{50} is the concentration that produces half of the maximum inhibition. The C_p value is the plasma drug concentrations).

Model 2 (Inhibition- k_{out})

Model 2 describes the drug response formed as a consequence of inhibition of the factors influencing the response variable k_{out} dissipation. The mechanism of inhibition with an IC_{50} is considered to function on k_{out} as shown in Eq. **Error! Reference source not found.** **Error! Reference source not found.** **Error! Reference source not found.** **Error! Reference source not found.** below:

$$\frac{dR}{dt} = k_{in} - k_{out} \left(1 - \frac{I_{max} \times C_p}{IC_{50} + C_p}\right) R \quad (4)$$

(I_{max} is the maximum fractional ability of the drug to influence the inhibition processes. IC_{50} is the concentration that produces half of the maximum inhibition. The C_p value is the plasma drug concentrations, R response).

Model 3 (Stimulation- k_{in})

Model 3 describes the drug response formed as a consequence of stimulating the factors regulating the response variable k_{in} development. The mechanism of stimulation process with SC_{50} is considered to function on the k_{in} as shown in Eq. **Error! Reference source not found.** below:

$$\frac{dR}{dt} = k_{in} \left(1 + \frac{S_{max} \times C_p}{SC_{50} + C_p}\right) - k_{out} \times R \quad (5)$$

(S_{max} is the maximum fractional capacity of the drug to affect stimulation. SC_{50} is the concentration which produces half of the maximum stimulation. C_p is the concentration of the plasma, R response).

Model 4 (Stimulation- k_{out})

Model 4 describes the drug response produced as a result of stimulating the factors influencing the response variable k_{out} dissipation. The mechanism of stimulation process with SC_{50} is considered to function on k_{out} as shown in Eq. **Error! Reference source not found.** below:

$$\frac{dR}{dt} = k_{in} - k_{out} \left(1 + \frac{S_{max} \times C_p}{SC_{50} + C_p}\right) R \quad (6)$$

(S_{max} is the maximum fractional capacity of the drug to affect stimulation. SC_{50} is the concentration which produces half of the maximum stimulation. C_p is the concentration of the plasma, R response).

1.7 Summary

In summary, Q.T. interval prolongation can lead to a life-threatening ventricular arrhythmia known as torsade de Pointes (TdP), resulting in sudden cardiac death. The extent of drug-induced Q.T. prolongation is highly variable in heart failure (H.F.) patients, and thus TdP is mostly unpredictable. Through in vitro and ex vivo examination, miR-362-3p has been identified as binding to the 3'UTR region of KCNH2, resulting in decreased KCNH2-mRNA and protein expression to lower IKr density. (Assiri A., et al. 2019) Therefore, developing a semi-mechanistic model to describe serum miR-362-3p expressions as a potential biomarker to predict susceptibility to drug-induced QT-interval Lengthening in patients with H.F. may represent a meaningful clinical diagnostic marker to identify high-risk patients.

We hypothesis that subject with higher level of serum miR-362-3 expressions are expected to be more susceptible to drug induced Q.T. interval lengthening .

1.8 Project Objective:

The primary objective was to develop a semi-mechanistic model to describe serum miR-362-3p expressions as a potential biomarker to predict sensitivity to ibutilide induced-QT interval prolongation in patients with H.F. Thus, we hypothesize that subjects with a higher level of serum miR-362-3 expressions are expected to be more susceptible to drug-induced Q.T. interval lengthening.

CHAPTER 2. METHODS

1.9 METHODS

The dataset utilized for the development of the population PK-PD model was acquired from a previously published clinical trial by our research group (Tisdale et al., 2020). This study was originally designed as a prospective, single, parallel comparative study to determine the impact of heart failure with reduced ejection fraction (HFpEF) on sensitivity to drug-induced Q.T. prolongation.

2.0 Ethical Aspect

The study was approved by the Indiana University Purdue University Indianapolis (IUPUI) Institutional Review Board (IRB). All patients provided written informed consent.

2.2 Study Subjects

Subjects were enrolled into one of the three study groups (1) heart failure with reduced ejection fraction (HFrEF), (2) HFpEF, and (3) Control group without documented H.F. that was matched to the HFpEF and HFrEF groups for age and sex. Subjects enrolled in the HFpEF group met the following criteria; symptomatic H.F., left ventricular ejection fraction (LVEF) $\geq 50\%$ within the previous 2 years, and 1 or more of the following: $E/A > 1.5$; $E/A < 0.5$; maximum left atrial volume

index of >40 mL/m²; and diastolic dysfunction grade 2 or greater. The subjects were enrolled based on the inclusion and exclusion criteria below.

2.3 Eligibility Criteria:

In order to qualify for the study, subjects had to weigh between 60 and 130 kg. Serum magnesium concentrations were required to be less than 1.8 mg/dL, and serum potassium concentrations less than 3.8 mEq/L. Qualified subjects had hepatic transaminases less than three times the normal upper limit, hematocrit levels above 26%, and creatinine clearances of at least 20 mL/minute. Subjects were excluded from the study if they were pregnant or lactating women, had an individual or family history of TdP, long Q.T. syndrome, or sudden cardiac death (not associated with a myocardial infarction), or had experienced New York Heart Association (NYHA) defined class IV heart failure. Subjects were also excluded for permanently paced ventricular rhythm or sustained arrhythmias, including atrial fibrillation. Patients who had been given Vaughan Williams Class 1a drugs or class III antiarrhythmic drugs within five half-lives of ibutilide infusion, had a baseline QTc interval above 450 ms, or had adjuvant use of Q.T. interval-prolonging drugs were also excluded.

2.3 Study Design

The design included two phases (1) a study day dedicated to a screening phase and (2) a study day dedicated to the treatment phase. During the screening phase, enrolled subjects remained for 12 hours at the Indiana Clinical Research Center (ICRC) for baseline Q.T. measures. Twelve lead ECGs (Marquette Mac 5500, G.E. Healthcare Biosciences, Pittsburgh, Pennsylvania) were serially collected in triplicate (approximately 1 minute apart) at the following pre-specified time points; 0, 15, 30, and 45 minutes, and 1, 2, 4, 6, 8 and 12 hours. In the treatment phase, subjects received a single IV dose of ibutilide (0.03 mg/kg) diluted in 50 ml of normal saline and administered slowly via infusion pump over 10 minutes at the ICRC. Following IV infusion, triplicate 12 lead ECG readings and venous blood samples (10 mL) were collected at baseline from all the subjects and at the following pre-specified times; end of infusion and at 5, 10, 15, 20, 30, and 45 minutes, and 1, 2, 4, 6, 8, and 12 hours post-infusion.

