IMPROVING QT CORRECTION METHODS THROUGH THE ANALYSIS OF PRECLINICAL SAFETY PHARMACOLOGY DATA

By

Nicholas David Ether

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Pharmacology and Toxicology – Doctor of Philosophy

2022

ABSTRACT

IMPROVING QT CORRECTION METHODS THROUGH THE ANALYSIS OF PRECLINICAL SAFETY PHARMACOLOGY DATA

By

Nicholas David Ether

Torsade de pointes is a polymorphic ventricular tachycardia that has been linked to sudden cardiac death. While typically rare and fleeting, increased risk for occurrence of and problematic outcomes from this arrhythmia has been attributed to prolonged QT intervals. These interval measurements of electrocardiogram waveforms represent the time between depolarization and repolarization of the ventricles. Prolongation of this period increases the likelihood of disruptions in ventricular cardiomyocyte conduction, which can lead to *torsade de pointes* events. Due to the potentially fatal outcomes associated with QT prolongation, it was not long until drugs found to induce this prolongation began to be removed from the market. These concerns led to the creation of safety pharmacology guidelines S7B and E14 that outlined suggested QT prolongation risk assessments to be performed during the preclinical and clinical stages of drug development, respectively. Studies based on these guidelines are intended to identify drug-induced changes to the QT interval, but must first control for the effect heart rate has on QT. To isolate drug effect QT correction methods are used to estimate corrected QT values as if the data was collected at a fixed heart rate. Using these values as a biomarker has led to highly sensitive assessments that have prevented any new drugs from reaching the market with unacceptable QT prolongation risk. However, these assessments are still in need of improvement as the pharmaceutical industry must deal with costly and time-consuming clinical safety studies despite the high sensitivity of preclinical assessments, leading to calls for the integration of preclinical and clinical guidelines. To achieve this, the translatability of preclinical results must be improved.

This dissertation aims to increase the reliability of preclinical results and improve their translatability by optimizing the QT correction methods they rely on. To do this, I analyzed large

ECG datasets from preclinical safety pharmacology studies obtained through Eli Lilly and Company (Indianapolis, IN). The central hypothesis of this dissertation is that through statistical analysis of these data, the collective understanding of QT correction methods will be expanded, and an improved method can be developed. In pursuit of this goal, the effectiveness of various preclinical QT correction methods was evaluated in simulated drug treatment scenarios, against 130,000+ bootstrap resampled ECG recordings of vehicle control treatments, and with study data from non-human primates treated with known QT prolonging drugs. The results of these evaluations provided evidence of how assumptions inherent to these methods affect the result of correction. Examples of such assumptions include what heart rate is relevant for the species, that a predetermined population-based estimate of the QT-rate relationship is appropriate for individuals, that this relationship will not change over time or between treatments, and that assuming this relationship before correction is appropriate in the first place. All of this led to the development of the novel Ratio QT correction method designed to be applicable to any scenario by dynamically responding to moment-to-moment changes in the relationship between timepoints. This novel method combines the simplicity and ease-of-use of population-based methods with the effectiveness of individual methods.

Taken together, this research has increased the collective understanding of QT correction methods and resulted in a novel method that is as effective as it is simple to use. The investigations presented in this dissertation have explored aspects of QT correction methods that have been in question for years. Optimizing these methods is now easier thanks to the information gained through these analyses of large preclinical ECG datasets. An integral step has been made towards the creation of a new industry standard of QT correction capable of bridging the gap between preclinical and clinical safety pharmacology studies. Such improvements can be used to help reduce the number of research subjects necessary for preclinical and clinical QT prolongation assessments.

To Faryal Mir, who opened my eyes to life's possibilities, and Brian Webster, who was not able to see enough of them.

ACKNOWLEDGEMENTS

The purpose of this dissertation is to demonstrate the knowledge and skills that I have collected over the past five years. I will always cherish it as evidence of what I am capable of when I work hard to achieve a desired outcome. However, none of this would have been possible without the assistance of those around me. This dissertation is also evidence for the incredible impact that you can have on someone's life through positive guidance and support. I would like to thank everyone in my life that has helped shape me into who I am today, but I will reserve this acknowledgement section for those that were instrumental in reaching this goal.

Dr. Adam Lauver, my Ph.D. mentor, hired me to work in his lab when I was at my lowest point as a scientist. I had just left an abusive work environment and had completely lost confidence in my research ability. Dr. Lauver provided a much-needed antithesis to that environment, with the space and support necessary to rekindle my love for science and rejuvenate my self-confidence. As I transitioned from MSU employee to MSU graduate student and began rotating through labs to select a Ph.D. mentor, it was Dr. Lauver's lab that I was comparing them to. So, when he told me about this new data analysis focused project, I jumped at the chance to pursue my Ph.D. in such an interesting topic under his supervision. Dr. Lauver, throughout this whole process, you have provided the support and guidance that any graduate student would be lucky to receive. I will be hard-pressed to find anyone that surpasses the example you set as both a mentor and a scientist. I will forever be grateful for the positive impact that you have had on my life and career.

My appreciation of my co-mentors, Dr. Marc Bailie and Dr. Derek Leishman, cannot be overstated either. Both of you brought your industry and research expertise which were vital to this project and taught me so much of what is contained within this dissertation. Beyond that, your perspectives and guidance have shaped my path from academia to industry. I'm grateful for all your support through this process and the laughs we've had along the way. Thank you to my committee members; Dr. Jason Bazil, Dr. Sudin Bhattacharya, and Dr. William Jackson. You all

provided valuable insight into this project through your questions and observations. I appreciate your suggestions and support through this journey, having helped direct me towards the finish line. To my fellow lab members, Barbara Christian and Dawn Kuszynski, thank you for your comradery and support over the years. If there is one regret I have about the primarily remote nature of my research, it is my limited time spent in the lab with such kind and talented people. Barbara, your consistently positive attitude created a welcoming environment, and your hard work always kept the lab running smoothly. Dawn, your seemingly never-ending motivation and numerous achievements were always inspiring to see. The experiences and educational resources that you shared at the most challenging points of this process were so valuable. I will never forget the feeling of relief I felt every time you offered some much-needed assistance.

The Department of Pharmacology and Toxicology was so entwined with my graduate experience that I cannot imagine having joined any other. I could not have asked for a more positive or supportive group of people to work alongside. Special thanks to Dr. Jamie Bernard, Dr. Anne Dorrance, and Dr. Stephanie Watts whom all helped guide me to Dr. Lauver's lab and always made time to provide valuable advice. I must also give thanks to all the office and support staff in the department, for always having all the answers. This work would not have been possible without the data and funding provided by Eli Lilly & Company. It is no exaggeration to say that this project and training has changed my life.

Of course, I could not have achieved this without the support I received from my friends and family. Each of you have helped me reach this point in your own unique ways. However, I don't know where I would be right now without my amazing wife, Faryal Mir. Your kindness, wisdom, and love were just some of your wonderful qualities that have had an indescribably positive impact on my life. Your unyielding belief in my abilities kept me going even when the obstacles seemed insurmountable. I am so fortunate to be your partner through life and I cannot wait to see how we navigate through the rest of it.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

KEY TO ABBREVIATIONS

APD	Action Potential Duration
BK9	Beagle Canine
Ca^{++}	Calcium Ion
Cl^{-}	Chloride Ion
CiPA	Comprehensive *in vitro* Proarrhythmia Assay
CPMP	Committee for Proprietary Medicinal Products
CYP3A4	Cytochrome P450 3A4
dRR	Change in RR Interval from Baseline Mean RR Interval
dQT	Change in QT Interval from Baseline Mean QT Interval
E14	ICH Guideline E14 Clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs
E14/S7B Q&As	ICH E14/S7B Implementation Working Group Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential – Questions and Answers
EAD	Early Afterdepolarization
ECG	Electrocardiogram
FDA	Federal Drug Administration
hERG	human Ether-à-go-go-Related Gene
HR	Heart Rate
hQTc	Hyperbolic Individual QT Correction
hQTcP	Hyperbolic Individual QT Correction – Pre-Treatment Sub-Method
hQTcPL	Hyperbolic Individual QT Correction – Pre-Treatment Lights Sub-Method
hQTcT	Hyperbolic Individual QT Correction – on-Treatment Sub-Method
hQTcTL	Hyperbolic Individual QT Correction – on-Treatment Lights Sub-Method
$I_{Ca\text{-}L}$	L-type Calcium Ion Channel

ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
I_{K1}	Inward Rectifying Potassium Ion Channels
I_{Kr}	Rapid Delayed Rectifier Potassium Ion Channels
I_{Ks}	Slow Delayed Rectifier Potassium Ion Channels
I_{Na}	Voltage-Gated Sodium Ion Channels
IQR	Interquartile Range
$I_{TO1,2}$	Transient Outward Current Channels 1 & 2
K^+	Potassium Ion
lQTc	Linear Individual QT Correction
lQTcP	Linear Individual QT Correction – Pre-Treatment Sub-Method
lQTcPL	Linear Individual QT Correction – Pre-Treatment Lights Sub-Method
lQTcT	Linear Individual QT Correction – on-Treatment Sub-Method
lQTcTL	Linear Individual QT Correction – on-Treatment Lights Sub-Method
LQTS	Long QT Syndrome
MDD	Minimal Detectable Difference
Na^+	Sodium Ion
NHP	Non-Human Primate
perE	Percent of Maximum Effect
QT	QT Interval
QTc	Corrected QT Interval
QTcB	Bazett's General QT Correction Method
QTcR	Ratio QT Correction Method
QTcT	On-Treatment Linear Individual QT Correction Method
QTcV	Vehicle Treatment Linear Individual QT Correction Method
QTcVa	Van de Water's General QT Correction Method

refR	Reference Rate
refRR	Reference RR Interval
RR	RR Interval
S7B	ICH Guideline S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals
SCD	Sudden Cardiac Death
StdDev	Standard Deviation
TdP	Torsade de Pointes
Yint	y-Intercept

CHAPTER 1:

INTRODUCTION

PART 1:

DRUG-INDUCED ARRHYTHMIA

Abstract

Sudden cardiac death is responsible for over 300,000 deaths in the US every year and is characterized as a cardiac related death occurring within an hour of the onset of symptoms. Due to the nature of such a diagnosis, the causes of these events are often unexpected or even unknown. Ventricular fibrillation is believed to be intrinsically linked to sudden cardiac death, which is a potential outcome of the polymorphic ventricular tachycardia *torsade de pointes*. This arrhythmia has been associated with anti-arrhythmic and non-cardiovascular drugs that prolong the QT interval of electrocardiogram waveforms. In the first half of this chapter, I provide the history behind these drug-induced arrhythmias that led to regulations requiring assessment of QT prolongation risk for novel pharmaceutical compounds. Then I discuss the electrophysiology behind QT prolongation and how it can increase the risk of *torsade de points* events. This overview of the history of drug-induced arrhythmias and the mechanisms behind it will serve as an introduction into the reasoning behind current safety pharmacology studies designed to prevent the approval of new drugs with unacceptable proarrhythmic risk.

Introduction

Sudden cardiac death (SCD) is one of the leading causes of death in western countries, with more than 300,000 occurring in the United States each year [1,2]. A sudden death, unrelated to trauma, occurring within an hour of new or worsening symptoms is the typical definition of SCD, though the agreed upon timing can vary [3,4]. Research has identified multiple causes of SCD, with the most common being ischemic heart disease [5]. However, no structural disease of note is required for SCD to occur. Electrical imbalances that can cause ventricular tachycardia, which devolve into ventricular fibrillation can cause unexpected SCD in otherwise healthy patients [2,3,5]. In 1923 MacWilliam described the role of ventricular fibrillation in SCD as a "misapplication of contractile energy thrown away in turmoil of fruitless activity" and that it can often occur "in a heart showing no failure of rhythmicity, excitability, or contractility" [6]. Dessertenne identified unique electrocardiographic (ECG) morphologies that seemed to represent a significant number of these unexpected cardiac events, which were named '*Torsade de Pointes*' (TdP) based on the French origin of Dessertenne's research and the accurate description of their morphology [7,8]. This term is roughly translated into English to mean 'Twisting of the Pointes', referring to the way the QRS complex appears to be twisting around the isoelectric baseline of the ECG recording [8,9]. Based on the ECG morphologies preceding a TdP event, as well as the cases in which they were commonly found, research focused on the role prolongation of ventricular repolarization played in the likelihood of TdP events [9–12]. Anti-arrhythmic drugs, which are often designed to prolong repolarization in an effort to interrupt an arrhythmic event, were quickly linked to many cases involving TdP [13–17]. However, studies on non-cardiac related drugs became increasingly important as a wide variety of drug classes were found to have unintended effects on the ventricular repolarization and were being associated with TdP events [18–23]. This unexpected risk of fatality from drugs as common as antihistamines and antibiotics encouraged researchers to better understand the molecular and electrophysiological mechanisms behind these effects [8,10,13,14,17,24,25]. As early as 1996, recommendations by public safety organizations were

released discussing the need for pharmaceutical companies to screen for the risk of novel compounds prolonging ventricular repolarization [26]. The first part of this chapter will explore the risk that drug-induced arrhythmias pose, how the arrhythmia of most concern (TdP) has been characterized, and the electrophysiological mechanisms that drive that risk.

Drug-Induced Sudden Death

Pharmaceuticals have been associated with sudden cardiac death for about half a century [27–29]. Typically, these drug-induced SCD events have been linked to ventricular arrhythmias, specifically TdP [30–33]. Initially the drugs investigated for these conditions were antiarrhythmics known to prolong ventricular repolarization, which can disrupt cardiac conduction, leading to arrhythmia [13–16,29,34,35]. However, these traits were soon found to be associated with non-cardiac related drugs like psychoactive treatments, macrolide antibiotics, diuretics, and antihistamines [19–23,28,32,36–38]. Because these types of drugs are so commonly prescribed and typically taken outside the supervision of cardiologists, unexpectedly induced arrhythmias present a concerning risk of sudden cardiac death [39–41]. Table 1 includes a list of some non-antiarrhythmic drugs that were removed from the market due to this risk, along with their therapeutic class, as detailed by Lester *et al* [42]. Despite decades of research into the role these drugs played in the risk of TdP and SCD, it was not until 1997 that the Committee for Proprietary Medicinal Products (CPMP) released recommendations about assessing the risk of non-cardiovascular drugs prolonging ventricular repolarization [43]. These were later replaced by guidelines from The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH), which were later integrated into governmental drug regulations [44,45]. A prime example of this evolving understanding of the risk these drugs pose and the regulations that followed is the antihistamine Terfenadine.

Drug	Therapeutic Class	Year Withdrawn
Prenylamine	Angina	1988
Terodiline	Urinary Incontinence	1991
Sparfloxacin	Antibiotic	1996
Terfenadine	Antihistamine	1998
Sertindole	Antipsychotic	1998
Astemizole	Antihistamine	1999
Grepafloxacin	Antibiotic	1999
Cisapride	Prokinetic	2000
Droperidol	Antipsychotic	2001
Levomethadyl	Narcotic Analgesic	2003
Propoxyphene	Analgesic	2015

Table 1: Non-antiarrhythmic Drugs Removed from US Market due to TdP Risk. Risk of inducing *Torsade de Pointes* is a well-known reason for non-antiarrhythmic drugs being removed from the market. Listed in this table are examples of such drugs, their intended uses, and the year they were removed from the US market [42].

Terfenadine as an example of drug-induced arrhythmia risk

Terfenadine was the first non-sedative antihistamine produced, marketed in the US in 1985 [46–48]. It's a prodrug that is metabolized by Cytochrome P450 3A4 (CYP3A4) into the active metabolite responsible for the antihistamine effects [48,49]. While the metabolite is not cardiotoxic, the un-metabolized terfenadine has been found to be cardiotoxic by prolonging ventricular repolarization through the inhibition of essential potassium ion channels [50,51]. While this wouldn't be a concern for the majority of people that can efficiently metabolize terfenadine, people taking contraindicated drugs that compete for CYP3A4, had a phenotype that metabolized it poorly, had liver damage, or even just consumed grapefruit juice (which can inhibit the metabolizing enzyme) were at risk of cardiotoxic concentrations of terfenadine in their system [49–53]. Any of these risk factors or a combination of them will reduce the rate of terfenadine metabolism, which causes higher plasma concentrations when the prodrug is absorbed faster than it can be metabolized. These higher systemic levels of terfenadine would prolong ventricular repolarization, and increase the risk of TdP events [50–53]. Due to these concerns, the US Federal Drug Administration (FDA) issued a report outlining these risk factors in June of 1990 and ordered the manufacturer to send out a warning letter to every practicing physician in August of 1990, which were later upgraded to a mandatory black box warning in July of 1992 [54,55]. These

measures led to a reduction in co-administration of terfenadine with contraindicated drugs but still terfenadine-related deaths continued [54–56]. Despite this continued risk the FDA approved a generic version of terfenadine, only to shortly after recommend that all terfenadine containing products be removed from the market [57]. In late 1997 terfenadine containing products finally began being removed from the market after being replaced with fexofenadine, the safer active metabolite of terfenadine [58]. The risk of TdP and SCD posed by these non-cardiovascular drugs led to the development of guidelines on the risk assessment of drug-induced ventricular repolarization prolongation by CPMP in 1997, and later by the ICH in 2005 [43–45]. These guidelines, endorsed by the FDA, increased the burden on pharmaceutical companies to assess the safety of their novel drugs and were foundational to the development of safety pharmacology studies [42,59].