2.4 QT Interval Measurements and Q.T. Interval Correction

Triplicate 12-lead ECGs were collected during both phases of this trial. The R.R. and Q.T. intervals were measured from leads II, V1, and V5 using electronic calipers in the the MUSE automated system (G.E. Healthcare Biosciences, Pittsburgh, PA). The triplicate ECG readings were collected approximately 1 minute intervals from pre-specified time points. In the second visit, (the treatment/ibutilide phase. The triplicate ECG readings were done at not later than 1-minute intervals from pre-specified time points, as described in the following: at 0 minutes, at the end of infusion, at 5, 10,15, 20, 30, and 45 minutes, and later on at 1, 2, 4, 6, 8 and 12 hours. The Q.T. intervals were corrected for the heart rate effect using the Fridericia formula, where the observed Q.T. interval is divided by the cube root of the R.R. interval, as shown in Equation **Error! Reference source not found. Error! Reference source not found.Error! Reference source not found.Error! Reference source not found.**(Piotrovsky 2005).

$$QT_{cF} = \frac{QT}{\sqrt[3]{RR}} \quad (7)$$

QT_{cF} is the Fredicria corrected Q.T. R.R. is the interval between two R wasves measured in milliseconds.

2.5Serum Ibutilide Concentration Assay

An assay of ibutilide serum concentration was determined from the blood (10 mL) samples obtained using an indwelling catheter in the arm contralateral to that of the ibutilide infusion. The serum was separated from whole blood and then stored (-70° F) until further analysis. Serum ibutilide concentrations were determined in the Indiana University Clinical Pharmacology Analytical Core (CPAC) Laboratory using reverse-phase high-performance liquid chromatography with mass spectrometry (HPLC-MS) detection (Agilent 1290 HPLC, Eksigent Autosampler, and AB Sciex 5500 MS).

2.6 Quantitative Reverse Transcriptase-Polymerase Chain Reaction (q-PCR) Analysis

To quantify the level of hERG mRNA expression in cell lines transfected for 48 hours using lipofectamine 2000, quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) analysis was used. According to the manufacturer's recommendation, a total of 500 ng RNA of each sample was reverse transcribed using the High Capacity RNA-to-cDNA Kit (Applied Biosystems, Foster City, CA, USA). The extracted RNA synthesized the cDNA by mixing the RNA with reverse transcriptase Buffer, enzyme mix, random sequence oligonucleotide primers, and nuclease-free water. RT-qPCR was performed using the TaqMan MicroRNA Reverse Transcription kit (Applied Biosystems; Thermo Fisher Scientific, Inc. Waltham, MA, USA). GAPDH or KCNH2 (Applied Biosystems, Taqman® assays, Foster City, CA, USA) was added for normalization of RT-qPCR threshold cycle (Ct). The relative miR-362-3p expression of the patients included in the analysis was performed using real-time quantitative polymerase chain reaction (RT-qPCR). The expression data of the 11 patients at baseline, end of infusion, 5, 10, 15, 20, 30, 45 min, 1, 2, 4, 6, and 8 hours post-ibutilide infusion was determined. RT-qPCR was performed using TaqMan® Gene Ex kit (Life Technology, Carlsbad, CA, USA)

1.9 Population Pharmacokinetic-Pharmacodynamic Analysis

1.9.1 Software

PK-PD modeling was performed using a nonlinear mixed-effect model approach (NONMEM, Version 7, ICON Development Solutions, Ellicott City, MD, USA). The population parameters were evaluated using the classical first-order conditional estimation method with interaction (FOCE-I) (Bauer 2019). The adjunct tools used to evaluate the population PK-PD model utilized during the modeling process were R (version 3.1.2) and XPose (version 4.5.3), along with the graphical interface Pirana version 2.9.0 (Lindbom, Pihlgren et al. 2005, Keizer, Karlsson et al. 2013, Keizer, Karlsson et al. 2013).

1.9.2 Structural P.K. Model

A non-compartmental analysis was performed to determine the preliminary pharmacokinetic parameters estimate to be utilized during the population P.K. modeling process. Several population P.K. models were investigated (two compartment and three compartment models). The selection of the final population P.K. base model was determined by multiple criteria including a likelihood ratio test, visual inspection of the goodness-of-fit (GOF) plots, precision in parameter estimates, the Akaike information criterion (AIC), and consistency with previously published ibutilide PK-PD parameters (Tisdale, Overholser et al. 2008, Zeng, Wang et al. 2017).

1.9.3 Structural PK-PD Model

QTc baseline from both phases was included as the dependent variable. To account for diurnal variation associated with baseline Q.T. interval, an inter-occasion term on the Q.T. baseline parameter was utilized according to the following equation:

$$CC=0, \text{ IF } (TIME.GT.12) = OCC1$$

$$IOV(1-OCC) * ETA (5) + OCC * ETA (6)$$

$$OMEGA \text{ BLOCK } (1) \ 0.05; IOV \text{ VARIANCE } OMEGA \text{ BLOCK } (1) \text{ SAME}$$

where IOV are random variables between occasion IOVs and OCC denotes each phase, with OCC0 representing the screening phase (visit 1) and OCC 1 representing the ibutilide phase (day 2).

The P.D. response model was developed using an indirect response model based on its mechanistic effects (I_{Kr} inhibition) with zero-order input parameter and output parameter predicting the Q.T. interval prolongation. The selected P.D. model provided the best fit to the observed data as described in equation

$$\frac{dQT}{dt} = Kin * 1 - \left(\frac{Emax * Conc}{EC50 + Conc} \right) - Kout \quad (8)$$

Kout is the 1st order rate constant. EC50 is the drug concentration producing 50% of the maximum effect. Emax is the maximal effect of ibutilide. Conc is the drug concentration.

1.9.4 Covariate Analysis

A structural base model was used to determine all the possible contributions of pre-specific covariates contribution to the observed variability in the P.K. model of ibutilide, and ibutilide-induced QT-interval (PK-PD model). The selection of covariates was initially based on the forward addition technique in which covariates are added to the base model one by one, and then the backward elimination technique was utilized in which they are removed one at a time. Covariates were considered statistically significant if the objective function value (OFV) decreased by > 3.84 units after the addition of each covariate. Similarly, only covariates that increased the OFV by > 6.63 units during backward elimination were kept in the model.

For continuous covariates, the following functional forms were utilized:

a. Linear

$$TV\theta = \theta + \theta_{cov} * (COV - COV_{median}) \quad (9)$$

NONMEM coding

$$TV\theta = THETA(1) * THETA(2) * (COV - COV_{median}) \quad (10)$$
$$\theta = TV\theta * EXP(ETA(1))$$

b. Power

$$TV\theta = \theta * \left(\frac{COV}{COV_{median}}\right)^{\theta_{cov}} \quad (11)$$

NONMEM coding

$$TC\theta = THETA(1) * \left(\frac{COV}{COV_{median}}\right)^{**} THETA(2) \quad (12)$$
$$\theta = TV\theta * EXP(ETA(1))$$

c. Exponential

$$TV\theta = \theta * EXP(\theta_{cov} * (COV - COV_{median})) \quad (13)$$

$$\begin{aligned} \text{NONMEM coding } TV\theta &= THETA(1) * EXP(THETA(2) * (COV - COV_{median})) \\ \theta &= TV\theta * EXP(ETA(1)) \end{aligned} \quad (14)$$

(TV θ reflects the population value of the parameter for a given covariate value and θ is the population value of the parameter for those individuals with a covariate value equal to the median value (COV_{median}) as for those individuals $COV - COV_{median} = 0$ or $COV / COV_{median} = 1$. θ_{cov} reflects a fractional change in population parameter value due to the covariate effect).

In NONMEM equations, TV θ is a standard value parameter with a covariate median value (COV_{median}). θ is the parameter estimate, ETA (1) is the parameter estimate difference between the person and the population. THETA (2) is a factor which describes covariate influence (COV).

1.9.5 Random Effect Model

The inter-individual variability of the P.K. and P.D. model parameters for each patient was best described by an exponential model (Equation **Error! Reference source not found.**) as shown below (Feng, et al., 2006):

$$Pi = \theta * \exp(\eta p) \quad (15)$$

where P_i is the individual parameter estimate for the subject; θ is the population mean estimate for parameter P ; and (ηp) denotes the difference between P_i and θ .

The intra-individual variability (residual error model, RUV) was best described by proportional error for the P.K. model and by additive error for the P.D. model. The proportional error equation (**Error! Reference source not found.**) is presented below (Choi, et al., 2016):

$$DV = IPRED + (IPRED * \varepsilon_1) \quad (16)$$

where DV is the dependent variable for the observed PK/PD model; IPRED is the P.K. and PKPD model predicted concentrations/effect for the individual; and ε_1 and ε_2 are the differences between the individual observed and individual predicted concentrations/effect.

1.10 Model Evaluation

The criteria for model evaluation included; objective function value (OFV), Akaike information criterion (AIC), visual assessment of the goodness of fit plots (e.g., predicted versus observed concentrations; weighted residual versus predicted concentration), and overall plausibility and precision of the P.K. and PK-PD parameter estimates. Furthermore, a drop-in $p < 0.05$ was considered a significant decrease in the objective of function value. The visual predictive check (VPC) was performed to further confirm the accuracy and predictive performance of both the P.K. and PK-PD output using the final parameter estimates to simulate a profile of 1,000 individuals by prediction of the 95 percentiles. The median and the fifth and ninety-fifth percentiles were determined and then overlaid with the original data points. The model was considered to appropriately represent the data if most of the observed data points fell within the fifth and 95 percentile intervals and were equally distributed around the median. (Baheti, Kiser et al. 2011, Park, Lee, et al. 2014).

CHAPTER 3 RESULTS

1.11 Clinical and Demographic Characteristics of all Subjects

A total of 22 subjects (15 women and 7 men), aged 18 to 80 years who met the inclusion and exclusion criteria were enrolled and divided into three groups: a group of heart failure with preserved ejection fraction (HFpEF, n=10), a group of heart failure with reduced ejection fraction (HFrEF, n=2), and ten healthy subjects in the control group were matched to subjects in the HFpEF for age and sex. There were no significant differences in terms of age and weight in all groups. Overall, total of 536 ibutilide serum concentrations, serum miR-362-3p expressions, and Q.T. interval data points included in the analyses. Table 1 shows the demographics and clinical characteristics of study subjects.

Table 1. Participant Demographics and Subject Characteristics

Characteristic	Matched Controls (n=10)	HFpEF (n=10)	HFrEF (n=2)
Male sex, n (%)	3 (30)	3 (30)	1 (50)
White race, n (%)	8 (80)	7 (70)	1 (50)
African American, n (%)	2 (20)	3 (30)	1 (50)
Age, years	67 ± 9	69 ± 8	67 ± 1
Weight, Kg	82.2 ± 12	89.7 ± 13	87.3 ± 3
Ibutilide dose, mg	0.25 ± 0.04	0.27 ± 0.04	0.26 ± 0.01
Serum miR-362-3p, n (%)	2 (20)	8 (80)	2 (100)
Concurrent diseases, n (%)			
- Hypertension	6 (60)	8 (80)	2 (100)
- Coronary artery disease	1 (10)	3 (30)	2 (100)
- Diabetes mellitus	2 (20)	5 (50)	1 (50)
- Hyperlipidemia	6 (60)	5 (50)	2 (100)
Concomitant medications, n (%)			
- ACE inhibitors/ARBs	3 (30)	7 (70)	2 (100)
- Loop diuretics	2 (20)	7 (70)	2 (100)
- Beta-blockers	2 (20)	8 (80)	2 (100)
- Statins	4 (40)	5 (50)	2 (100)

Note: HFpEF = Heart failure Time preserved ejection fraction; HFrfEF= Heart failure with reduced ejection fraction; Data presented as n (%) or mean $\pm$ S.D.

1.12 Population P.K. Modeling

The population P.K. model was developed to describe ibutilide serum concentration versus Time. The P.K. model was estimated using the first-order conditional estimation method with an interaction term (FOCE-I). The P.K. parameters of ibutilide were evaluated by both two- and three-compartment P.K. models (implemented via ADVAN3, TRNAS4, AVAN11, and TRANS4 subroutines receptively) with first-order elimination from the central compartment. The final population P.K. base model was selected based on the objective function value (OBJ), visual inspection of the goodness of fits plots, individual and population parameter estimates, and reduction in both residual and interindividual variability.

Following noncompartmental analyses, the selection of the base P.K. model was initially 2-compartment model with first-order elimination from the central compartment were compared to a 3-compartment model where there was a clear differences in volume of distribution of the central compartment resulted in a poorly estimated parameter with a high shrinkage values (unreliable) that does not precisely align with acceptable fit. Based on evaluation of the goodness of fit plots (GOF) presented in Figure 3 and the plausibility of the two compartment model parameters estimate listed in Table 2. Accordingly, the structural population P.K. model was best described by a 3-compartment model with first-order elimination rate constants from the central compartment, as illustrated in Figure 4. While other residual error model did not significantly improve the model fit (Δ OFV < 3.84) as shown by the GOF in Figure 5, A proportional error model best described the residual variability. The mean parameter's value of ibutilide clearance (CL) was 199 L/hr., and the mean volume distribution of the central compartment (V.C.) was 28.6 L; the mean volume distribution of peripheral compartment 1 (VP1) and that of peripheral compartment 2 (VP2) was 80.2 L and 1020 L respectively.

1.12.1 Covariate Analysis—P.K. Model

In analyzing the covariate relationships, each covariate was added univariately in the base model using centered, linear, exponential, or power relationships, as presented in Table 3. Covariate

resulted in a decreased of the OBJ by more than 3.7 (if any) were considered as significant covariates. The tested covariates did not have significant effect on any of the P.K. parameters. Hence, the base structural model concluded as the final P.K. model.