Torsade de Pointes

Torsade de Pointes (TdP) is an atypical ventricular tachycardia that is characterized by its unique ECG morphology, an example of which can be seen in Figure 1 [7,8]. The undulating QRS waves rotating around a isoelectric baseline demonstrated in this figure are the reason this type of event was named *"torsade de pointes"* which is a French phrase that roughly translates to English as 'twisting of the points/peaks' [8]. TdP is a rare cardiac event that typically resolves on its own, however if left untreated for too long it does run the risk of devolving into ventricular fibrillation and then sudden cardiac death [60–62].

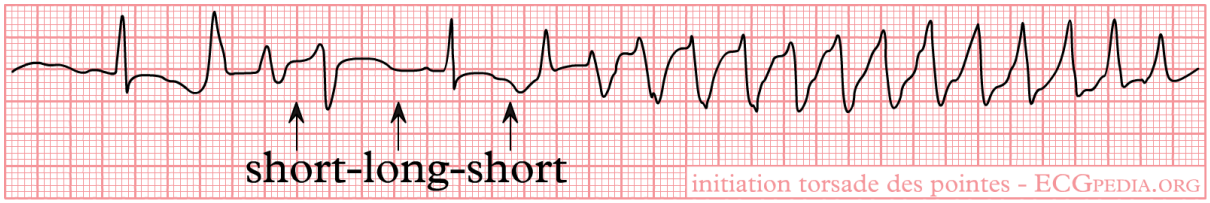

Figure 1: ECG Recording of Torsade de Pointes Event. The waveforms in this lead II ECG recording depict an example of a TdP event. These polymorphic waves changing direction around the hypothetical baseline are the twisting peaks the arrhythmia is named after. Typically preceded by a short-long-short group of beats signifying prolonged ventricular repolarization, electrically measured using the QT interval. This ECG recording was obtained from ECGpedia, made available through the creative commons license CC BY-NC-SA 3.0 [63].

Preceding electrocardiographic characteristics

A lot about the causes of TdP have been determined by studying the characteristics of ECG waveforms immediately preceding the events [7,10,11]. As exemplified in Figure 1, the polymorphic oscillations attributed to TdP are often preceded by a grouping of three beats (short-long-short) initiated by a premature ventricular beat, followed by a beat demonstrating prolonged ventricular repolarization, then finally a supraventricular beat of relatively short length [10,13,17,64]. Ventricular repolarization is electrically measured by the QT interval (QT), which is the time between the start of the Q wave (ventricular depolarization) and the end of the T wave (ventricular repolarization) on an ECG waveform [65]. Figure 2 contains a simplified representation of a typical waveform that would be seen on a lead II ECG recording, along with the direction and phase of cardiac conduction related to each wave. Research has continuously shown that this prolonged ventricular repolarization represented by QT is closely linked to TdP events and sudden death [56,61,62,66–69].

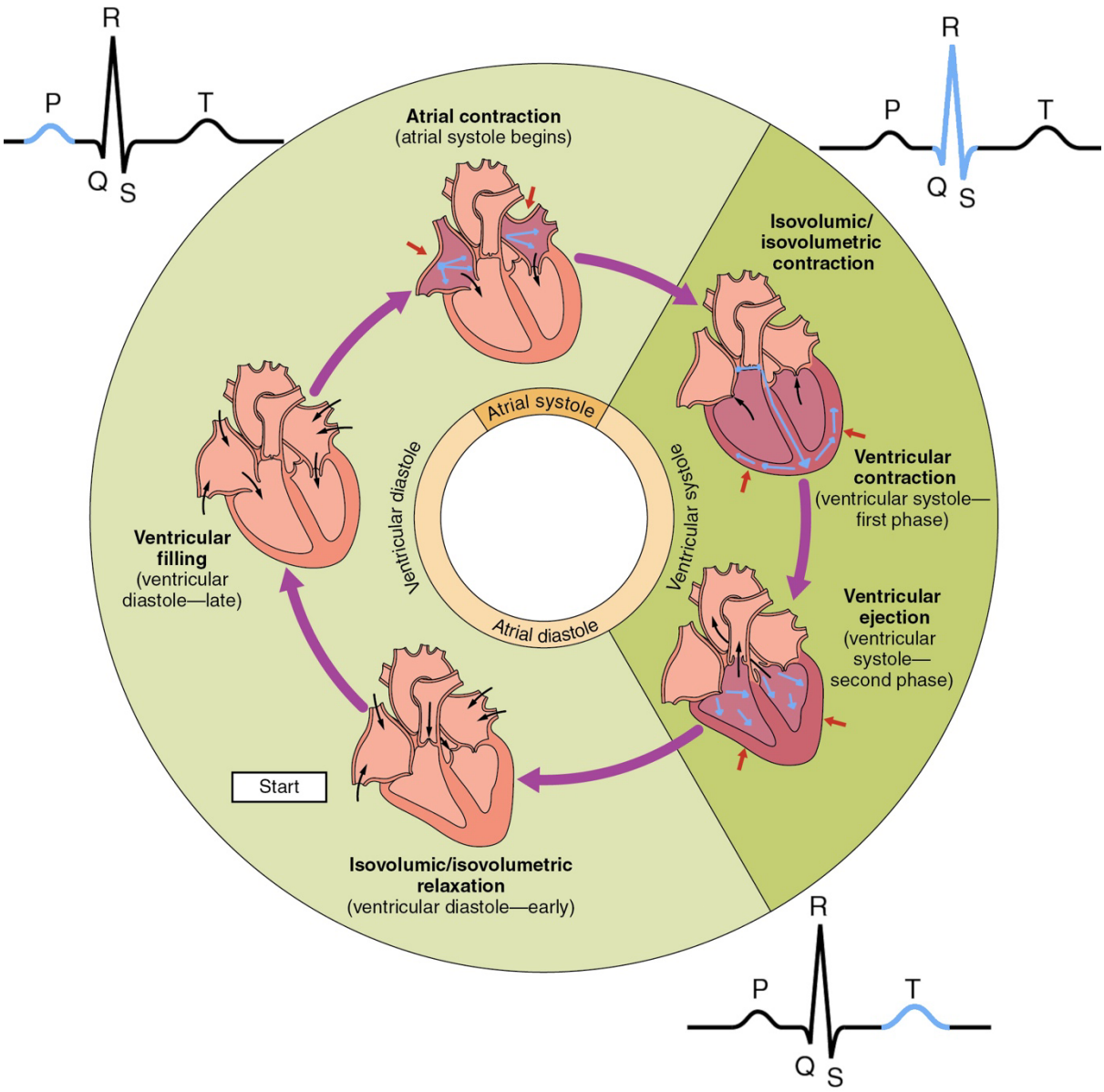

Figure 2: The ECG Waveform and Cardiac Conduction. This diagram depicts the cardiac cycle depicting the physiological phases of the heartbeat (diastole and systole) and how the signal conduction relates to contraction and the ECG waveform. The black arrows represent the direction of drug flow, the red arrows represent the direction of contractile force, and the blue arrows represent the direction of electrical signaling. These blue arrows correlate with the blue highlighted region of the lead II ECG waveform. The lead II ECG measures current from the top left of the depicted hearts to the bottom right. Signaling traveling in this same direction is represented as a positive wave on the ECG, signals traveling in the opposite direction is represented by a negative wave, and current traveling perpendicular to the lead will show no ECG change. This figure was modified for clarity from one made available on Wikimedia through the creative commons license CC BY 3.0 [70].

Risk factors associated with Torsade de Pointes

As the relationship between QT prolongation and TdP became more widely understood, more focus was placed on researching the specific risk factors that increase the chance of TdP events. These risk factors were generally tied to their ability to prolong ventricular repolarization or disrupt conductance distribution. For example, cardiac and noncardiac drugs that have been found to prolong QT through intended or unintended mechanism [56,60–62]. The rate of TdP events observed from these drugs can range from up to 5% for a high risk antiarrhythmic like quinidine to 1/100,000 for a relatively low risk drug like terfenadine [40,71]. These drugs typically interact with the ion channels responsible for managing the action potential during cardiomyocyte conduction, prolonging cell repolarization [72]. In addition to drugs that can affect these essential ion channels, disruptions in the extracellular ion balance due to diuretics or pathological disruptions can also prolong cardiomyocyte repolarization and increase the risk of TdP [32,73]. There are also genetic risk factors such as congenital Long QT Syndrome (LQTS), which increases the baseline QT interval and makes patients more susceptible to other QT prolonging triggers and increases the risks of TdP events [66,74,75].

Electrophysiology of Prolonged Ventricular Repolarization

Ventricular repolarization, electrically measured by QT, typically refers to the time a cardiac signal takes to conduct from the atrioventricular node of the heart, through the Purkinje fibers that rapidly disperse the signal throughout the ventricles and then back down through the remaining ventricular myocytes [76,77]. A simplified depiction of the relationship between cardiac conductance and a lead II ECG wave is provided in Figure 2. Mechanically speaking, QT relates to the ventricular systole of the heart which is the time between peak volume in the ventricles until they finish their contractions ejecting the blood from the ventricles [65,78,79]. Prolongation of this repolarization process can cause conduction disruptions and out of sync signaling, which can lead to ventricular tachycardia like TdP [69,80,81]. If these tachycardic events go uncorrected, they can devolve into ventricular fibrillation which can result in sudden cardiac death due to the heart

being unable to properly pump blood [6,30]. The risk of fatality such events pose is why ventricular repolarization is so commonly monitored in patients through the QT interval of the ECG [3,10,15,18,82]. This method of monitoring electrophysiological changes in the heart is used to represent the driving force of those changes, which are the effects on cardiomyocyte action potential duration (APD) [43,72].

Ventricular action potential duration

Ventricular cardiomyocytes react to signaling from nearby cells to trigger depolarization, which rapidly brings the membrane potential up and beyond a neutral 0mV beginning a cascading effect of opening and closing ion channels called repolarization, which slowly returns the cell to its original resting membrane potential. This process results in the activation of the next cell, while the original cell enters a refractory period during which it is less likely to respond to additional excitatory signaling [83]. The cardiac action potential is typically described in 5 phases, which have been illustrated in Figure 3 along with the ion channels that influence each phase [84]. Changes to ion concentration or function of their channels can alter the duration of the action potential, which affects the refractory period, can alter the relationship between cell excitation and muscle contraction, and change the proarrhythmic status of the tissue [85]. Thus, monitoring the conduction of the heart through the electrocardiogram is essential to clinical risk assessment and management [86]. Understanding the phases of ventricular cardiomyocyte action potential allow for more precise identification of the causes of increased arrhythmia risk and is vital to preventing or reversing them.

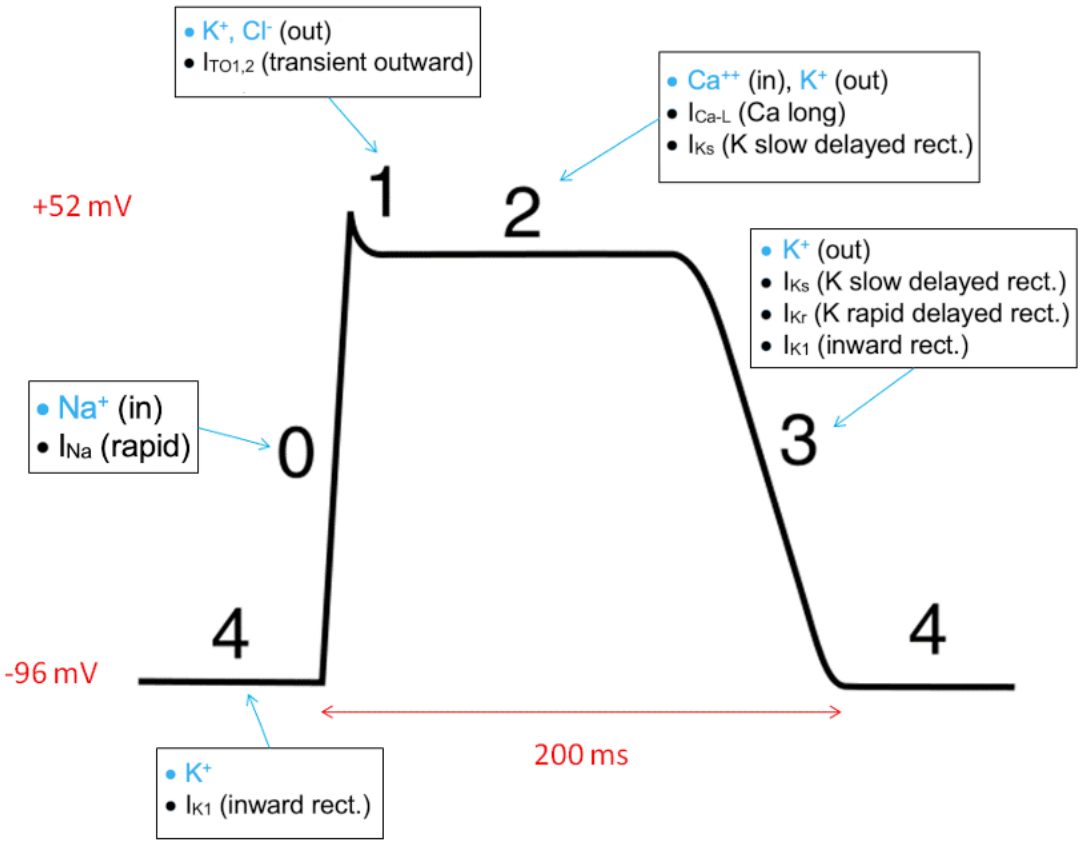

Figure 3: Ventricular Cardiomyocyte Action Potential. An illustration representing the phases of the action potential of ventricular cardiomyocytes responding to an excitatory signal. The ion channels associated with each phase has been included, with the direction the ion is moving in relation to the cell (in or out) in parenthesis next to the ion. Examples of resting membrane potential (-96 mV) and peak potential (+52 mV) have been included for reference. This figure was obtained from Wikimedia through the creative commons license CC BY-SA 3.0 [84].

Phase 0 is the part of the action potential when rapid depolarization begins and is triggered when the resting action potential of the cell increases slightly due to the electrochemical activity of the neighboring cell, transmitted through the gap junctions between the cells [87]. When the membrane potential climbs to about -70 to -55 mV from the resting potential of -90 to -80mV, voltage gated sodium ion (Na^+) channels (I_{Na}) are opened [88]. This allows Na^+ to rapidly enter the cell over the course of about 2ms until the cell reaches Na^+ electrochemical equilibrium and the membrane potential is around +50 mV [88]. During this period, L-type calcium channels (I_{Ca-L}) are also allowing calcium ions (Ca^{++}) to enter the cell, though the effect this has a relatively small impact on depolarization [89]. This voltage-dependent step of the action potential is an all-

or-nothing situation, as membrane potential of the cell must reach the necessary voltage for I_{Na} to open and begin depolarization. Anything that interferes with reaching this voltage, changes the target voltage, or blocks these channels can prevent the propagation of the action potential or prolong the time depolarization takes [90]. Changes affecting this part of the ventricular action potential would most likely be reflected in the widening of the ECG QRS complex which is linked to ventricular activation [91].

Phase 1 is the slight but steep decrease that occurs after the peak of depolarization. It is at this point that most of the sodium channels that were opened during phase 0 become rapidly inactivated [92]. This limits the flow of Na^+ into the cell and allows repolarization to begin. The early rapid repolarization seen during phase 1 is due to a combination of this reduced Na^+ flow and the activation of the transient outward current channels ($I_{TO1,2}$) that rapidly open and then rapidly close for a quick release potassium (K^+) and chloride (Cl^-) ions [93].

Phase 2 has been referred to as the plateau phase, as the membrane potential remains steady while the delayed rectifying currents slowly activate [91]. This balance is maintained by the ions entering and leaving the cell. The L-type calcium channels that were activated in phase 0 are still allowing Ca^{++} into the cell, which bind to ryanodine receptors on the sarcoplasmic reticulum, which releases more Ca^{++} [94]. All these calcium ions are positively affecting the membrane potential which should cause an uptick on the action potential, but the plateau is maintained by counteracting this increase in cations through cation transfer out of the cell through the Na^+/Ca^{++} exchange, Na^+/K^+ pumps, and most importantly the increasing activation of the slow delayed rectifier K^+ current (I_{Ks}) releasing K^+ from the cell [88,95–97]. I_{Ks} activation will continue through phase 3 and has had conflicting reports about its role in the action potential duration. However, this channel has been linked to certain mutations related to Long QT Syndrome (LQTS) and may be essential to mediating APD prolongation due to sympathetic signaling [94,95,97,98].