1.12.2 Model Evaluation

The dependent variable (observed ibutilide serum concentration) vs Individual predicted serum concentration (**IPRED**) vs Time along with the GOF plots indicated no misspecifications in the model (Figure 5&6). The predictive performance was determined to be acceptable, as most of the observed data points contained in the model's 90% confidence interval of the predictions reflect the 5th–95th percentiles as displayed in Figure 7. The final population P.K. parameter estimates for both the fixed-effect and random-effect parameters were in close agreement with the corresponding median estimates derived from the bootstrap. Table 4

Table 2. A 2-Compartmental population P.K. model parameter estimate for ibutilide

Parameter	Population Estimates (RES%)
Cl_s, L/hr.	264 (7%)
Q, L/hr.	566 (12%)
V_c, L	62.5 (12%)
V_{p1}, L	940 (7%)
ω Cl	0.104 (52%)
ω Q	0.0633 (43%)
ω V_c	0.015 (39%)
ω V_p	0.109 (100%)
σ PROP	0.0611 (14 %)

Note. **Cl_s** = systemic clearance, **Q**= Intercompartmental clearance, **V_c** = Volume distribution of central compartment, **V_p** = Volume distribution of peripheral compartment, **ω** = Inter-individual variability (omega), **σ** = Residual variability (sigma), **PROP** = Proportional, **RSE** = Residual standard error.

Figure 3. Goodness-of-fit plots for the 2-compartmental population P.K. model of ibutilide

Note. (A) observe ibutilide concentration vs. Individual predicted ibutilide serum concentration (B) observed concentration vs. Population predicted concentration. (C) Conditional weighted residuals versus Population predicted concentration. (D) Conditional weighted residuals versus Time.

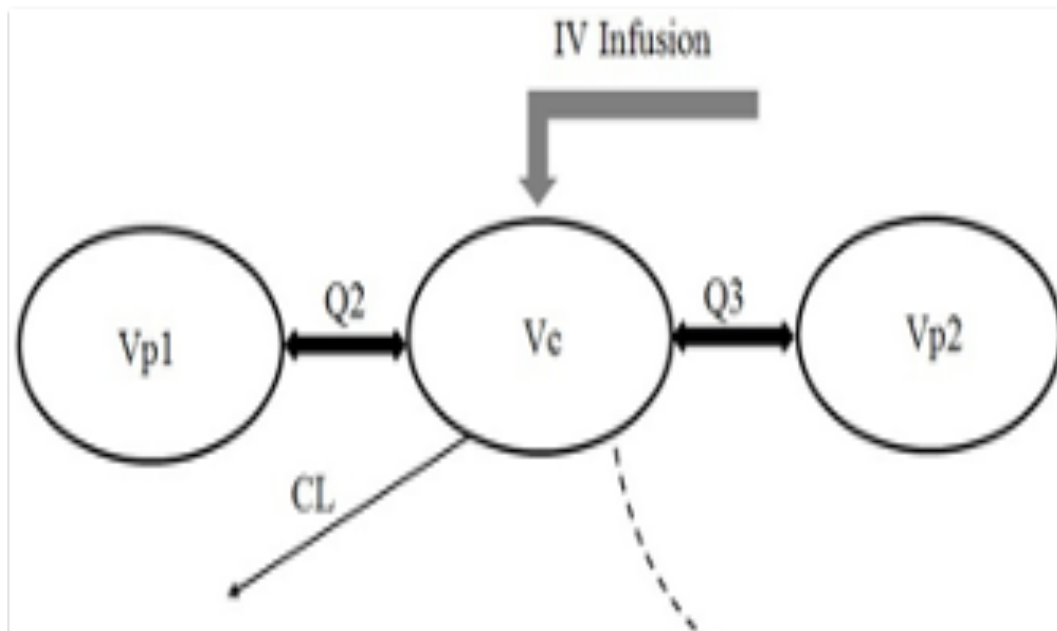


Figure 4. Structural picture for ibutilide pharmacokinetic model

Note. CL = systemic clearance, Q_2 = Intercompartmental clearance 1, Q_3 = Intercompartmental clearance 2, V_c = Volume distribution of central compartment, V_{p1} = Volume distribution of peripheral compartment 1, V_{p2} = Volume distribution of peripheral compartment 2.

Table 3. Summary of pop-PK model covariate analysis

Model Parameter	Covariate	OBJ	Δ OBJ	P-value	Functional Form
CL	Weight	-1280.914	0	N.S.	Power
CL	Weight	-1280.914	0	N.S.	Additive
CL	Weight	-1280.914	0	N.S.	Exponential
Vc	Weight	-1280.914	0	N.S.	Power
Vc	Weight	-1280.914	0	N.S.	Additive
Vp1	Weight	-1280.914	0	N.S.	Power
Vp1	Weight	-1280.914	0	N.S.	Additive
Vp1	Weight	-1280.914	0	N.S.	Exponential
Vp2	Weight	-1280.914	0	N.S.	Power
Vp2	Weight	-1280.914	0	N.S.	Additive
Vp2	Weight	-1280.914	0	N.S.	Exponential
CL	AGE	-1280.914	0	N.S.	Power
CL	SEX	-1280.914	0	N.S.	Power
CL	RACE	-1280.914	0	N.S.	Power

Note. **OBJ** = The objective function value; Δ **OBJ** = the difference in objective function values between the base and the added covariate model', **Vc**= Systemic clearance from the central compartment; **Vc**= Volume distribution of

central compartment, **Vp1**= Volume distribution of peripheral compartment 1, **Vp2**= Volume distribution of peripheral compartment 2; **WT** = Weight; **NS**= Non-significant.

Figure 5. Goodness-of-fit plots for the final population PK model of ibutilide

Note. **(A)** observed concentration vs. Individual predicted concentration. **(B)** observed concentration vs. Population predicted concentration. **(C)** Conditional weighted residuals versus Population predicted concentration. **(D)** Conditional weighted residuals versus time.

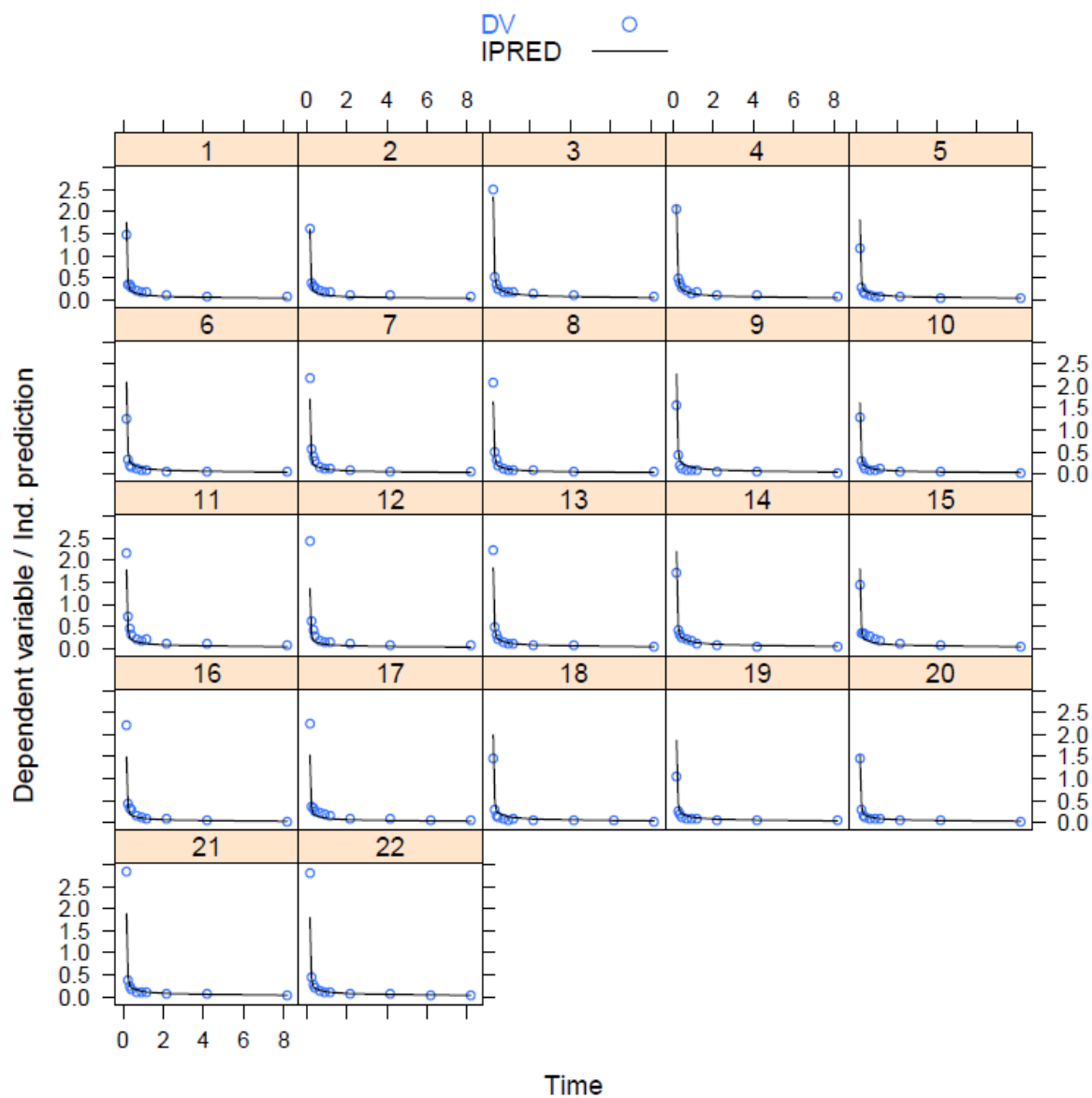


Figure 6. Individual plots for the final P.K. model outputs

Note. Open circles are observed ibutilide serum concentrations. Solid lines are individual predicted ibutilide serum concentrations.

Figure 7. Visual predictive checks (VPC) of final pharmacokinetic model

Note. Ibutilide serum concentrations (ng/mL) are represented by open circles. The observed 5% and 95% percentiles are presented with dashed red lines, and the 95% confidence intervals for the corresponding model predicted percentiles are described by semitransparent blue fields.

Table 4. Final population pharmacokinetic model and the bootstrap median parameter estimate for ibutilide

Parameter	Population Estimates (RES%)	Bootstrap Median (95 CI%) *
Cl, L/hr.	199 (8.7%)	203.18 (165.09 - 232.90)
Cl_{ic1}, L/hr.	209 (8.1%)	207.92 (175.87 - 242.12)
Cl_{ic2}, L/hr.	316 (5.5%)	327.01 (281.70 - 350.30)
V_c, L	28.6 (7.9%)	29.86 (24.19 - 33.01)
V_{p1}, L	80.2 (0%)	80.20 (80.20 - 80.20)
V_{p2}, L	1020 (7.9%)	1075.58 (862.41 - 1177.58)
ω Cl	0.103 (30.8%)	0.109 (0.0514 – 0.175)
ω Cl_{ic1}	0.055 (45%)	0.057 (0.0136 – 0.104)
ω Cl_{ic2}	0.039 (34.1%)	0.036 (0.0134 – 0.0649)
ω V_c	0.01 FIX	-
ω V_{p1}	0.01 FIX	-
ω V_{p2}	0.146 (36.8%)	0.116 (0.0255 – 0.204)
σ PROP	0.026 (11.2 %)	0.0255 (0.02 – 0.0316)

Note. **Cl**_s = systemic clearance, **Cl_{ic1}** = Intercompartmental clearance 1, **Cl_{ic2}** = Intercompartmental clearance 2, **V_c** = Volume distribution of central compartment, **V_{p1}** = Volume distribution of peripheral compartment 1, **V_{p2}** = Volume distribution of peripheral compartment 2, **ω** = Inter-individual variability (omega), **σ** = Residual variability (sigma), **PROP** = Proportional, **RSE** = Residual standard error, **CI** = Confidence interval.

1.13 Population PK-PD Model

For the P.D. model, 444 QT intervals record from all subjects included as the dependent variable. To characterize the relationship of ibutilide serum concentration and its effects based on IKr inhibition induce Q.T. interval prolongation. Individual ibutilide serum concentrations' time profile was then used in an indirect response model where ibutilide serum concentrations are indirectly driving the Q.T. interval prolongation throw inhibition of the output (Kout) parameters. The indirect PK-PD model is schematically illustrated in Figure 8. A high inter-occasion variability due to day-to-day variability in the Q.T. baseline was observed thus. We estimated inter-occasion terms on the baseline Q.T. parameter. The addition of IOV resulted in results in a significant reduction seen (OBJ dropped by 69). Hence the addition of the IOV term improves model fit. The visual inspections of the GOF plots for the structural PK-PD model displayed in Figures 9 & 10. The objections of functions values and the Akaike Information Criterion (AIC) were evaluated. The structural parameters estimated from the indirect PK-PD model were for the Emax and EC50 0.115 ms and 0.36 ng/mL, respectively. Table 5 shows the parameter estimates and bootstrap analysis of the structural PK-PD parameters.

Figure 8. Schematic representations of ibutilide pharmacodynamic/pharmacokinetic model effects

Note. K_{in} = denotes the zero-order rate constant; K_{out} = denotes the first-order rate constant assumed to be equal in the absence of ibutilide.