Phase 3 is known as the rapid repolarization phase which occurs after $I_{Ca\text{-}L}$ closes, allowing the steady stream of K^+ out of the cell through the still open I_{Ks} to reduce the cell membrane potential

[94]. It is at this point the another voltage gated potassium channel begin to open, the rapid delayed rectifier K^+ channel (I_{Kr}), and the inward rectifying K^+ channels (I_{K1}) release K^+ from the cell [88,99]. The release of positive potassium ions from the cell quickly returns the membrane potential to a resting range, at which point the delayed rectifiers are inactivated while I_{K1} remains open to manage the membrane potential during phase 4 [99,100]. The summation of phase 3 duration for all ventricular cardiomyocytes is represented by the T wave of the ECG, and alterations to this phase have been linked to problematic prolongation of the QT interval [91]. Mutations to the human Ether-à-go-go-Related Gene (hERG), which encodes the protein for the alpha-subunit of the I_{Kr} channel ($K_v11.1$), have been linked to a longer baseline QT interval in cases of hereditary LQTS [100–102]. Similar conditions can be acquired through drug treatments that target this channel or pathologies that interfere with ion availability [43,61]. Actions on these channels are the main concern during initial safety pharmacology screenings due to the risk of TdP when QT is prolonged [26,71,103,104].

Phase 4 is the resting phase of the action potential and is the state the cell begins in before phase 0 occurs. This equilibrium at the resting potential of about -90 to -80 mV is largely attributed to the continued activity of I_{K1} which allows K^+ in at potentials below the equilibrium range, and releases K^+ when above that range [88].

Long QT Syndrome

Long QT Syndrome is often the clinical diagnosis attributed to increased risk of TdP, and thus increased SCD as well. As the name suggests, LQTS is a condition in which a patient's baseline QT interval is longer than normal, increasing the chances for ventricular tachycardia [105]. This can be due to genetic mutations (hereditary LQTS) or environmental factors (acquired LQTS). Hereditary LQTS comes in multiple variations, depending on the genes responsible for the condition, while acquired LQTS can be due to drug treatments or ion imbalances that affect action potential duration [61]. Mutations in potassium ion channels are most related with hereditary LQTS, with specific attention given to LQT2 which is linked to mutations in hERG which codes for

the alpha subunit in the I_{Kr} channel [66]. Identification of the role hERG plays in LQTS was essential in furthering understanding of drug-induced QT prolongation [102]. A variety of drug classes have been found to inhibit I_{Kr} by binding inside the open pore of the hERG channel. This high binding affinity to such different chemical structures has been linked to the larger pore size compared to other K^+ channels, along with the aromatic residues found on the S6 domain (Y652 and F656) [106]. However, mutations in other genes related to ion channels can also contribute to LQTS such as I_{Ks}, I_{K1}, and I_{Na} [91]. Through studying these genetic causes of LQTS, research has been able to narrow down causes of acquired LQTS. QT prolongation can be due to ionic imbalances like hypokalemia, hypomagnesemia, and hypocalcemia [66]. Most importantly, from a drug regulation perspective, are the drug-induced incidents of acquired LQTS which often are due to inhibition of the same ion channels affected by hereditary LQTS [14,38,106]. Regardless of the cause of LQTS, the increased risk of TdP stems from the prolonged repolarization period of the action potential. This increases the risk of an early afterdepolarization (EAD) occurring, which is when the membrane potential rises unexpectedly during phase 2 or 3 of the action potential [11,64,107]. EADs can disrupt the cardiac rhythm and lead to tachycardia, especially if these alterations to repolarizations are dispersed through the ventricles. The risks associated with LQTS become more pronounced in the presence of other factors that may prolong QT or increase EAD risk, such as reduced heart rate, sympathetic activity, or the longer QT intervals observed in female patients [66,98,108]. It is because of these risks that assessment of novel drugs for their potential to prolong QT has become such a vital aspect of the drug-development process [26,71,109].

Discussion

Torsade de pointes is a rare and short-lasting ventricular tachycardia that is potentially fatal due to its link to sudden cardiac death [62]. The risk of TdP increases in the presence of inherited or acquired Long QT Syndrome, which are caused by alterations to cardiomyocyte ion channel activity due to genetic conditions or drugs effects, respectively [61,105]. These changes to ion

channel activity prolong the repolarization phases of the action potential, which is reflected on the ECG as prolonged QT intervals [85]. Of particular note is the effects of mutations to hERG and drugs that block the channel related to its encoded protein (I_{Kr}) [102,110,111]. Effects on this ion channel are associated with increased TdP risk, such as the hERG blocking terfenadine which was removed from the market due to its risks [38,55,57]. Pharmaceutical companies are incentivized to identify drugs with such risk early in the development process to prevent unnecessary harm and avoid more drugs being removed from the market. Due to the rarity of TdP events and wide range of risk factors that would be difficult to capture in clinical trials, QT prolongation has been adopted as a biomarker of TdP risk [112]. Guidelines provided by the ICH have become essential to the assessment of this QT prolongation risk [44,45]. Though further improvements are still being made to these guidelines along with alternative assessment strategies based on increased understanding of the interconnected roles played by the ion channels involved with the cardiomyocyte action potential [113–116].

PART 2:

ASSESSING QT PROLONGATION RISK DURING DRUG DEVELOPMENT

Abstract

Due to the danger posed by unexpected drug-induced arrhythmias, safety pharmacology studies have been developed to assess this risk. These studies rely on QT prolongation as a biomarker for proarrhythmic risk due to its close relationship to arrhythmias such as *torsade de pointes* and the rarity of such events. Pharmaceutical companies design their safety assessments in accordance with regulator expectations based on guidelines released by the ICH. Safety guidelines ICH S7B and ICH E14 detail the best practices for preclinical and clinical safety assessment of drug-induced QT prolongation risk, respectively. As a result of these guidelines, drugs with unacceptable QT prolongation risk no longer make it to market in the US. However, they are not without their shortcomings and pharmaceutical companies are pushing for improvements that will lighten the burden of these assessments. In this section of the chapter, I provide the history and context for these safety studies and present examples of how they can be improved. Most relevant to this dissertation are the suggestions for improving QT correction methods. In doing so, the increased reliability of study results may increase the chance of preclinical and clinical study integration. This integration would reduce the time, money, and subjects necessary for such studies.

Introduction

Due to the risk posed by drug-induced arrhythmia events like TdP, especially in non-cardiac intended drugs, pharmaceutical companies must screen for this risk during drug development [31,117]. However, the incidence rate of TdP events can vary from up to 5% of cases in high-risk drugs like quinidine to a rate of 1/100,000 for relatively low-risk drugs like terfenadine [40,71]. This rarity makes detecting TdP events an unreliable metric during preclinical and clinical safety studies, which has led to QT prolongation being adopted as a biomarker for TdP risk. ICH E14 detailed the suggested methods of clinically assessing QT prolongation using 'Thorough QT' studies [45]. Similarly, ICH S7B outlined preclinical safety studies for assessing arrhythmia risk *in vivo* and *in vitro* [44]. The *in vivo* studies focused on testing drug candidates in appropriate preclinical animal species at relevant drug concentration, while the *in vitro* studies encouraged the testing of drug effect on the hERG-related ion channel responsible for I_{Kr} [116]. These guidelines have since been endorsed and utilized by regulatory agencies, such as the US FDA [118]. This section of the chapter provides an overview of why QT prolongation is used, the importance of heart rate correction, the common safety pharmacology studies that have been adopted, and how they can still be improved.

Prolongation of the Heart Rate Corrected QT

Prolongation of the ECG QT interval has a long history of association with TdP and SCD events. Even back when TdP was first being investigated, the role of QT prolongation was identified as a relevant factor [10,16]. Additional research into the incidence rate of TdP in the presence of QT prolonging drugs and genetic conditions have further cemented their relationship [14,15,19,23,103,119]. However, drug-induced QT prolongation alone is not enough to assess proarrhythmic risk, as QT has long been known to have an inverse relationship with heart rate [79]. Accounting for heart rate effects is vital to isolate drug effect on QT prolongation, especially because the proarrhythmic risk increases when the relationship between repolarization and heart rate becomes decoupled [120]. To address this, methods have been developed to correct

measured QT datapoints based on their associated heart rate. These QT correction methods aim to produce corrected QT values (QTc) to replace the raw measured QT with estimates of what QT would have been had the subject been paced at a fixed heart rate. Prolongation of this QTc value compared to vehicle treatment and/or baseline data is then used to assess proarrhythmic risk [72].

QT Correction Methods

Correcting QT for rate goes back to when the relationship was first mathematically determined by Bazett, who found that the relationship between the two could generally be accounted for by dividing QT by the square root of rate [79]. The Bazett's correction method was commonly used for decades and is still used by some today [121]. However, another popular method by Fridericia from around the same time found that using a cube root instead of a square root provided more accurate correction at the extreme ends of rate [78]. Though these two methods are the most well-known human QT correction methods, other studies have attempted to improve on them using different methods and study populations. This includes Mayeda, who examined four times as many subjects as either Bazett or Fridericia and determined that raising rate to the power of 0.604 was more appropriate than using a square or cube root [122]. Other popular QT correction methods include those from Hodges and the Framingham Heart Study, which are based on the linear equation using slopes obtained from linear regressions performed on data from their respective study populations to represent the QT-rate relationship [123,124]. These types of fixed formulae corrections have been referred to as universal or general corrections, and are designed from human data, for human data. However, preclinical species are important parts of the risk assessment process and typically have different heart rate ranges and QT-rate relationships than humans [112]. Despite this, these human-based general correction methods are still used in some preclinical animal studies [125]. Though work has been done to produce more relevant general corrections for preclinical species. Examples include Van de Water's method developed for canines and Nakayama's method developed using cynomolgus monkeys [126,127]. Table 2

contains a list of some QT correction methods, along with their formulae and what study populations were used to derive them. While this general correction method approach has been the standard for decades and was continually recalibrated by attempting to use more appropriate study populations, all general methods rely on the same pivotal assumption: that the QT-rate relationship observed in a study population will accurately reflect that of an individual subject. However, many studies have shown that this is not the case and that ignoring this variability in the rate relationship between subjects and over time adds unnecessary error to study results [128–133]. Recent advancements in computing have allowed for the regular use of individualized correction methods, which typically use linear regressions to identify the QT-rate relationship for an individual subject and then use the slope of that relationship as a correction factor [129,134,135]. These individual methods are taking similar approaches as Hodges and Framingham took, but thanks to the ability to quickly evaluate large amounts of data, these values can be determined for each subject shortly after study completion. Despite the ability of these newer individualistic correction methods to account for inter-subject variability, regulatory bodies like the FDA still find the use of general correction methods to be acceptable during safety pharmacology studies [42].

Method	QTc Formula	Study Species	Number of Subjects
Bazett [65,79]	$\frac{\mathrm{QT}}{\mathrm{RR}^{1/2}}$	Human	39
Fridericia [78]	$\frac{\mathrm{QT}}{\mathrm{RR}^{1/3}}$	Human	50
Mayeda [122]	$\frac{\mathrm{QT}}{\mathrm{RR}^{0.604}}$	Human	200
Hodges [123]	$\mathrm{QT} + 1.75(\mathrm{HR} - 60)$	Human	607
Framingham [124]	$\mathrm{QT} + 0.154(1 - \mathrm{RR})$	Human	5,018
Van de Water [127]	$\mathrm{QT} - 87(60/\mathrm{HR} - 1)$	“mongrel” dogs	10
Nakayama [126]	$\frac{\mathrm{QT}}{\mathrm{RR}^{0.576}}$	Cynomolgus monkey	353
Individual	$\mathrm{QT} - \mathrm{m}(\mathrm{rate} - \mathrm{refR})$	Any	1

Table 2: QT Correction Method Examples. Included are examples of QT correction methods, their formulae, and the study populations used to derive them. QT is the QT interval in ms, RR is the interval between two ECG waves in seconds, and HR is the heart rate in beats per minute. Each method provides a QTc value in ms. The individual correction is similar to those used for Hodges, Framingham, and Van de Water. In it, m is the correction factor for individual subject QT-rate relationship, typically determined from the slope of a linear regression of QT and either HR or RR, depending on which provides a more linear relationship. The rate value is the associated rate value (either HR or RR) that is related to the QT value being corrected, while refR is the reference rate value the method is correcting to.

Safety Pharmacology Studies

Research linking TdP risk and QT prolongation goes back to the 1980s [10,13–15,19,64]. Investigations into QT prolongation being used as assessments of TdP and SCD risk continued through the early 1990s [11,23,31,38,117,119,136]. However, it was not until 1997 that the CPMP released Note CPMP/986/96; ‘Points to Consider: The Assessment of the Potential for QT Interval Prolongation by Non-cardiovascular Medicinal Products’ [43]. These relatively early guidelines were some of the first to encourage pharmaceutical companies to assess their drug candidates for the risk of prolonging QT as a substitute for evaluating TdP risk directly. Since then the CPMP suggestions have been wholly replaced in 2005 by the ICH efficacy guideline E14 titled ‘The Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs’ and safety guideline S7B titled ‘The Non-Clinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals’

[44,45]. Which the FDA later implemented in late 2005, though with reservations about the translatability of preclinical assessments [137,138].

Preclinical Safety Studies

Preclinical safety studies described by ICH safety guideline S7B includes a combination of *in vitro* and *in vivo* experiments to assess the mechanistic and physiological risk of a drug candidate of inducing QT prolongation. The role of the *in vitro* safety assessments described by ICH S7B is to evaluate the ability of human pharmaceuticals to affect ionic current flow and action potential duration, specifically in relation to repolarization. While multiple ion channels are involved in the repolarization process, ICH S7B specifies inhibition of hERG I_{Kr} as the most common mechanism responsible for drug-induced QT prolongation in humans. The guidelines recommend assessing action potential duration and patch clamp measurements of hERG ionic current in the presence of test article. While this guideline allows for the use of animal cardiac myocytes in these assessments, using human cardiac myocytes and/or models with cloned human ion channels would be preferable.

For *in vivo* assessment, ICH S7B recommends obtaining ECG recordings from conscious or anesthetized animals treated with the test article. These ECGs should be examined for prolongation of corrected QT values and/or the presence of arrhythmic waveforms. The QT correction methods recommended are Bazett's or Fridericia's, leaving it up to the researcher to justify which they select. However, the guidelines note that if there is a large enough difference between the rates of control and drug treatments, both methods may be ineffective for assessing risk. In these cases, using individual correction methods may be more appropriate, though they do not elaborate further. Larger animal species typically used for electrophysiology studies are recommended for use such as dog, monkey, swine, rabbit, ferret, and guinea pig. As with the selection of QT correction method, the guidelines leave the justification of species selection up to the researcher. They do emphasize the importance of considering the sensitivity and reproducibility of the chosen test system, as well as factors that may introduce error or confound

results. These include data acquisition and analysis methods, selection of dose period and measurement timepoint, heart rate effect, inter-species and gender differences (affecting electrophysiology, hemodynamics, and/or metabolism), and the fact that drugs interacting with multiple ion channels may increase the difficulty of interpreting the results. The results obtained from preclinical *in vitro* and *in vivo* experiments are intended to be combined with relevant information from other studies and the history of risk from other drugs in the same class. This evidence of risk should be used to inform pharmaceutical companies and regulators about the potential risk of prolonging QT in humans [44].

Clinical Thorough QT/QTc Studies

The clinical evaluations of drug-induced QT prolongation risk described in ICH E14 revolve around what they call the 'Thorough QT/QTc Study. The ICH prefaces these guidelines by acknowledging that QT prolongation is an imperfect biomarker, and that it is unclear whether uncorrected or corrected QT are more likely to be associated with TdP risk. However, as the measurement of QT prolongation prior to TdP events have been prevalent in cases with recalled torsadogenic drugs, it is important to assess the QT prolongation risk of new drugs before they enter the market. The intention of these guidelines is to encourage the clinical evaluation of non-arrhythmic drugs that are systemically distributed for off target effects on cardiac electrophysiology. It is recommended to exclude any volunteers that may be at increased risk due to longer baseline QT until early clinical assessments have been completed. These Thorough QT/QTc studies are vital to the understanding of risk in the human population and are encouraged to take all precautions possible when it comes to eliminating bias and variability. The threshold of drug effect on QTc that is of regulatory concern is 5ms (as evidenced by the upper bound of the 95% confidence interval of a 10ms mean QTc effect). To validate the ability of the study to identify this level of effect, a positive control that is expected to result in a 5ms change in QTc should be used as well. If a drug effect crossing this threshold is detected, the study is deemed a 'positive Thorough QT/QTc study'. For a study to be considered negative, the highest degree of effect

must not have a 95% confidence interval that crosses 10ms. If deemed negative, the typical ECG monitoring in accordance with typical regulatory practices for that therapeutic class are considered sufficient for regulatory purposes. However, if the study is deemed positive or if it deemed negative when the preclinical investigation showed substantial risk there must be further evaluations into the drug effects. As with the preclinical assessments, the clinical recommendations for QT correction methods include Bazett's and Fridericia's, which are more appropriate for use in humans. Though the guidelines acknowledge their shortcomings and suggest submitting QT, rate, Bazett's corrected QT, Fridericia's corrected QT, and an individually corrected QT based on a linear regression method. While they don't go as far as endorsing the individual correction, the do recognize the importance of evaluating its ability in these studies [45].