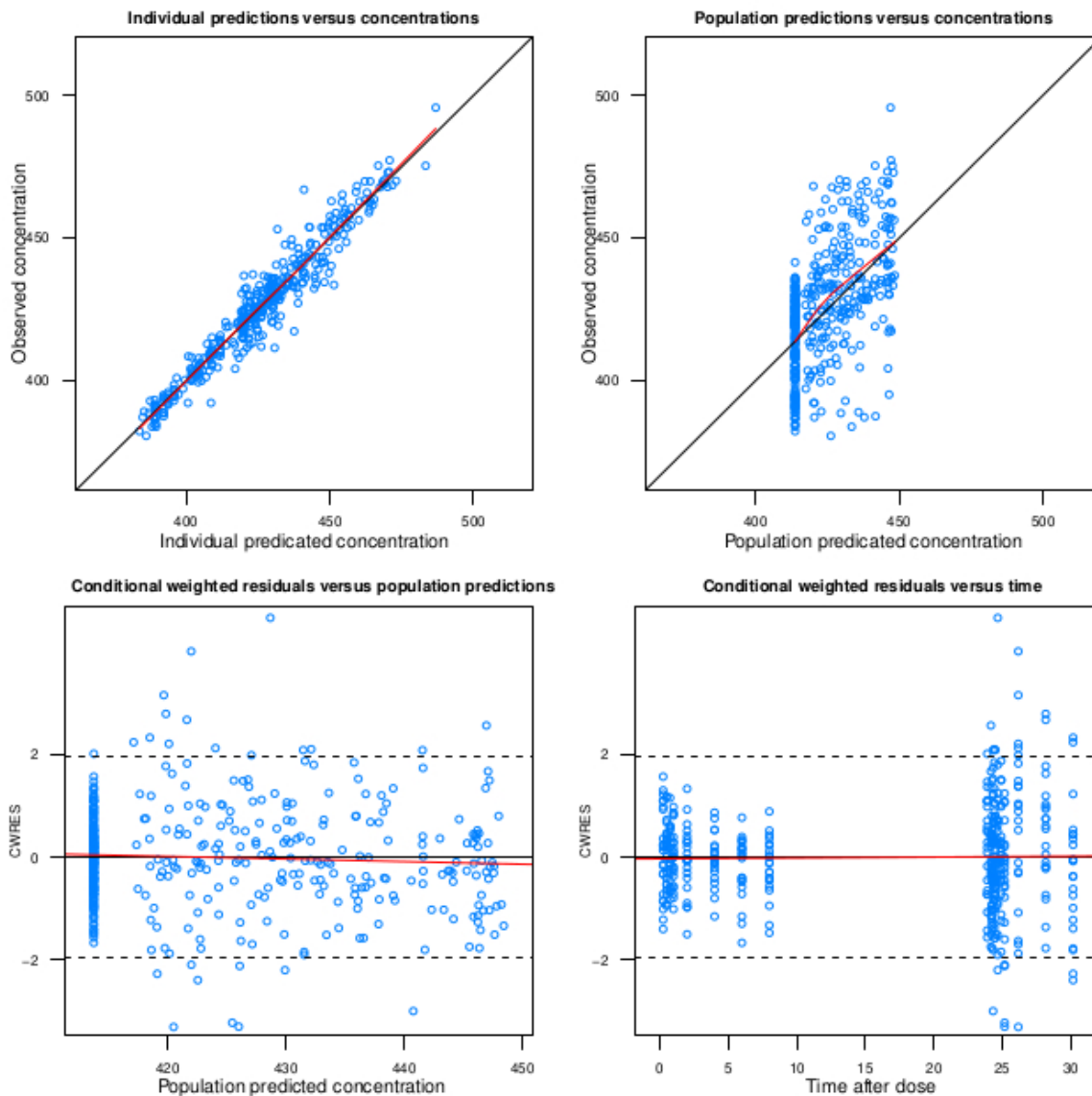


Figure 9. The goodness-of-fit plot of structural pharmacokinetic pharmacodynamic model of ibutilide

Note. (A) Observed QTF interval versus individual predicted QTF interval (ms) (B) observed QTF interval versus Population predicted. (C) Conditional weighted residuals versus Population predicted concentration. (D) Conditional weighted residuals versus time.

Figure 10. Individual plots for final PK-PD model outputs.

Note. Open circles are observed QT interval. Solid lines are individual predicted ibutilide serum concentrations.

Table 5. Summary of estimated population pharmacokinetic/pharmacodynamic parameters from the base model

Parameter	Population Estimates (RES%)	Bootstrap Median (95 CI%)*
E_{max} _(ms)	0.115 (11.8%)	(0.088 – 0.142)
EC₅₀ (ng/mL)	0.36 (8.4%)	(0.301 - 0.419)
Baseline QT (ms)	414 (0.5%)	(409.59 - 418.41)
K_{out} (hr ⁻¹)	9.65 (3.4%)	(9.005 - 10.295)
ω K_{out}	0.324 (31.2%)	-
ω EC₅₀	1.46 (9%)	-
ω Base QT	0 FIX	-
IOV Variance	0.0009 (5.8%)	-
IOV Variance	0.0009 (14.2%)	-
σ PROP	0.0002 (8.7%)	-

Note. * Based on Percentiles. Base, pre-ibutilide dose QT interval; E_{max} = Maximum effect of ibutilide on QT; IC₅₀ = Serum ibutilide concentration required to achieve 50% of the maximum effect QT occurs; K_{out} = first order rate constant; IOV, Inter-occasion variability; ω = Inter-individual variability (omega), σ = Residual variability (sigma), PROP = Proportional, RSE = Residual standard error, CI = Confidence interval.

1.13.1 Biomarker Model

Preliminary efforts to describe the influences of serum miR-362-3p in the circulation on the P.D.PD model parameters were not successful. A sub-set of a linear model, direct and indirect reopen model initially were attempted. The indirect response model was attempted, as illustrated in Figure 11, to describe the expression patterns of serum miR-362-3p parameter observed within a group of heart failure subjects with preserved ejection fraction vs. a group of heart failure subjects with reduced ejection fraction, as compared to the control group as displayed in Figure 12. Due to the small sample size, availability of the baseline of serum miR-362-3, and collections time was limitations for the dataset, this approached failed. Therefore, a sub-set of the liner and direct model was attempted, but none of the serum miR-362-3p model parameters were predicted by the model; thus, a covariate analysis approach was performed.