Improving Translation Between Non-Clinical and Clinical Safety Studies

The ICH guidelines E14 and S7B have been largely successful, and since their implementation no drug has been approved with an unacceptable risk of TdP [139]. However, concerns have been raised about placing so much emphasis on imperfect biomarkers like QT prolongation and hERG blockade. Drugs like amiodarone are known to do both and yet aren't associated with TdP risk, likely due to its effects on multiple ion channels [136]. Such methods guarantee high sensitivity of risk detection, but the specificity is unknown, and may prevent useful and otherwise safe drugs from being marketed. There are attempts at improving the specificity of risk assessment through better understanding of the role each ion channel plays into TdP risk [114]. Such considerations are the foundations of collaborative efforts by The Comprehensive *in vitro* Proarrhythmia Assay (CiPA) initiative, which aims to design more through mechanistic models for accurately assessing TdP risk [115,116].

In addition to these improvements to specificity, there have been attempts to better integrate the preclinical results into the clinical decision-making process [140]. Assessing the ability of preclinical studies to identify compounds that have demonstrated clinical TdP risk is a common research topic and typically supports their ability to do so [141–146]. Regardless of the outcomes

of these preclinical studies, pharmaceutical companies are required to proceed with Thorough QT/QTc studies during the clinical phases of drug-development [45]. As many pharmaceutical industry researchers would eagerly point out, this disconnect between the results of S7B studies and the decisions made for E14 studies wastes time and money on Thorough QT/QTc studies for low-risk compounds [139].

In an effort to address concerns about the E14 and S7B guidelines, including requests for the integration of the two, the ICH recently published the 'ICH E14/S7B Implementation Working Group Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential – Questions and Answers' (E14/S7B Q&As) [113]. In this document the ICH addressed a wide range of questions about specific techniques, best practices, and the integration of preclinical and clinical risk assessment. While these responses do encourage the use of additional ion channel assays to better assess TdP risk and clarifies when enough nonclinical assessments have been made, they do little to integrate the use of preclinical studies with clinical decision making. The exception being when Thorough QT evaluation is not viable, in which case an integrated approach using preclinical and clinical data can be used. It seems further improvement to preclinical techniques and additional evaluations of the translatability of preclinical results are needed before preclinical studies are sufficient to prove Thorough QT/QTc studies unnecessary. Important for the topic of this dissertation is the discussion about QT correction methods within the E14/S7B Q&As. Accurately correcting QT for heart rate is a vital aspect of improving the accuracy of the preclinical study results. Making these improvements could reduce variability, in turn increasing the power associated with the methods, and allow for a reduction in the number of subjects needed per study. Additionally, by reducing the variability of the results the preclinical data is more likely to be accepted for integration with the clinical assessments. The E14/S7B Q&As provide important guidelines on how to pursue this goal. Unlike the original guidelines, these responses encourage the use of individual methods over the general correction methods, describing them as more accurate and sensitive. They also state that the decision to choose a

specific QT correction method should be supported by demonstrating the independence of QTc in relation to RR intervals. This should be done using QTc vs RR plots accompanied by additional numerical data to represent their relationship. Another way to evaluate the method chosen is assessing the sensitivity by calculating the minimal detectable difference and testing against positive controls. While these are straightforward suggestion on how to justify chosen QT correction methods, they're the first to be endorsed by the ICH and therefore are likely to become standard throughout the industry.

Discussion

The connection between QT interval prolongation and *Torsade de Pointes* events have been thoroughly investigated since Dessertenne first describe the polymorphic tachycardia back in 1966 [7,8,10,11,19,41,80,103]. Understanding this link and the mechanisms behind them have led to the development of drug-development guidelines designed to limit the approval of torsadogenic drugs by assessing their risk of prolonging QT [44,45,113]. These guidelines from the ICH outlined suggested practices for the preclinical assessment (S7B) and clinical Thorough QT studies (E14) that pharmaceutical companies still follow. An important aspect of both the clinical Thorough QT studies and the preclinical *in vivo* studies is the need to correct QT for the influence of heart rate [25]. However, the QT corrections selected for use can vary by species or by researcher preference. Only recently has the ICH released updated guidelines that encourage the use of individual based correction methods and describe methods for justifying the correction method selected [113]. These updated guidelines recognize the inherent problem of general correction methods that use fixed population-based correlation coefficients [132]. Many researchers had hoped that these updated guidelines would provide more substantial integration of the preclinical and clinical assessment process [139]. Unfortunately, the ICH fell short of providing full integration of the E14 and S7B guidelines. However, they have provided a clearer path forward in terms of improving QT correction methods by standardizing how they are justified. This will allow for more meaningful comparisons of traditional and novel correction methods.

Through the improvement of such a vital aspect of these risk assessments it is possible to reduce the variability and increase the accuracy of these studies. In this pursuit it is possible to increase the power of the studies, allowing for a reduction in the number of subjects needed for assessment of drug effect on QTc. By improving our understanding about how these methods work and increasing their reliability, we come closer to the day when the translatability of preclinical results is accepted by regulators.

Overview of Dissertation Aims

There is a critical need to determine the best use of QT correction methods and identify ways to improve them. Doing so could produce a method that is adopted as an industry standard and increase the translatability of preclinical safety study results. Such improvements to our understanding of QT correction methods can streamline the drug development process and reduce the number of subjects required for investigations. My overall goal for this dissertation was to identify factors that influence QT correction methods and how best to control for them. I also sought to create a novel method of QT correction based on this in-depth research into their function. Below is an overview of the aims for this project which I will cover thoroughly in the following chapters.

Aim 1: Compare assumptions inherent in QT correction methods and how they affect detection of relevant drug-induced QT prolongation.

The second chapter of this dissertation examines how various QT correction methods function and what assumptions are inherent to them. This begins with tutorial figures demonstrating how different approaches to QT correction adjust QT based on rate. Then the methods are compared using simulated drug data with pre-specified effects on QT and rate. By comparing them this way the scenarios in which they produce the most accurate results can be assessed.

Aim 2: Evaluate factors contributing to variability in the QT-rate relationship.

The third chapter of this dissertation explores inter- and intra-subject factors that affects the QT-rate relationship by comparing different QT correction methods designed to account for them. By

identifying which methods most consistently reduce the correlation between QTc-rate and produces the results with the least variability, the important factors influencing the deviation of the QT-rate relationship can be assessed. This chapter focuses on using bootstrap simulated data from vehicle treated non-human primates and beagle canines. Through these methods the importance of accounting for the differences between subject, occasion, and environmental light status is made evident.

Aim 3: Create and evaluate a novel QT correction method using drug-treatment data.

In the fourth chapter of this dissertation, I further evaluated QT correction methods using non-human primate subjects treated with known QT prolonging drugs (dofetilide and moxifloxacin). Additionally, a novel correction method created by myself with the help of my project mentors is compared to traditional methods. Through these evaluations, the importance of individual correction is emphasized, and the benefits of the new more dynamic method are highlighted.

This introductory chapter has described the history of drug-induced arrhythmia, why they are dangerous, the mechanisms behind them, and the safety assessments developed to prevent further harm to patients. It also highlighted areas that still need improvement, specifically the QT correction methods used. In summary, drug-induced arrhythmias are dangerous and must be screened for during the drug development process. However, the safety assessments take time, are costly, and may be eliminating useful drugs from the market. All this while the results of preclinical evaluations have little bearing on the Thorough QT process. Improving QT correction methods is one way to increase the trust in preclinical results and further translatability.

CHAPTER 2:

UNDERSTANDING QT CORRECTION METHODS

Abstract

QT correction methods are used clinically and preclinically to isolate changes to the QT interval, irrespective of the effects of heart rate changes. This is a vital aspect of the safety pharmacology studies that occur during drug development. Accurate QT correction is necessary to compare the effects of drug treatments on QT between subjects and treatments. Without an accurate QT correction, the everchanging heart rates, which have their own effects on QT, may obfuscate the drug effect and increase variability. To address this, QT correction methods estimate what each QT value would be if the rate was fixed, based on an understanding of the QT-rate relationship. The accuracy of a QT correction method depends on the QT-rate relationship it uses to correct and what rate the data is being corrected to. Typically, these methods fall into one of two categories: general methods that use fixed correction coefficients derived from historical population data to represent the QT-rate relationship, or individual methods that use a regression technique to calculate a correction coefficient unique to the QT-rate relationship of each subject. This chapter explores the effects these approaches have on the resulting corrected QT values. A novel Ratio-based correction method is also introduced which attempts to combine the convenience of a general method with the accuracy of an individual method by using a reference point to estimate the rate relationship for each datapoint. The assumptions inherent to each of these methods are explored and compared against simulated drug treatment data designed to emulate drug effect on heart rate, QT, or the QT-rate relationship directly. Results of these comparisons confirm our hypothesis that the novel method provides more effective correction than a general method, more like that of individual methods. Additionally, these results show how assuming the QT-rate relationship before correction may hinder the detection of drug effect that alters this relationship in a dose-dependent manner. Information gained from this study will increase our understanding of QT correction methods and assist in their optimization.

Introduction

As discussed in the previous chapter, QT correction methods play an important role in safety pharmacology assessments. They are crucial in isolating drug effect on QT prolongation in the presence of changes to heart rate [147]. Historically this has been done using general methods which assume that their understanding of the rate relationship based on a limited number of subjects is sufficient to correct each subject of a study. Bazett's correction was one of the first QT correction methods developed and is still in use today, despite being based on only 39 humans back in 1920 [79]. However, there have been countless attempts at improving these general methods, including suggestions that using linear regression produced a more appropriate correction coefficient and method [123,124,148]. Regardless of the QT correction method selected, the goal and process remain the same; to minimize the relationship between QTc and rate by estimating what QT would be at a fixed rate based on the method's assumptions about the QT-rate relationship. This is where general correction methods fall short, as they base their relationship assumptions on limited sample populations (usually human) and are often designed to correct to a rate of 60 bpm. The problems with these methods are already criticized when used in humans, especially for those with cardiovascular conditions that make 60 bpm an unrealistic reference [123,131,149]. Furthermore, these methods ignore inter- and intra-subject variability in the QT-rate relationship [128,130,132,149]. This problem is further exacerbated when these human-based correction methods are used in preclinical studies on species with very different ranges of heart rates and QT-rate relationships than those seen in humans [126,127,150–153]. Since this dissertation focuses on QT correction methods in the context of preclinical animal species, specifically non-human primates (NHP), the more appropriate RR interval (RR) will be used to represent rate instead of heart rate. This interval between the R waves of two ECG beats is simpler to measure and typically has a more linear relationship with QT in NHPs [134]. With RR being the inverse of heart rate, and heart rate having an inverse correlation with QT, this means the QT-RR relationship is a positively correlated linear relationship. For context, the equivalent to

a heart rate of 60 bpm is an RR of 1000 ms. With that clarification made, it is important to note that the assumptions made by general correction methods can introduce unnecessary error to preclinical studies, which is why the pharmaceutical industry is moving towards the use of individual-based correction methods [113,132,135]. Such methods are designed to estimate QTc more accurately by evaluating the QT-rate relationship of each individual subject and use that relationship to correct to a more species appropriate reference rates (refR). However, even the effectiveness of these can vary based on the assumptions made and how successfully they account for inter- and intra-subject variability. The foundations of an effective correction method are an accurate representation of the QT-rate relationship present in the data and the correction to a biologically relevant refR.

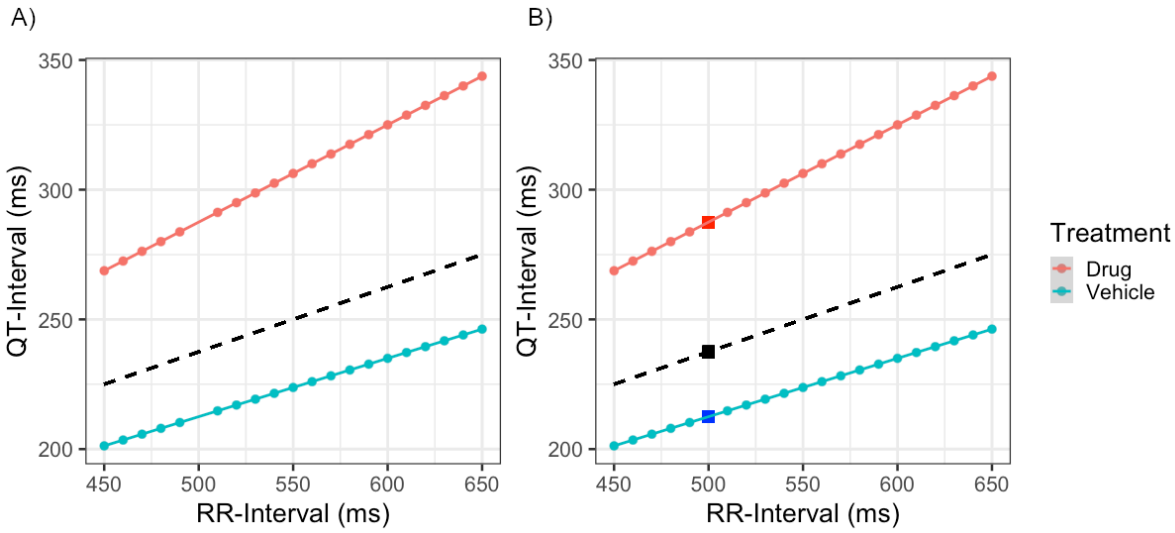

Figure 4: Using Different QT-RR Relationships to Estimate Missing Data. This figure contains simplified hypothetical data representing data from a 'vehicle control' (blue) and 'drug treatment' (red) that have resulted in different relationships between QT and RR. Also included is a dashed black line to represent a QT-RR relationship derived from a hypothetical population. In both data sets presented in (A) a value for QT is missing at 500 ms RR. In (B) the estimates of what those missing values should be are represented by color-matched squares. The red square was estimated using the relationship of the hypothetical drug treatment data, the blue square was estimated using the hypothetical vehicle treatment data, and the black square was estimated using the population-based relationship.

To demonstrate the importance of using an accurate representation of the QT-rate relationship, Figure 4 has been included with two demonstrative QT vs RR plots. In both plots the blue points represent a hypothetical vehicle control treatment data and the red points represent a hypothetical

drug treatment data, and both datasets have a color-matched regression line through the data representing their QT-RR relationship. A black dashed line without datapoints has also been included to represent the linear QT-RR relationship determined based on a hypothetical population, similar to a general correction method. In both datasets presented in Figure 4A, a datapoint is missing for an RR of 500 ms. If a researcher collecting this data was trying to estimate the QT at those two missing datapoints, they would most likely use the relationship between QT and RR from a dataset to predict the datapoint missing from that dataset. Figure 4B shows the results of trying to estimate what that QT value should be based on the three relationship options available. The color-matched square datapoints represent the estimated QT values, and each fall on the line used to estimate them. While the results presented may be easy to predict, it demonstrates the necessity to use an accurate estimation of the QT-rate relationship when correcting. Had the population-based relationship been used to fill in the gaps it would have underestimated the treatment datapoint and overestimated the vehicle datapoint. Similarly, if the vehicle-based relationship was used to estimate the missing datapoint of the treatment data; the value would have been severely underestimated, and vice versa. The point of this demonstration is to raise a vital question about QT correction methods: if we expect to correct QT based on the QT-rate relationship, is it appropriate to use a relationship from a different dataset?

General Correction Methods

So how do these QT correction methods effect the datapoints to produce the QTc values? Well, each varies slightly, but when it comes to general correction methods like Bazett's they have a fixed correction coefficient based on the historical population used to develop it [65]. The rate (HR or RR) value is raised to the power of this fixed coefficient (0.5 in the case of Bazett's) and then QT is divided by the result to shift it along the y-axis proportionally to the distance the rate value is from the reference rate (60 bpm / 1000 ms for Bazett's). Figure 5 demonstrates the effect of Bazett's QT correction method (QTcB) on the data presented in Figure 4. In the figure the axis and linear relationship lines have been extended out to 1000 ms to demonstrate the effect that

correcting to this inappropriate rate has on the data. In Figure 5A, color-matched arrows have been included to show where the data is being shifted to and how the magnitude of that shift increases the further the data is from the reference rate. Figure 5B illustrates the results of using QTcB, which has varying effectiveness in reducing the relationship slope depending on the hypothetical treatment. This is likely due to the QT-RR relationship from the hypothetical vehicle data more closely matching those seen in the population used by Bazett.

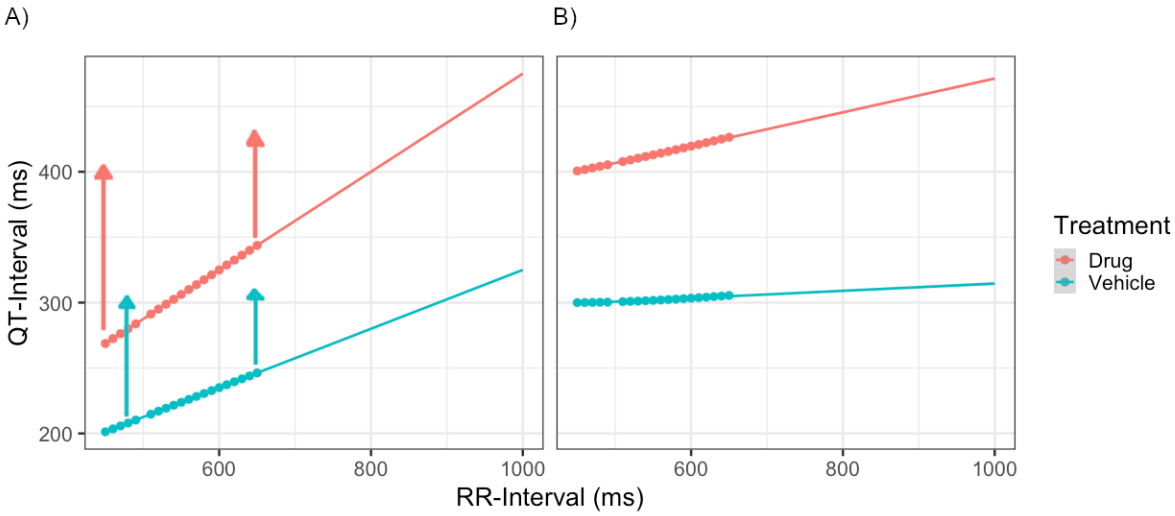

Figure 5: Emulating the Effect of General QT Correction. The data in this figure is the same simplified hypothetical data used in Figure 4. Hypothetical vehicle treatment data are represented by the blue dots and hypothetical drug treatment data are represented by red dots. Color-matched linear regressions are included to represent the QT-RR and QTc-RR relationships. These regression lines and the x-axis have been extended out to 1000 ms to demonstrate the effects of a general correction method that uses an outlying reference rate. (A) contains the original data points, with color matched arrows demonstrating the direction and magnitude of data shift that the correction will cause. (B) contains the results of the data after Bazett's QT correction.

It is important to note how the closer to the 1000 ms reference rate, the less shift seen in the resulting line. Commonly used preclinical animal have faster heart rates and therefore lower RRs than humans. Examples include cynomolgus monkeys and beagle canines which typically have heart rates closer to 120 bpm (RR of 500 ms) and 80 bpm (RR of 750 ms), respectively. This means that preclinical data corrected with QTcB will always be influenced by this biologically irrelevant rate, further reducing the effectiveness of correction. The plots in Figure 5 also highlight

the overestimation of QTc compared to the raw QT values. Not a single QTc value falls within the range of the original data.

Individual Correction Methods

These inherent flaws in general correction methods, along with the understanding that the QT-RR relationship will vary between subjects and occasions has led to an increased interest in linear regression-based individual correction methods [128–131,135]. Individual corrections do not assume that historical data sets are sufficient for understanding the rate relationship in an individual subject. Instead, they assume that using data from an individual subject is the best way to represent the QT-RR relationship of that subject on study. This is typically done using a linear regression calculation to identify the line that best fits through the QT-RR data from a subject's ECG recording. Taking the slope from this resulting line to represent the relationship between the two variables, the following formula can be used: QTc = QT – m*(Rate-RefR). This is a modification of the standard linear equation: y = mx + b. On a QT vs RR plot, this linear equation is equal to QT = m*RR + b, where "m" is the slope of the regression line through the data and "b" is the y-intercept value of that line. In the individual correction method, the distance between a rate value and the reference rate is used in place of x. This sets the equation to be relative to the reference rate, setting a pseudo-axis to x = RefR, which means "b" would now refer to an intercept at this pseudo-axis by a line with slope "m" that passes through the datapoint being corrected. By calculating this intercept, the QTc value at RefR is determined. This is a concept that is easier to demonstrate than describe, so Figure 6 has been included to help visualize this process.

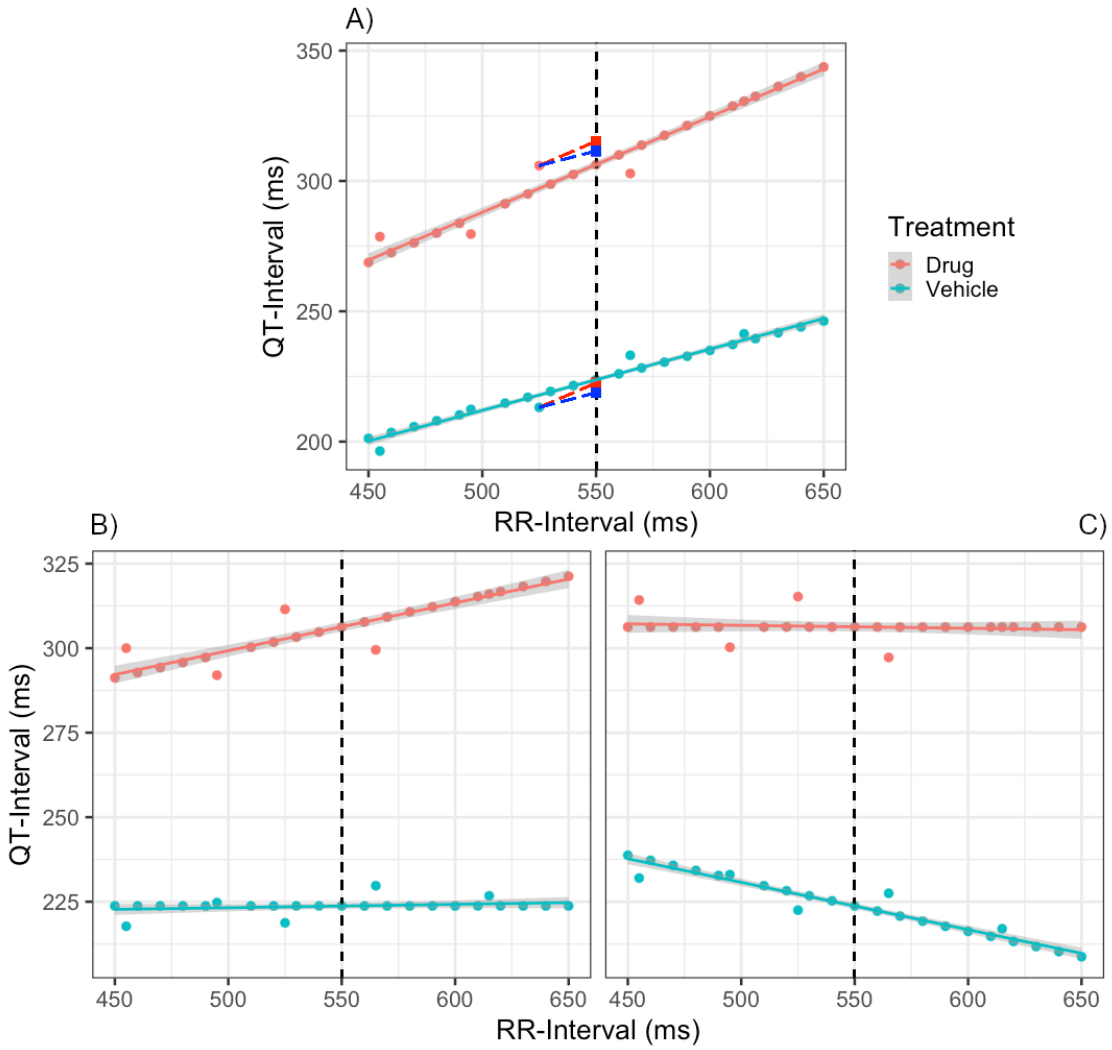

Figure 6: Emulating the Effect of Individual QT Correction. The data in this figure is the same simplified hypothetical data used in Figures 4 and 5, except some randomly generated outlier datapoints have been added so the confidence intervals around the color-matched linear regression lines are now visible as grey shading. The vertical black dashed lines represent the pseudo-axis at the reference rate (550 ms) that the individual correction methods calculate the intercept of to estimate QTc. (A) contains the uncorrected data, with examples of how two data points will be affected by the individual methods depending on the relationship utilized. The blue squares represent the QTc values determined for the datapoints they are connected to using the slope of the hypothetical vehicle data. The red squares represent the QTc values determined for the datapoints they are connected to using the slope obtained from the hypothetical treatment data. The color-matched dashed lines represent the slope used to calculate the QTc they're connected to. (B) contains the QTc results using individual correction with the slope from the hypothetical vehicle data. (C) contains the QTc results using individual correction with the slope from the hypothetical treatment data.

In this figure, the same data from the previous two figures has been include in addition to some randomly generated outlier data that more easily demonstrate how the method works. In Figure

6A the corrected data is presented along with a vertical dashed line through the reference rate used (550 ms). Two datapoints from each dataset are selected to demonstrate the effects of correction, both associated with RR values of 525 ms. As described above, the individual correction method will find the intercept of this vertical pseudo-axis based on a theoretical line that passes through the datapoint with the slope selected by the researcher. Therefore, the result of the individual correction is based on two factors, the RefR and slope used. The effects of choosing different slopes are demonstrated in Figure 6A by the red and blue dashed lines representing slopes derived from the hypothetical drug treatment and vehicle treatment data, respectively. The color-matched squares connected to these lines represent the calculated QTc value for the datapoint they are linked to. While the QTc values determined by the different methods don't appear to have provided QTc results that are too different for these datapoints, the true effect of each method is presented in Figures 6B and 6C. Figure 6B contains the results from using an individual correction method based on the QT-RR relationship observed in the vehicle data. This provided near perfect correction of the hypothetical vehicle data, resulting in a flat relationship between QTc and RR values. However, it seems to have under-corrected the hypothetical drug treatment data, based on the positive slope of the regression line. The results of the individual correction using the slope of the hypothetical drug treatment data seems to have had the opposite outcomes (Figure 6C). In this plot, the hypothetical drug treatment data has been corrected to a QTc-RR slope that is near zero, while the hypothetical vehicle treatment data has been over-corrected (as evidenced by the negative slope). Figure 6 demonstrates how individual correction methods correct the data around the reference rate and provides examples that support the need to use slopes that accurately represent the QT-RR relationship of the data being corrected.

The individual correction methods can be used to represent the QT-RR relationship more accurately and therefore more effectively correct the data by minimizing the relationship between QTc and RR. However, this requires the careful consideration of which data to use for the

determination of the QT-RR relationship. As the simplified examples in Figure 6 suggest, it may be most appropriate to use an on-treatment individual correction that calculates the QT-RR relationship for every dataset it corrects. Doing this would provide more accurate estimation of the relationship for that day, but this method is not without its own limiting assumptions. Regardless of whether you use a single linear regression slope per subject, per day, or per treatment, the individual correction method assumes that an estimate of the relationship through the whole dataset is sufficient to represent the relationship of QT and RR at every measurement timepoint. Additionally, it pre-assumes the QT-RR relationship before correction even begins. This could be problematic if the drug effect being investigated is altering this relationship between QT and RR. The burden of using this method must also be considered as it requires computationally intense calculations that must be performed *post-hoc*, preventing researchers from evaluating QTc during the study. An issue that is especially limiting for clinical use where physicians often do not have the luxury of collecting the large amount of data necessary for regression or waiting for that data to be collected.

The QT Ratio Method

To address the issues with the individual correction methods, while maintaining the efficacy they provide, our research group developed a novel QT correction method designed to combine the ease of using a general correction with the dynamic adjustment of using an individual method. Most importantly, it avoids making assumptions about the rate relationship before correction so that it can adapt to changes to the relationship over time. This novel correction method is called the Ratio method and it focuses on using a relevant reference point as an assumption instead of presuming the slope of the relationship. Currently, it relies on a species-specific y-intercept value of 100 ms for NHPs. This was determined using linear regression on 42 NHP subjects to find a mean y-intercept values. The way this method works is by using the slope formula to find a ratio between the datapoint being corrected and the y-intercept. (y2-y1 / x2-x1), which also functions as a slope between the two points. Using this datapoint specific slope within the same formula as

the individual correction means that no slope assumptions are being made for any data point, only the assumption that the y-intercept chosen was appropriate. This provides the following formula adapted from the individual QT correction method: QTc = QT – ((QT – Yint) / (rate – 0))*(rate-RefR). The Ratio method can provide similar results as the individual methods without the need for *post-hoc* computational analysis or large data sets, as this dissertation will explore further in Chapter 4. For now, it seems appropriate to introduce this novel method while discussing how these other more traditional methods function. Figure 7 contains the same data used in Figure 6 to demonstrate how the Ratio method works.

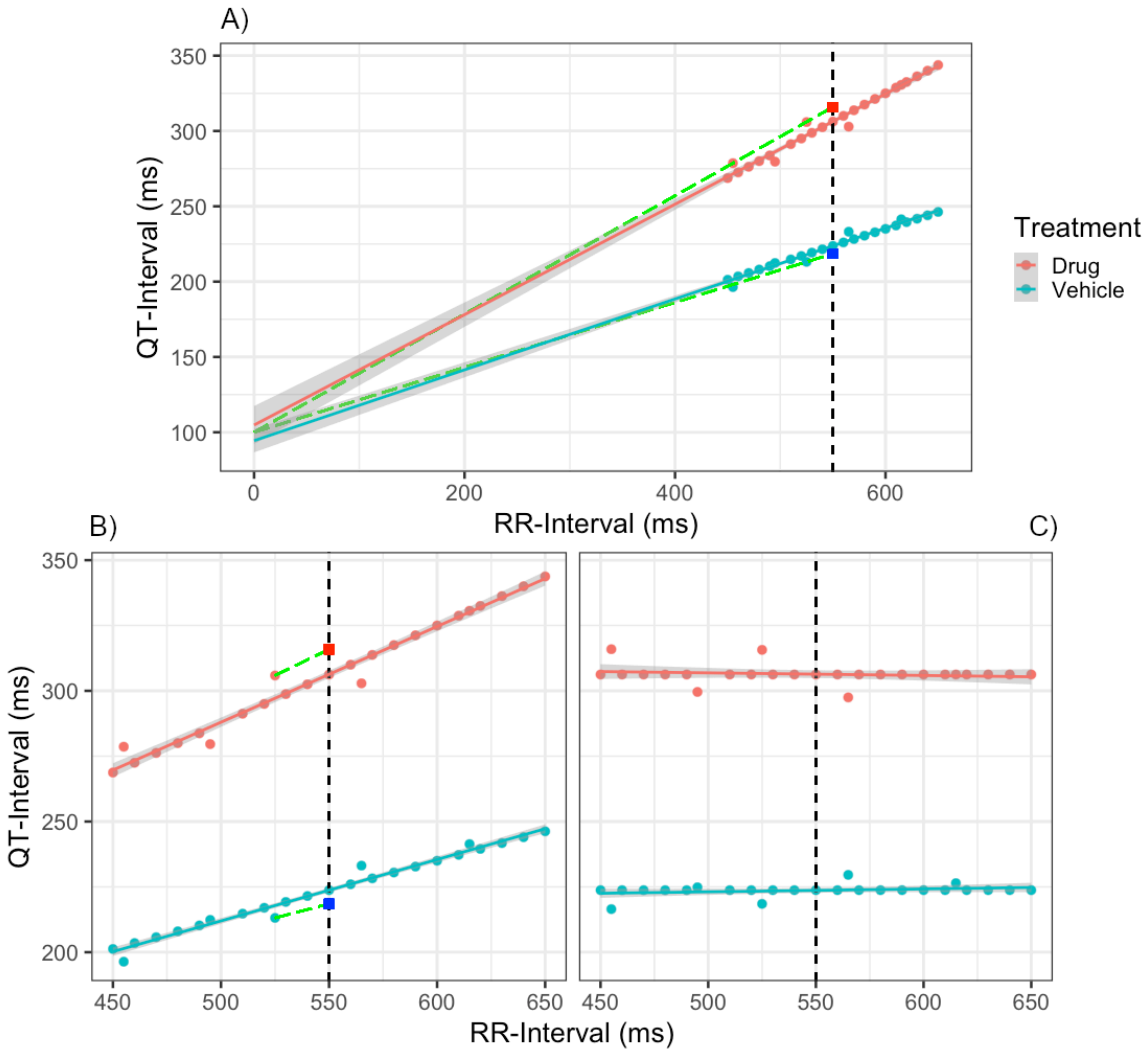

Figure 7: Emulating the Effect of the Novel Ratio QT Correction. The data in this figure is the same used in Figure 6. Data from a hypothetical vehicle treatment are still represented by the blue dots and hypothetical drug treatment data are still represented by red dots, while color-matched lines represent the linear regression of the data. The vertical black dashed lines represent the pseudo-axis at the reference rate (550 ms) that the individual correction and novel Ratio methods calculate the intercept of to estimate QTc. (A) contains the uncorrected data, with the x-axis extended back to 0 to present the y-intercepts of both linear regression lines. In this plot the green dotted lines represent the Ratio method estimating the slope between the same datapoints previously used and a fixed y-intercept value of 100 ms. This slope will then be used in the same formula used for the individual corrections, except each datapoint will have a uniquely calculated slope. The green dotted line has been extended through the data point until it intersects with the pseudo-axis at the reference rate. Color-matched squares represent the calculated QTc values from this method. (B) contains the same uncorrected data, but at the same scale used for the individual corrections for better comparison. The slope estimated for each datapoint is represented as a green dashed line connecting to the calculated QTc (C) contains the resulting QTc values calculated by the Ratio method for every datapoint of both datasets.