Figure 11. Schematic picture for the biomarker model linked to the PKPD model

Note. K_{in} = denotes the zero-order rate constant; K_{out} = denotes the first-order rate constant

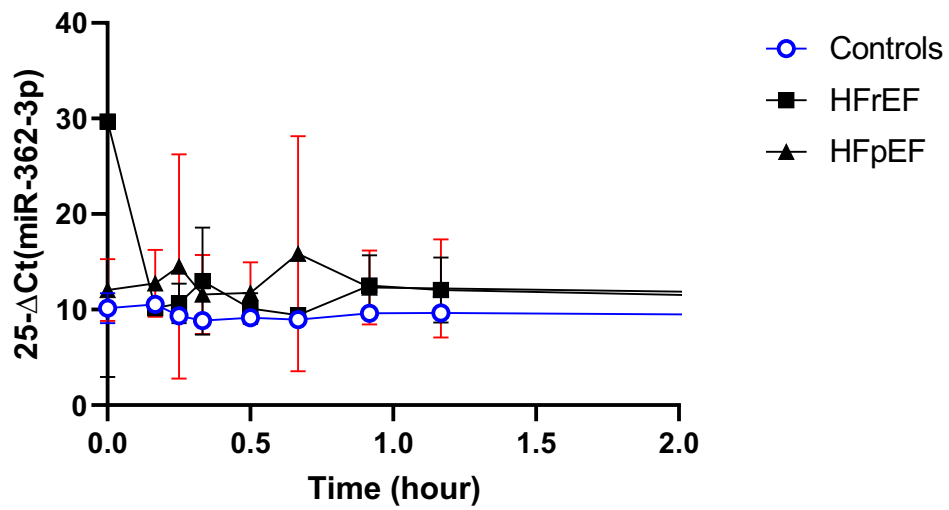
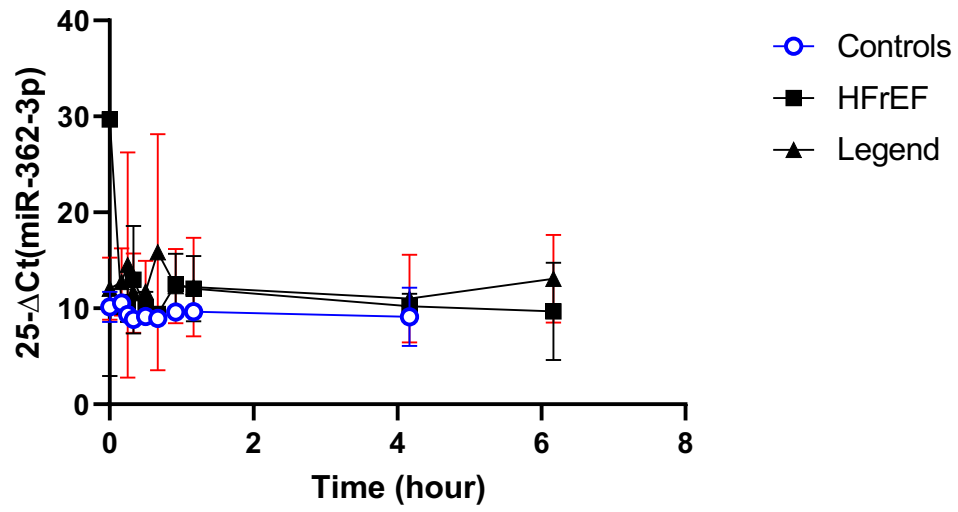


Figure 12. The expressions of serum miR-362-3p (CT-25) vs Time (hrs.) in three groups of heart failure subjects

1.13.2 Covariate Analysis— Model

An alternative approach was to test all subjects' covariate that potentially influences the PK-PD model parameters, as listed in Table 5. The covariate selection process was done using stepwise selections procured on Emax and EC50 parameters, where forward inclusion resulted in the PK-PD model identifying circulating miR-362-3p expression in serum and myocardial infarction as a significant covariate influencing Emax parameter (ΔOFV -16.03, -15.09, $p < 0.05$).

Overall, the constructed PK-PD model's performance was improved compared to the base PK-PD model, and the included covariates demonstrated good portions of the un-explained variability in the population. Compared to base model the unexplained inter-patient variability in the final covariate PK-PD model decreased from 11.8% to 9.7% for Emax which significantly reduced the OBJ -5.2. Overall, the performance of the constructed covariate PK-PD model was acceptable compared to the base model. The final PK-PD model estimates and covariate-parameter relationships are summarized in Table 6.

Table 6. Summary of covariates in the pharmacokinetic/pharmacodynamic (PK/PD) modeling

	OBJ	Delta OBJ	EC50	E _{max}	P Values
BASE MODEL	2243.65				
miR-362-3p on E _{max}	2227.62	-16.04	0.32	0.10	<0.05
miR-362-3p on EC50	2235.87	-7.76	0.48	0.12	<0.05
HFG on E _{max}	2243.65	0.00	0.35	0.11	ns
HFG on EC50	2252.39	8.74	0.25	0.10	ns
SEX on E _{max}	2243.57	-0.08	0.35	0.12	ns
SEX on EC50	2243.62	-0.04	0.38	0.11	ns
RACE on E _{max}	2241.35	-2.30	0.43	0.13	ns
RACE on EC50	2243.64	-0.01	0.36	0.11	ns
Weight on E _{max}	2243.63	-0.02	0.35	107.00	ns
Weight on EC50	2243.65	0.00	0.38	0.11	ns
AGE on E _{max}	2243.65	0.00	0.35	0.11	ns
AGE on EC50	2242.80	-0.85	1.57	0.11	ns
DM on E _{max}	2239.15	-4.50	0.38	0.13	<0.05
DM on EC50	2241.99	-1.67	0.47	0.11	
MI on E _{max}	-15.97	-15.97	0.30	0.15	<0.05
MI on EC50	2243.61	-0.04	0.38	0.11	ns
LVEF on E _{max}	2243.65	0.00	0.35	0.11	ns
LVEF on EC50	2243.65	0.00	0.35	0.11	ns
BB E _{max}	2243.65	0.00	0.11	0.35	ns
BB EC50	2243.65	0.00	0.29	0.11	ns
LD on E _{max}	2243.65	-1.25	0.12	0.37	ns
LD on EC50	2243.65	-3.72	0.36	0.12	ns
ACE on E _{max}	2243.65	-0.36	0.24	0.11	ns
ACE on EC50	2242.07	-1.59	0.24	0.11	ns

Note: ΔOFV is the difference in objective function values between base and covariate model; DM= Diabetes mellitus; MI= Myocardial infarctions; LVEF= Left ventricular fractions BB= Beta-blocker; ACE= ACE inhibitors; LD=Loop

Table 7. Forward Selections

Base Model 1	OBJ	Delta OBJ	EC50	E_{max}
miR-362-3p + MI on E _{max}	2222.10	-5.52	0.26	0.09
miR-362-3p + MI on E _{max} + miR-362-3p on EC ₅₀	2221.91	-0.20	0.24	0.09
miR-362-3p MI + DM on E _{max}	2222.10	0.00	0.26	0.09

Note. Most significates were miR-362-2p which were kept in the model while testing others.

Model Evaluation

Several individual plots of the GOF were examined closely (Figure 13) showed a good descriptive of model predictions and no mis-specifications' predictive performance of the final PK-PD model was evaluated using visual predictive check. The VPC plots determined to be acceptable as the most observed data points contained in the 90% prediction interval (5th-95th percentiles) of the model (Figure 14). Moreover, the final covariate PK-PD parameter estimates for both fixed and random-effect agreed with the corresponding median estimated obtained from bootstrap analysis indicates the high predictive performance of the final model parameter estimates (Table 8).