In Figure 7A, the same datapoints corrected in Figure 6 are once again being corrected, except the slopes used are those calculated between the selected y-intercept value (100 ms) and the datapoint (represented by the green dashed lines). The QTc value is calculated as the point that a line with this slope (green), passing through this datapoint, intercepts the pseudo-axis (RR = 550 ms). The slopes of the green lines are unique to each datapoint being corrected. Figure 7B contains the same uncorrected data but at a scale comparable to Figure 6. Figure 7C contains the results of Ratio correction, showing that this method corrected both hypothetical datasets near perfectly. Of course, no method is without any flawed assumptions. The Ratio method relies on the assumption that the selected y-intercept value is sufficiently representative of the data being corrected. By assuming the y-intercept value instead of slope this method should be more effective at correcting QT in the presence of a drug effect changing the QT-RR relationship, but less effective if the effect is directly on QT.

Testing the Effectiveness of Each Correction Method with Simulated Drug Data

Understanding how these methods work is necessary to improve QT correction. So far, this chapter has demonstrated the function of each method using very simplified hypothetical data. However, using more realistic simulated data would allow for deeper understanding. Linear regression between QT and RR was performed using a 24-hour ECG recording from a NHP treated with vehicle control. The resulting linear equation was used in conjunction with the that subject's RR values to estimate the QT values. This acted as a simulated vehicle control treatment that was very similar to the original data (Data A). The equation was then modified to simulate the effects of three hypothetical drug treatments that cause 10ms of QT prolongation by affecting QT, RR, or the QT-RR relationship (Data B, C, and D, respectively). In the remaining pages of this chapter, each of the previously mentioned correction methods are compared by their ability to correct these four sets of simulated data. Using this simulated data with predetermined drug effect, the effectiveness of each correction method is compared by the ability to minimize the relationship between QTc and RR, the accuracy of detecting proarrhythmically-relevant QTc

prolongation, and the variability of the resulting QTc values. Based on the assumptions each correction method relies on, I hypothesize the following: 1) general Bazett's correction method (QTcB) will perform the worst in each category, 2) the dynamic adaptation to changes in the QT-RR relationship will allow the Ratio method (QTcR) to provide the lowest variability and best detection of drug effect in Data D, 3) the presumption of slope will benefit the individual methods when detecting drug effect in Data C, with the on-treatment method (QTcT) performing better than the vehicle only method (QTcV), and 4) each method will avoid detecting drug-induced QT prolongation in Data B.

Methods

Subject Data

Original subject ECG data is from a randomly selected NHP implanted with a L21 model telemeter device (Data Science International) and monitored over 24-hours during vehicle treatment. This data was provided by Eli Lilly and Company and all animal procedures were approved of by the Institutional Animal Care and Use Committee before collection. The ECG data from this subject was used as a template to create a linear model to simulate drug effect.

Generating Simulated Data

A linear regression was performed between the QT and RR from the subject's ECG data. This produced a linear equation (QT = 0.2773*RR + 95.5776) that was then used to calculate the simulated QT values using the subject's original RR values. These simulated QT values were used as simulated vehicle control treatment data (Data A). This linear equation was then modified to produce QT values representing the simulated effects of three hypothetical drug treatments. Each simulated treatment was designed to cause a 10ms increase in QT at its maximum effect. To achieve this change in effect strength a variable was used to modify the percent of effect applied in relation to time from dose (perE). This variable was designed to increase the effect rapidly in a linear fashion until the maximum effect (10 ms change in QT) was reached after 120 mins. The variable was then designed to slowly decrease the effect linearly until the end of the

recording. First, it was modified to simulate a drug effect that acted on heart rate (Data B QT = 0.2773*(RR + 36.07*perE) + 95.5776). Second, it was modified to simulate a drug that acted directly on QT (Data C QT = 0.2773*RR + (95.5776 + 10*perE)). Both methods successful altered the QT by the exact amount intended. However, the final simulated drug effect was on the QT-rate relationship so the modifications to slope have a rate-dependent effect. Thousands of modifying constants were tested to identify one that minimized this rate-dependent effect. This final modified equation was used to simulate this drug effect on the QT-RR relationship (Data D QT = (0.2773 + 0.015942*perE)*RR + 95.5776).

Analysis

QTc vs RR plots were used to gauge the ability of the correction methods to reduce the QTc-RR relationship. Change in QT from vehicle was plotted over time to demonstrate their ability to detect simulated drug effects. These plots also allowed for the comparison of variability.

QT Correction Methods

Methods	Formulae	Assumptions
General Correction (QTcB)	$\frac{\mathrm{QT}}{\sqrt{RR/1000}}$	Using a rate relationship from humans and correcting to 1000 ms RR is relevant for NHPs in all scenarios.
Individual Correction (QTcV, QTcT)	$\mathrm{QT} - \mathrm{m}(\mathrm{RR} - \mathrm{refRR})$	Deciding the rate relationship before correction is appropriate for all time points.
Ratio Correction (QTcR)	$\mathrm{QT} - \mathrm{z}(\mathrm{RR} - \mathrm{refRR})$	Deciding the y-intercept before correction and using the slope between it and each datapoint will provide more dynamic correction

Table 3: QT Correction Method Assumptions. Included are the QT correction methods used, their formulae, and the assumptions intrinsic to each. QT: QT interval (ms), RR: RR interval (ms), refRR: reference RR (550 ms), m: slope of linear regression, z: slope between reference y-intercept (100 ms) and datapoint being corrected (RR, QT).

Bazett's general correction method (QTcB) was used to represent methods that rely on inappropriate populations and fail to account for changes to rate relationships. Both individual corrections used the slope of a linear regression as a subject-specific correction coefficient. One

used vehicle treatment data to find a single coefficient (QTcV) and the other used data from each treatment to find a coefficient specific to each (QTcT). These methods both assume predetermining the slope of the relationship will not hinder detection of drug effect. Only QTcV assumes no change in this relationship between treatment. The Ratio method (QTcR) assumes this relationship is inconsistent, instead using a y-intercept value assumed to be relevant. Table 3 contains each method, their formulae, and their inherent assumptions.

Results

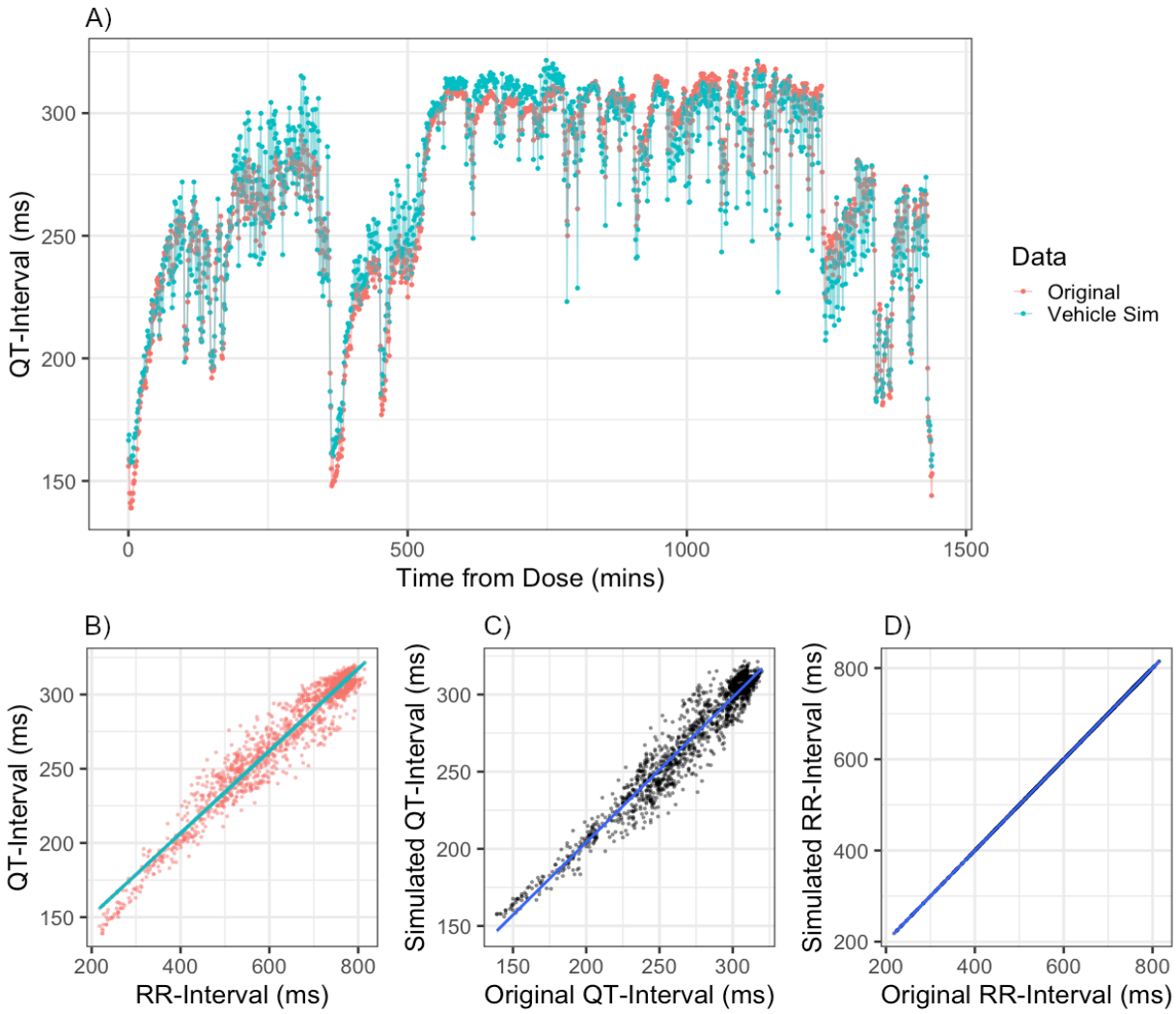

Figure 8: Simulated Vehicle Data from a Linear Model. These plots demonstrate the similarities between real and simulated vehicle control ECG data. (A) QT vs Time from Dose comparing the 1-minute mean datapoints of the original data (red) and simulated data (blue). Sharp declines in QT at ~375mins correspond to scheduled blood draws. (B) QT vs RR plot comparing the QT-RR relationships of the two datasets. Because the simulated data is created using a linear model, all blue points fall along the blue linear regression line representing the relationship. Also, since this model was created from the linear regression of the original data, the simulated data perfectly overlaps the red linear regression line of the original data. (C) Simulated QT values vs original QT values to demonstrate their correlation. (D) The RR values used for the simulated data are the same as those from the original data, represented in this plot as a perfectly straight correlation.

Before addressing the results of the simulated drug treatments and evaluating the effectiveness of the correction methods, the validity of such simulations must first be demonstrated. In Figure 8 the vehicle control data simulated (Data A) by the linear model is compared to the original vehicle

control ECG data. Figure 8A contains the 1-minute means from the ECG (red) plotted along with the simulated 1-minute means (blue) over time from dose. This plot demonstrates the similar pattern of distribution between the two datasets and, though the simulated has more variability than the original, the two are closely related. As expected by the method the simulated data was created, the two datasets have the same QT-RR relationship in Figure 8B. The correlation between the original QT values and the simulated ones are confirmed in Figure 8C, while the fact that both datasets use the same RR values is demonstrated in Figure 8D.

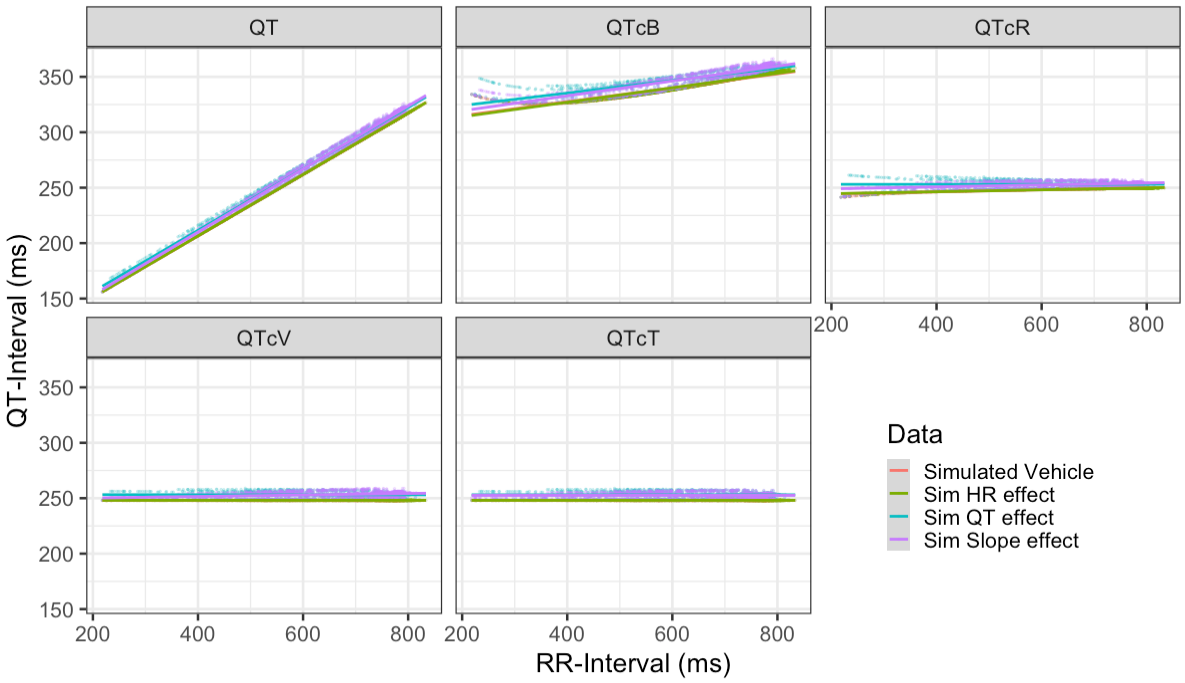

Figure 9: QTc vs RR Plots of Simulated Treatment Data. These plots show QT or QTc vs RR as indicated by the title boxes at the top of each plot. Each datapoint represents a simulated 1-minute mean. The QT plot uses uncorrected QT values. The QTcB plot uses QT values corrected with Bazett's correction method. QTcR refers to QT values corrected with the novel Ratio method. QTcV uses QT values corrected with an individual correction method that uses the slope of the QT-RR relation obtained from the simulated vehicle data. QTcT uses QT values that were also corrected with an individual correction, but the slopes used to correct each dataset were obtained from the linear regression of that dataset.

With the simulation process justified, the ability of the correction methods to reduce the relationship between QTc and RR can be compared. This is done using QT or QTc vs RR plots, as presented in Figure 9. Each plot in this figure contains the results of different QT correction methods, except for the first "QT" titled plot which uses uncorrected data. As demonstrated before

in the hypothetical data of Figure 5, QTcB under-corrects and overestimated the QTc value. Interestingly, this plot shows that the tail end furthest from the 1000 ms reference used by QTcB has curved upwards. Perhaps it is reaching the limits of its correction ability at such low RRs or this is an artifact from the fact that Bazett's method was based on a logarithmic assessment of relationship instead of linear. The rest of the methods have similar reductions in the QT-RR relationship as each other, though QTcT appears to perform the best with nearly flat relationships. Meanwhile, QTcR is also demonstrating some curvature at the lower end of the RR range.

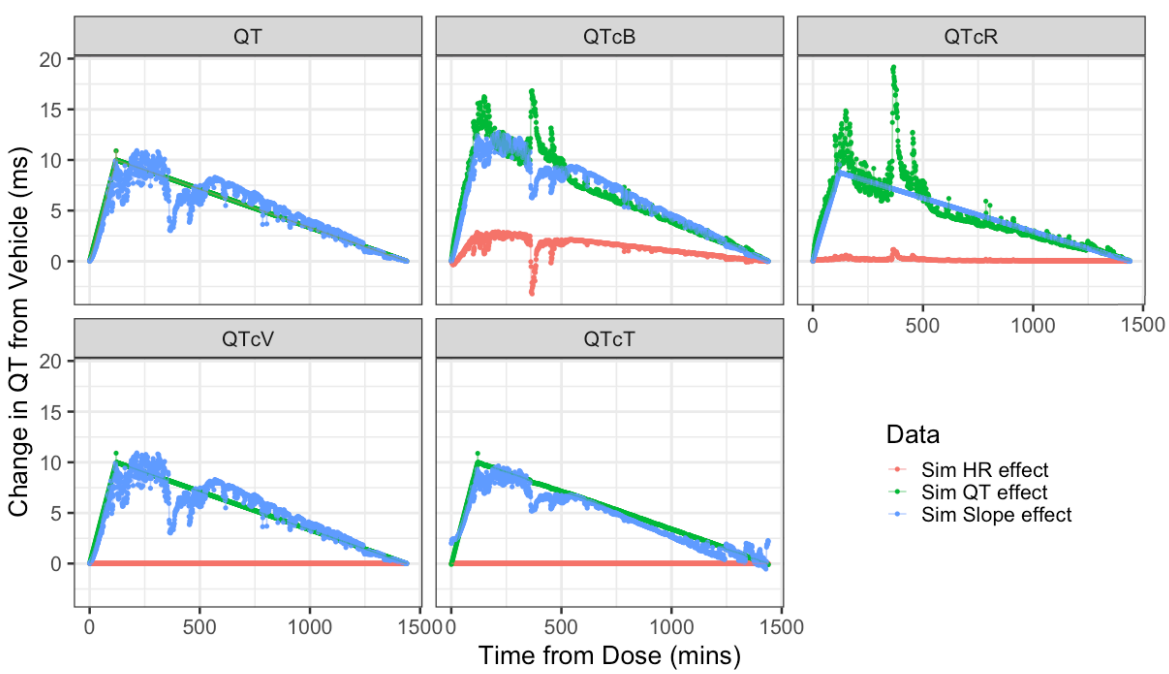

Figure 10: Change in QTc from Simulated Vehicle Data Over Time. These plots contain the difference between the simulated 1-minute mean QT or QTc data for each simulated drug effect and the time match simulated vehicle data. Sharp declines in QT at ~375mins correspond to scheduled blood draws. The first plot "QT" containing the uncorrected QT appears to be missing the simulated for the HR effect treatment, but it is perfectly covered by the data for the simulated QT effect treatment. This is because, unlike the rate-dependent slope effect in Data D, both the QT and HR effects were precisely applied. The other plots contain the results of the QT correction methods in their titles. QTcB: Bazett's QT correction, QTcR: Ratio QT correction, QTcV: Individual correction using the QT-RR relationship from the simulated vehicle data, QTcT: "On-treatment" individual correction using the QT-RR relationships of each dataset being corrected.

The most informative part of this analysis is how each method handles these different simulated drug effects. Figure 10 contains the results of each correction method as the change from their results on the simulated vehicle data. Each simulated drug effect was intended to produce a

maximum change of 10 ms at 120 minutes from dose. The first plot of Figure 10 for “QT” demonstrates that this was successfully performed by the HR effect simulation (red) and QT effect simulation (green); so much so that the green line completely eclipses the red. Because of the rate-dependent effectiveness of the slope effecting simulation (blue) the resulting data is much more variable but still follows the same trend as the other two datasets. In the QTcB plot the effects of each treatment has been overestimated and is variable. It even detects around 2.5 ms of QTc prolongation from the simulated drug only designed to affect rate. QTcB did result in less variability for the QT effect simulation than QTcR and less variability for the slope effect simulation than QTcV. Other than a small blip around 375 minutes, QTcR did not mistake simulated drug effect on rate for relevant QT prolongation. It also was able to successfully detect the QT prolongation produced by the other two simulated drug effects, but it often overestimated datapoints for the QT effect simulation, giving it the highest variability for that dataset. However, it produced nearly perfect estimation of the QT prolongation created by the simulated effect on slope, even eliminating the variability present in the uncorrected data. The only benefit QTcV demonstrated overusing uncorrected QT was its ability to completely rule out any QT prolongation from the HR effecting drug simulation. QTcT had similar results as QTcV, except it was better able to reduce the rate-dependent variability in the slope effecting simulation.

Discussion

The results of this comparison confirmed many of my original hypotheses based on the nature of each correction method. QTcB did indeed perform the worst in most categories, but it did produce lower variability in its correction of the QT effecting simulation and the slope effecting simulation compared to QTcR and QTcV, respectively. QTcR was confirmed to provide the lowest variability and the best detection of drug effect in the slope effecting simulation. It also successfully corrected away most of the rate effect from the HR effecting simulation. While QTcR did result in the highest variability for the QT effecting simulation, the variability was in the form of overestimation of effect, which is preferable to underestimation in this scenario. And finally, both individual correction

methods were able to perfectly estimate the QT prolonging effect of the QT effecting simulation, and QTcT outperformed QTcV in the other comparisons. I did expect each method to avoid attributing the HR effects simulations of Data B to relevant QT prolongation. However, QTcB incorrectly estimated it to have some effect on QT. These results support the fact that, while general methods are easier to use, they are not as accurate as individual methods. However, individual methods require a lot of data and computational power and are useless to someone attempting a cursory glance at the data or a clinician trying to track a patient's reaction to a drug treatment in real time. The Ratio method takes the best of both options and provides an easy-to-use method that can dynamically adjust to changing rate relationships. Not only does this ability provide better correction than the general methods in most comparisons, but it was also the only method capable of accurately dealing with a simulated drug effect acting directly on the QT-Rate relationship. It's also so simple that a clinician could even use it while on rounds. It's important in safety pharmacology studies to select the most appropriate correction method. The ICH recommends that this is done by checking the resulting correlation between QTc and rate, the variability of the QTc values, and sensitivity to detect drug effect [113]. The limited simulations used for comparison were examples of how to compare correction methods to justify which is selected and provided a promising start to this evaluation by demonstrating some of the benefits and drawbacks associated with each method. However, understanding the factors that influence these methods more, and comparing them using real drug treatment data is still required. These aspects of the investigation will be tackled in chapters 3 and 4 of this dissertation, respectively.

CHAPTER 3:

IDENTIFYING UNCONTROLLED FACTORS THAT INFLUENCE QT CORRECTION

Modified from: Ether ND, Jantre SR, Sharma DB, Leishman DJ, Bailie MB, Lauver DA. Improving corrected QT; Why individual correction is not enough. J Pharmacol Toxicol Methods. 2022 Jan-Feb;113:107126. doi: 10.1016/j.vascn.2021.107126. Epub 2021 Oct 13. PMID: 34655760.

Abstract

The use of QT-prolongation as a biomarker for arrhythmia risk requires that researchers correct the QT interval (QT) to control for the influence of heart rate (HR). QT correction methods can vary but most used are the general correction methods, such as Bazett's or Van de Water's, which use a single correction formula to correct QT intervals in all the subjects of a study. Such methods fail to account for differences in the QT-HR relationship between subjects or over time, instead relying on the assumption that this relationship is consistent. To address these changes in rate relationships, we test the effectiveness of linear and non-linear individual correction methods. We hypothesize that individual correction methods that account for additional influences on the rate relationship will result in more effective and consistent correction. To increase the scope of this study we use bootstrap sampling on ECG recordings from non-human primates and beagle canines dosed with vehicle control. We then compare linear and non-linear individual correction methods through their ability to reduce HR correlation and standard deviation of corrected QT values. From these results, we conclude that individual correction methods based on post-treatment data are most effective with the linear methods being the best option for most cases in both primates and canines. We also conclude that the non-linear methods are more effective in canines than primates and that accounting for light status can improve correction while examining the data from the light periods separately. Individual correction requires careful consideration of inter-subject and intra-subject variabilities.

Introduction

An important aspect of the drug development process is the screening of drug candidates for their risk of inducing arrhythmias, such as TdP, in the clinical population [41,67,154]. Concerns about drug induced arrhythmias are one of the leading causes of discontinued development and use restrictions due to their potentially fatal outcomes [155]. Preclinical assessments of a candidate's risk of producing these arrhythmias are vital to protect patients from adverse events and reduce excess investment in drug candidates that may eventually fail in the clinic due to this liability. While drug induced arrhythmias are potentially fatal, they can also be quite rare in many patient populations [140]. For example, TdP has an incident rate ranging from 1 in 20 for drugs considered highly proarrhythmic to 1 in 100,000+ in non-cardiac related drugs that are still considered unsafe [40]. Due to the rarity of incidence, relying on identification of arrhythmias in preclinical and clinical study populations is not enough to protect the much larger patient populations. This is why safety pharmacology studies focus on a compound's effect on the electrocardiogram (ECG) QT interval (QT) which, when prolonged, can lead to early after depolarizations in cardiomyocytes that can propagate arrhythmias [40,120]. For these reasons, the International Conference on Harmonization (ICH) published recommendations to use QT prolongation as a biomarker for drug induced arrhythmia risk [44,45]. However, the QT interval is highly variable and correction methods must be used to control for that variability. Heart rate (HR) is the principal driver of QT variability due to their inverse relationship; the slower the heart rate, the longer the QT interval. Because of this, QT correction methods typically focus on controlling for that relationship to reduce variability [148,150]. While these correction methods are useful due to their high sensitivity, their specificity is lacking [68]. To increase specificity and better translate methods between species, ICH is currently evaluating how best to improve arrhythmia risk predictions [139].

Many of the ICH recommendations involve improving the selection and validation of QT correction methods used in studies on arrhythmia risk. It is important to select a method that accurately

accounts for sources of QT interval variability, which differs by species and likely even by subject [151]. Without selecting an appropriate correction method, the variability of QT can hinder the measurement of drug effects on QT prolongation. Multiple methods have been developed for correcting QT for heart rate which can be categorized as either general QT correction methods or individual QT correction methods. General correction methods are fixed formulae that adjust the QT interval based on the related heart rate. These fixed formulae are based on limited populations and assume a fixed relationship between QT and heart rate. Inherently, the effectiveness of each general correction method varies by species and perhaps even by subject. Using a general QT correction method fails to account for differences in the QT-HR relationship between subjects. They also assume that this relationship is fixed, when literature reports have demonstrated that it can vary over time [128]. Other variables may also influence this relationship, such as environmental factors and drug effects [156,157]. Recently there is increased interest in individual QT correction methods, which have the potential to account for more influences on QT and address variations in the QT-HR relationship between subjects [135,158]. Individual correction methods use data from a subject to identify a more specific rate relationship, which is then used in the correction formula. This provides a way to account for the inter-subject differences in rate relationship. In contrast, general correction methods use a single formula for all subjects, regardless of their specific QT-HR relationship.

General QT correction methods are commonly used in clinical and preclinical safety studies, likely due to their ease of use and widespread literature support. However, as evidence of variable QT-HR relationships increases, and data analysis becomes easier, individual QT correction methods have become more viable and important. The variability of this rate relationship is not limited to inter-species differences, as inter-subject differences have also been demonstrated [130]. Additionally, intra-subject variations in this QT-HR relationship can occur over time [128,159]. The presence of these variations in the QT-HR relationship calls into question the viability of general QT correction methods, which cannot account for these factors and therefore cannot be equally

effective for all subjects. Outside of the general variations between subjects, the light cycle and time of day have also been associated with changes in the QT-HR relationship, explained in part by physiological differences linked to circadian rhythm and autonomic tone [160,161]. It is also possible that this relationship may be affected by the treatments being studied [25,162]. Using general correction methods that fail to account for these variations could result in different correction results depending on the subject, the day, the time, or even the drug plasma concentration at the time of recording. Without accounting for these variations, general correction methods may be unsuitable for use when comparing results between subjects and treatments, which is often the case during arrhythmia risk studies. For these reasons we hypothesize that using QT correction methods based on linear and non-linear regressions of individual QT-HR relationships will result in more consistent correction and reduced variability in QTc values. To illustrate the importance of accounting for these variations in the QT-HR relationship we will use canine and primate ECG recordings to test multiple general and individual correction methods by comparing their abilities to correct for the QT-HR relationship and reduce variability. Comparing these methods and identifying the factors that influence their accuracy is the first step to promoting a standardized protocol for selecting QT correction methods and validating their results.

Methods

Data used in this study were provided to us by Eli Lilly & Co. (Indianapolis, IN) from eight toxicology studies that were performed on 34 cynomolgus non-human primates (NHP) and 36 beagle canines (BK9) fitted with jacketed telemeters. Subjects were age matched and both species included an equal number of males and females. The housing rooms utilized a controlled light cycle with 12 hours of lights on followed by 12 hours of lights off. After allowing the subjects to acclimate to the jacketed telemeters, a 24-hour ECG recording was collected on one pre-treatment day and two post-treatment days for each subject. These raw recordings were processed, and the resulting 1-minute mean summarization of ECG intervals were used for further analysis. Summarizations included the 1-minute means for QT and RR intervals, analysis time,

and subject information for each minute of an ECG recording. For each subject, a pre-treatment recording day was used for the pre-treatment correction methods and two post-dose recording days were used for analysis.

QT Correction Methods

Correction Methods	Formula
General Methods	
Bazett's Correction (QTcB)	$QT / (RR / RefRR)^{1/2}$
Van de Water's Correction (QTcVa)	QT – 0.087 (RR - RefRR)
Individual Methods	
Linear Correction (lQTc)	QT - (m * RR + b) + (m * RefRR + b)
Hyperbolic Correction (hQTc)	QT - (a / RR + c) + (a / RefRR + c)

Table 4: QT Correction Method Formulae*.* Two general and two individual correction methods are presented. Bazett's and Van de Water's correction methods were selected as the general methods because they are typically used for primates and canines, respectively. The first individual correction (lQTc) is based on the linear regression of the QT and RR values from an ECG recording. The second individual correction (hQTc) method is based on the hyperbolic regression of the QT and RR values determined using nonlinear least squares estimates. For both individual corrections, the relationship formulas are used to estimate a QT value based on RR and RefRR. To find the corrected QT value (QTc) the difference between the actual QT and the RR-estimated QT value for that minute is added to the RefRR-estimated QT value. QT: 1-minute mean QT interval (ms), RR: 1-minute mean RR interval (ms), RefRR: Species specific reference RR interval (NHP: 550ms, BK9: 750ms), m: linear variable representing slope, b: linear variable representing y-intercept, a: hyperbolic variable affecting foci distance, c: hyperbolic variable affecting position along y-axis

The correction formulae used in this study are outlined in Table 4. These formulae contain representations of the heart rate as RR, which is a measure of the period between ECG waveforms. The time between waveform beats is directly linked to the number of beats per minute and using RR allows for direct comparisons between 1-minute means of QT and RR. This also allows for consistent use of the milliseconds unit in the formulae. In these formulae, QT is the 1-minute mean QT interval (ms) value being corrected, RR is the 1-minute mean RR interval value for the same minute as QT, and RefRR is the species-specific reference RR interval. The RefRR used for NHPs is 550ms, equivalent to a HR of about 109bpm, and the RefRR used for BK9s is

750ms, equivalent to 80bpm. The general correction methods used were based on Bazett's [QT / (RR / RefRR)1/2] and Van de Water's [QT – 0.087 (RR - RefRR)] corrections which were used for NHP and BK9 subjects, respectively [79,127]. Typically, these methods would divide RR by 1000 to convert into seconds, based on an assumed 60 bpm reference rate. As we have found this causes the general methods to overcorrect their QTc estimates, they have been modified to instead divide by the reference RR to provide a more appropriate comparison. The individual linear (lQTc) and hyperbolic (hQTc) corrections are based on the following formulae, respectively: [QT - (m * RR + b) + (m * RefRR + b)] and [QT - (a / RR + c) + (a / RefRR + c)]. In the linear formula 'm' refers to the slope of the linear regression, while 'b' refers to the y-intercept of that regression. In the hyperbolic formula, 'a' and 'c' are variables in the hyperbolic regression that affect the foci distance and position along the y-axis for the hyperbola.

As described in Table 5, these methods are divided into sub-methods based on the time period used for the correction, e.g. baseline day, treatment night, etc. In the Pre-treatment correction methods (lQTcP, hQTcP), we test the assumption that the QT-RR relationships of a subject's pre-treatment data are indicative of the QT-RR relationship in a post-dose recording. Several lines of research seek to implement this type of individual correction [135,149]. For these Pre-treatment sub-methods, each subject had a single correction formula based on the linear or hyperbolic regression of the QT-RR relationship throughout the whole pre-treatment recording. Expanding on this method, the Pre-treatment Lights sub-method (lQTcPL, hQTcPL) was used to explore the impact of differences in the QT-RR relationship between lights-on and lights-off in the pre-treatment method.

Individual Correction Sub-Methods	Regression Data Source
Pre-treatment (lQTcP / hQTcP)	Subject's ECG recording taken before treatment (24-hours)
Pre-treatment Lights (lQTcPL / hQTcPL)	Subject's ECG recording taken before treatment (12-hours based on light status)
Treatment (lQTcT / hQTcT)	Subject's ECG recording taken during treatment (24-hours)
Treatment Lights (lQTcTL / hQTcTL)	Subject's ECG recording taken during treatment (12-hours based on light status)

Table 5: Individual QT Correction Sub-Methods. The two individual correction methods use different regression formulae to represent the relationship between QT intervals (ms) and RR intervals (ms), but they use the same data set to determine that relationship. There are multiple options for data sources when it comes to individual correction, so sub-methods are defined by which period of data is used to determine the relationship for the individual correction. The Pre-treatment sub-methods use a subject's ECG recording obtained before treatment began, providing one relationship formula (per method) to be used for that subject's corrections. The Pre-dose Lights sub-methods use that same recording but splits it into two 12-hour segments based on the light status at the time of each measurement. This results in two relationship formulae (per method) for each of that subject's corrections, which is determined by the light status when the QT interval being corrected was measured. The Treatment sub-methods use the same ECG recording that contains the QT interval being corrected. Similarly, the Treatment Lights sub-method uses the ECG recording that contains the QT interval being corrected, but only the12-hours of that recording that were measured during the relevant light status.

With these sub-methods, each subject had two correction formulae used, with both formulae using the subject's pre-treatment data but two different regressions performed based on light status. The third set of individual correction sub-methods is the Treatment method (lQTcT, hQTcT) which assumes that using the QT-RR relationship observed on the day of recording (on-treatment) is most appropriate. These methods assume that the pre-treatment sub-methods fail to account for day-to-day changes to, and treatment effects on, the QT-RR relationship. For these methods, the formula has a unique regression for each recording day. Similar to how the pre-treatment lights sub-methods expanded on the pre-treatment sub-methods, the Treatment Lights sub-methods (lQTcTL, hQTcTL) splits the QT-RR relationships based on light status. In this scenario, each recording has two formulae for correction, determined by the light status at the time of QT interval measurement.