Figure 13. Individual plots for the final PK-PD model outputs

Note. Open circles are observed interval. Solid lines are individual predicted ibutilide serum concentrations.

Figure 14. Visual predictive checks (VPC) of final the PK-PD model

Note. Ibutilide plasma concentrations(ng/mL) are represented by open. The observed 5% and 95% percentiles are presented with dashed red lines, and the 95% confidence intervals for the corresponding model predicted percentiles as described by semitransparent blue fields.

Table 8. Summary of the final Covariate Pharmacokinetic/Pharmacodynamic parameters estimate

Parameter	Population Estimates (RES%)
E_{max}(ms)	0.09 (8.9%)
EC₅₀ (ng/mL)	0.26 (8.4%)
Baseline QT (ms)	414 (0.5%)
K_{out} (hr⁻¹)	11.2 (1.4%)
ω K_{out}	0.324 (31.2%)
ω EC₅₀	1.46 (9%)
ω Baseline QT	0 FIX

IOV Variance	0.00107(0%)
IOV Variance	0.00107(5%)
σ PROP	0.00019 (8 %)

Note. * Based on Percentiles. Base, pre-ibutilide dose QT interval; Emax = Maximum effect of ibutilide on QT; IC₅₀ = Serum ibutilide concentration required to achieve 50% of the maximum effect QT occurs; Kout = first order rate constant; IOV, Inter-occasion variability; ω = Inter-individual variability (omega), σ = Residual variability (sigma), PROP = Proportional, RSE = Residual standard error, CI = Confidence interval.

CHAPTER 4 DISCUSSIONS

In the present study, the contribution of serum miR-362-3p to drug-associated Q.T. interval changes in patients with heart failure was investigated. For this purpose, a semi-mechanistic model was developed to describe the relationship between ibutilide concentration and QTc changes and determine the contribution of miR-362-3p expression and other potential covariates' variability with ibutilide-induced Q.T. interval lengthening.

The P.K. and P.D. data used in the current project were obtained from a previous clinical trial that assessed patients' susceptibility with HFpEF and HFrEF to ibutilide induced Q.T. interval prolongation (Tisdale et al., 2020). Based on a nonlinear mixed effect model approach, the population pharmacokinetic model described the profile of ibutilide serum concentrations vs. Time was best described by a three compartmental model with a first-order elimination rate constants

from the central compartment, thus confirming previously published population P.K. model (Zeng et al., 2017).

The present study's outcome demonstrated that subjects with heart failure had a greater degree of ibutilide-induced QTc, with a mean Emax increased by 39% compared to the control group. Accordingly, H.F. patients (either HFrEF or HFpEF) had a higher sensitivity to ibutilide-induced Q.T. interval lengthening. In general, a QTc of higher than 500ms or an increase in the QTcTimeerval greater than 60ms is usually considered to confer an increased risk of TdP in an individual patient (Heist & Ruskin, 2005, Barnes & Hollands, 2010). The current study's outcome agrees with those of previously published research by Tisdale et al. (2012). The authors observed that the median area under the effect curve for QTF vs. Time was significantly higher in H.F. patients. Simultaneously, EC50 was lower, indicating that H.F. patients have enhanced sensitivity to drug induced QTc.

The effect of miR-362-3p on ibutilide-induced QTc was also evaluated as a potential covariate. Undoubtedly, serum miR -362- 3p expression effects on the P.D. model parameter were identified as a predictor of ibutilide with an EC50 (ng/mL) 0.26 (8.4%) as compared to ibutilide base model parameters with an EC50 (ng/ml) 0.36 (8.4%); notably, they were negatively correlated, as patients with higher expression of miR-362-3p demonstrated a lower EC50. The specific mechanism determining the association between miR-362-3p expression and ibutilide remains unclear. However, the possible explanation is reducing expressed channels due to high miR- 362-3p expression, leading to lessor available targets for ibutilide, thereby enhancing its effect.

Another possible explanation is the changes mainly driven by ibutilide-associated Q.T. changes; subsequently, the more precise effect of miR-362-3p on Q.T. interval lengthening could be

evaluated several hours (approximately >12 hours) following ibutilide administration. Furthermore, ibutilide might have a possible involvement in regulating miR-362-3p expression, supported by a recent study by Rodrigues et al. (2011). They concluded that several drugs might be associated with alteration of miRNA expression. The authors evaluated 19 drugs' involvement in expressing ten different miRNAs in four different cell lines. It was found that all ten miRNAs were differentially expressed, depending on the type of drug administered. Lastly, the changes noticed in ibutilide response could be related to either a significant increase in releasing cellular miRNAs to circulation or significant disposition of miRNAs from circulation, resulting in an alteration of circulating miR-362-3p.

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The baseline of the Q.T. (ms) model parameter was 414 (0.5%). This work's results relieved that a high level of serum miR-362-3p expressions and a history of myocardial infarction are significant predictors of enhanced ibutilide-induced QT-interval prolongation. Subject with a history of MI had an Emax(ms) of 0.09 (8.9%) compared to the base parameters Emax 0.115 (11.8%). This indicates subjects with a history of myocardial infarction and high expressions of serum miR-362-3p are a predictor of subjects with an increased risk to ibutilide - induced Q.T. interval prolongation compared to subjects with no history of myocardial or and with a low serum miR-362-3p expressions.

The study had a few limitations; foremost, the inability to recruit the required sample size, the study analysis is underpowered to detect clinically relevant statistical differences in some outcome

measures. Secondly, inter-intra variations between H.F. subgroups concerning elapsed between the initial diagnosis and the impact of other morbidities were not evaluated. Furthermore, miR-362-3p expression levels may be influenced by another pathway, pathophysiological signaling, and underlying mechanisms not assessed in the present study. Finally, the potential use of miR-362-3p as a biomarker warrant assessment for the prevention of drug-induced QT-interval prolongation in heart failure. Indeed, serum miR-362-3p expression and MI were identified as a predictor.

This is supported by previous lab work data, which has demonstrated that miR-362-3p expression reduced hERG regulation and may mediate the involvement in drug-induced Q.T. interval lengthening (Ref). However, the definite mechanism of the association between miR-362-3p expression and the drug target remains inconclusive. Consequently, future studies should be directed towards understanding the association between microRNA serum expression and Q.T. interval changes. The assessment of such an association would explain the exact mechanism and contribution of miRNA in drug-induced Q.T. interval lengthening.

Conclusion

Within the study's limitations, the following conclusion was drawn; serum miR-362-3p expression was transformed during H.F. and was significantly responsible for the variations associated with the sensitivity in Q.T. interval prolongation following ibutilide administration. This was in agreement with previous findings demonstrating that high expression of serum miR-362-3p expression correlates with a higher sensitivity to ibutilide induced Q.T. interval Lengthening.

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