QT Relationships and Variability

For the linear individual corrections, we performed linear regressions on 1-minute means for QT intervals and their corresponding RR intervals to characterize the relationship from an ECG recording. The slopes and y-intercepts of these regression lines were then used in the individual correction methods. These regression lines will be represented by straight lines through any scatterplots involving QT and rate. For hyperbolic individual corrections, we used non-linear least squares to perform a regression on the QT-RR data using a hyperbolic formula. This provided us with values for the two variables of the hyperbolic formula that resulted in the best fit through the QT-RR relationship. Since the goal of QT corrections is to remove the influence of heart rate on the QT interval, the success of a method is in part determined by its ability to produce corrected QTc values independent of heart rate. To test this, the correlation between QTc values and HR were determined using Spearman rank correlation coefficients. This was chosen over the Pearson correlation coefficients due to the non-parametric nature of continuous biological recordings [163]. This non-parametric attribute of the data was confirmed using the Shapiro-Wilk test of normality [164]. Correlation coefficients results are in a range between 0 and 1, with 0 representing no correlation and 1 representing complete correlation. For a single recording, the correlation between HR and the QTc values from each method was determined. The method with the lowest correlation coefficient was considered to have the strongest correction for that recording. This provides insight into how well a method can correct to a reference rate, but it is also necessary to compare how much variability each method produces. Theoretically, if a correction method introduced high variability into the resulting QTc values the correlation between those values would be minimal, but the high variation would make detecting drug effect on QT improbable. Standard deviation of QT and QTc values were compared to identify the correction method with the greatest reduction in the range of QTc values. Low variability in QTc reduces the noise when measuring drug effect and increases our confidence in the results of the correlation comparison.

Bootstrap Resampling and Statistical Analysis

Bootstrap methods are resampling techniques which we used to quantify and validate the results observed in the individual subjects [165]. These methods provide more data by randomly sampling with replacement from an original ECG recording to simulate thousands of potential sample recordings from each post-dose recording. Each recording was first separated into lights on and lights off data sets to ensure the difference in environment was maintained in the simulated recording. Then, for every minute of the simulated recording, a minute from the original recording was randomly sampled with repeat sampling allowed. Once this is done for both lights on and lights off data sets, the resulting samples were combined into one simulated recording. The process was repeated 1000 times for both post-dose recordings from each subject, resulting in 68,000 and 72,000 24-hour simulated recordings for primates and canines, respectively. The standard deviation and the correlation to HR was then calculated for the QTc values from each correction method as well as for the original QT values. This provided two data points for each method per simulated recording which were used to identify the method that resulted in the lowest correlation and the method that resulted in the lowest standard deviation for each recording. For each method we tallied how many recordings it resulted in the lowest correlation and how many recordings it resulted in the lowest standard deviation. We then compared these results using the pairwise.prop.test() function from the R stats package to identify significance in the difference between methods [166]. Additionally, the correlation coefficients and standard deviations were used to create boxplots to compare the variation and means of these results. The statistical significance of these differences was determined using the pairwise.t.test() function from the R stats package. In this study, significance was defined by P-values less than 0.05.

Results

QT-RR Relationship Over Time

Examples of QT vs RR graphs for an individual subject on multiple days are shown for NHP and BK9 in Figure 11 and Figure 12, respectively. In these figures the relationship between the

intervals is represented by three regression lines; one line for the QT-RR relationship while lights are on (blue, long-dashes), one for the relationship while the lights are off (red, solid), and a third to represent the relationship over the entire 24-hour recording (black, short-dashes). Over the course of the study, the relationship between QT and RR is inconsistent and even varies by light status. We found a varying relationship to be observable throughout the data regardless of the subject's sex or species but found that it was typically the QT-RR relationship while lights are on that varies the most. Noting these differences during previous studies is what first led to doubts about the usefulness of general QT corrections, such as Bazett's and Van de Water's methods.

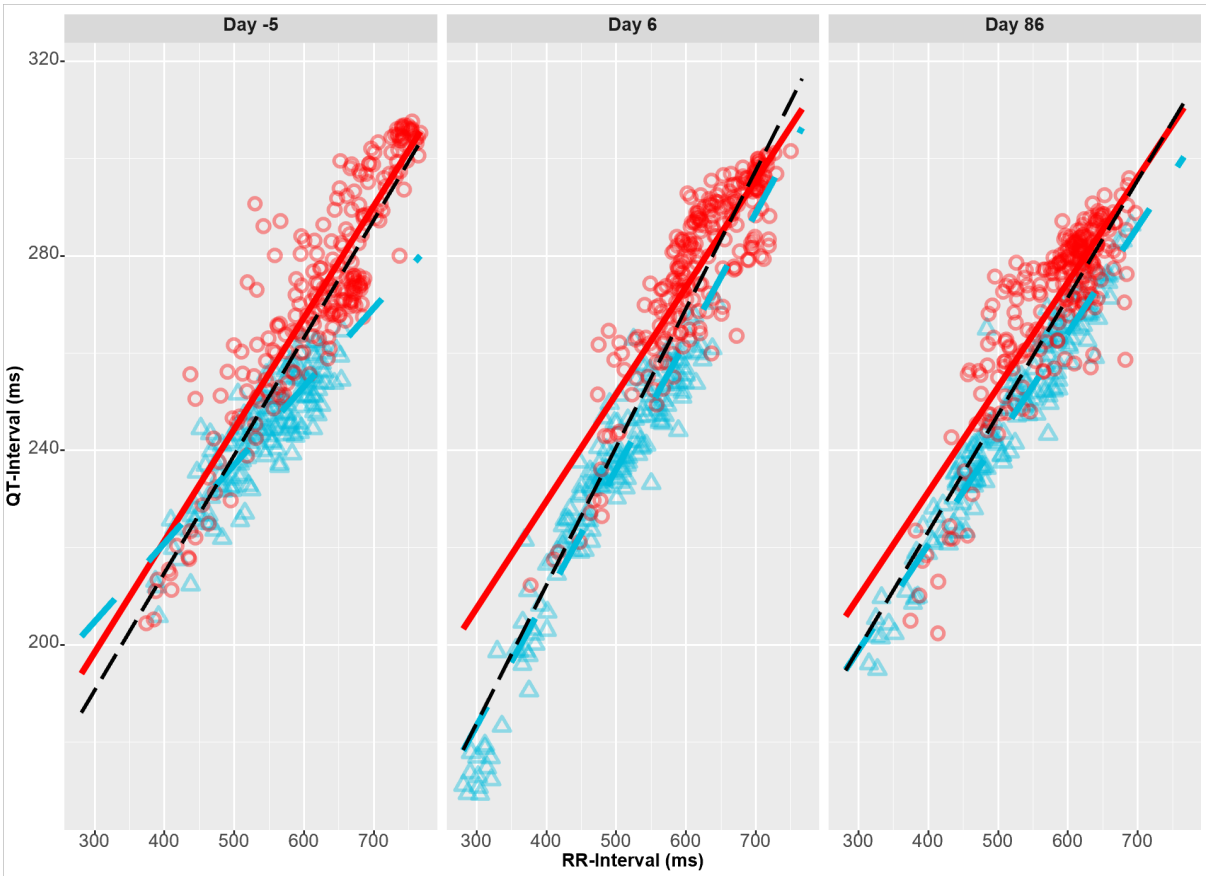

Figure 11: NHP Example of the Change in QT-RR Relationship Over Time. QT vs RR plots from a representative male non-human primate over three days. The day number at the top of the graph is relative to the day vehicle control was administered. The data are separated based on the light status at each time point. The lines represent the slope of the linear regression and have been extended for visibility. The blue long-dashed line and triangular points represent data recorded while lights were on. The red solid line with the circular points represents data recorded while the lights were off. The short-dashed black line represents the QT-RR relationship for the entire 24-hour recording. Unique subject ID: S01G01P0002M

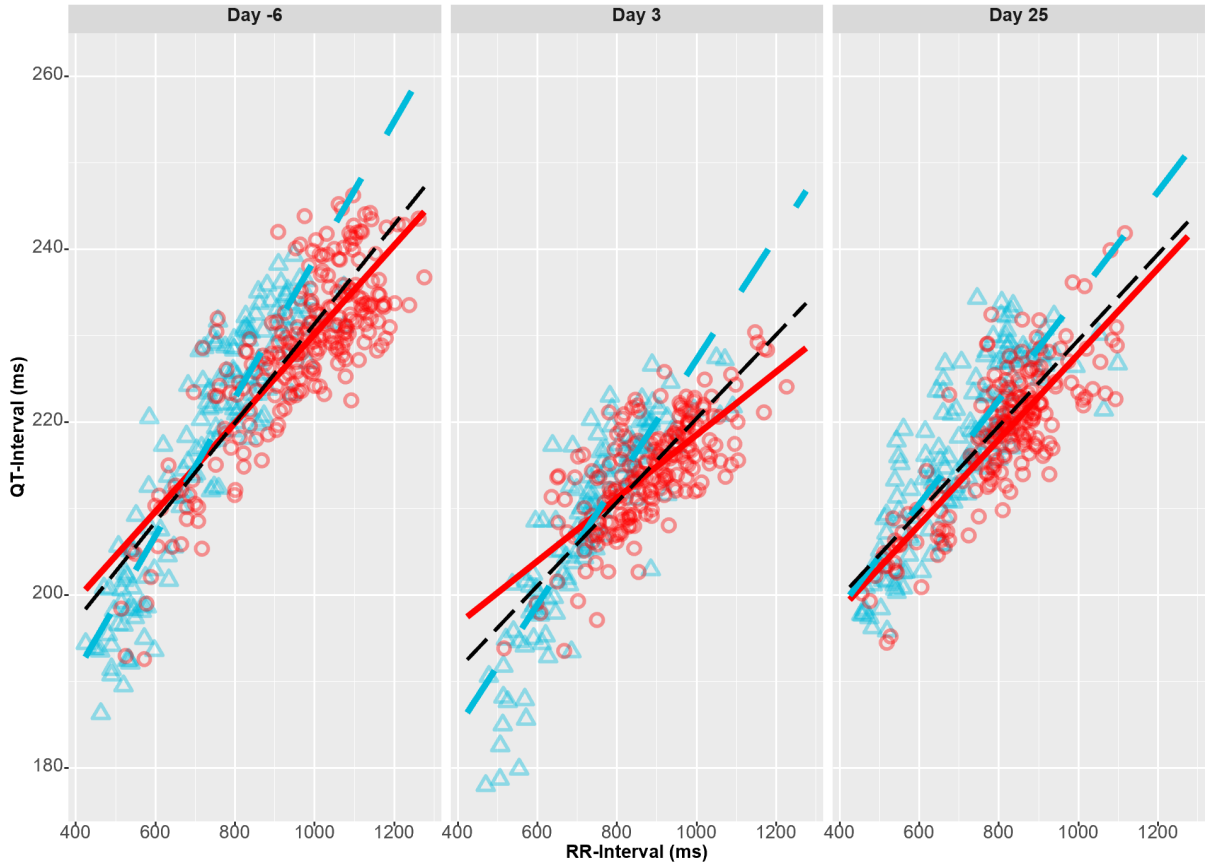

Figure 12: BK9 Example of the Change in QT-RR Relationship Over Time. QT vs RR plots from a representative male beagle canine over three days. The day number at the top of the graph is relative to the day vehicle control was administered. The data are separated based on the light status at each time point. The lines represent the slope of the linear regression and have been extended for visibility. The blue long-dashed line and triangular points represent data recorded while lights were on. The red solid line with the circular points represents data recorded while the lights were off. The short-dashed black line represents the QT-RR relationship for the entire 24-hour recording. Unique subject ID: S04G01D0001M

Bootstrap Sampling and Method Comparison

To simplify the presentation and increase the accuracy of these comparisons, bootstrap sampling was used to simulate 68,000 NHP and 72,000 BK9 sample recordings. These were then used to determine how frequently each method resulted in the lowest QTc-HR correlation and how frequently each method resulted in the lowest standard deviation as described in the methods section. The NHP results of these comparisons are presented in Table 6, in which lQTcT has the lowest QTc-HR correlation significantly more often than all other methods when examining the entire 24-hour period of each sample.

QT Correction Method	Lowest Heart rate Correlation			Lowest Standard Deviation		
	24-hour	*12-hour Lights On*	*12-hour Lights Off*	*24-hour*	*12-hour Lights On*	*12-hour Lights Off*
	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)
None (QT)	0 (0.0) *	0 (0.0) *	0 (0.0) *	0 (0.0) ~	0 (0.0) ~	0 (0.0) ~
Bazett's (QTcB)	4,670 (6.9) *	1,958 (2.9) *	3,124 (4.6) *	7,820 (11.5) *	1,949 (2.9) *	7,464 (11.0) *
Linear Pre-treatment (lQTcP)	4,373 (6.4) *	3,501 (5.1) ~	4,974 (7.3) ~	0 (0.0) ~	0 (0.0) ~	0 (0.0) ~
Linear Pre-treatment Lights (lQTcPL)	3,114 (4.6) *	3,637 (5.3) ~	3,954 (5.8) *	2,933 (4.3) *	0 (0.0) ~	0 (0.0) ~
Linear Treatment (lQTcT)	39,396 (57.9) *	3,954 (5.8) *	3,412 (5.0) *	30,322 (44.6) *	0 (0.0) ~	0 (0.0) ~
Linear Treatment Lights (lQTcTL)	3,839 (5.6) *	38,715 (56.9) *	27,054 (39.8) *	13,619 (20.0) *	45,023 (66.2) *	37,842 (55.7) *
Hyperbolic Pre-treatment (hQTcP)	2,646 (3.9) *	1,072 (1.6) *	4,988 (7.3) ~	0 (0.0) ~	0 (0.0) ~	0 (0.0) ~
Hyperbolic Pre-treatment Lights (hQTcPL)	2,891 (4.3) *	3,057 (4.5) *	2,404 (3.5) *	2,512 (3.7) *	0 (0.0) ~	0 (0.0) ~
Hyperbolic Treatment (hQTcT)	5,747 (8.5) *	1,629 (2.4) *	5,342 (7.9) *	7,453 (11.0) *	0 (0.0) ~	0 (0.0) ~
Hyperbolic Treatment Lights (hQTcTL)	1,324 (1.9) *	10,477 (15.4) *	12,748 (18.7) *	3,332 (4.9) *	21,028 (30.9) *	22,694 (33.4) *

Table 6: NHP Bootstrap Comparison*.* Two post-treatment ECG recordings from 17 male and 17 female NHP subjects were bootstrap resampled 1,000 times to create 68,000 simulated ECG sample recordings. For each sample, the Spearman correlation coefficient was determined between heart rate and the results of each QT correction method. The standard deviation of each method's results was also determined for each sample. For each method, the number of samples in which that method had the lowest correlation and the number of samples in which that method had the lowest standard deviation were counted. The counts were then used to find the proportion of the 68,000 samples for pairwise comparison. These analyses were performed on both light periods of each recording and the entire recording. Each column refers to the same 68,000 samples, only varying in the time-period being examined and/or the comparison being made. Percentages are rounded up to the nearest tenths place. Significance is defined as P-value ≤ 0.05.

* results are significantly different from all other results in that column.

~ or # results are significantly different from all other results in that column, except for the other results that share the same symbol.

QT Correction Method	Lowest Heart rate Correlation			Lowest Standard Deviation		
	24-hour	*12-hour Lights On*	*12-hour Lights Off*	*24-hour*	*12-hour Lights On*	*12-hour Lights Off*
	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)	Count (%)
None (QT)	0 (0.0) *	0 (0.0) *	147 (0.2) *	0 (0.0) ~	0 (0.0) ~	0 (0.0) ~
Van de Water's (QTcVa)	989 (1.4) *	3,715 (5.2) *	310 (0.4) *	0 (0.0) ~	0 (0.0) ~	0 (0.0) ~
Linear Pre-treatment (lQTcP)	7,602 (10.6) *	1,422 (2.0) *	3,096 (4.3) ~	0 (0.0) ~	0 (0.0) ~	0 (0.0) ~
Linear Pre-treatment Lights (lQTcPL)	1,301 (1.8) *	2,528 (3.5) ~	5,317 (7.4) *	656 (0.9) *	0 (0.0) ~	0 (0.0) ~
Linear Treatment (lQTcT)	24,660 (34.3) *	3,209 (4.5) *	2,999 (4.2) ~	1,191 (1.7) *	0 (0.0) ~	0 (0.0) ~
Linear Treatment Lights (lQTcTL)	2,824 (3.9) ~	21,315 (29.6) *	17,851 (24.8) *	5,059 (7.0) *	9,375 (13.0) *	8,439 (11.7) *
Hyperbolic Pre-treatment (hQTcP)	4,695 (6.5) *	5,066 (7.0) *	5,813 (8.1) *	1 (0.0) ~	0 (0.0) ~	2 (0.0) ~
Hyperbolic Pre-treatment Lights (hQTcPL)	2,834 (3.9) ~	4,203 (5.8) *	6,669 (9.3) *	4,327 (6.0) *	0 (0.0) ~	1 (0.0) ~
Hyperbolic Treatment (hQTcT)	21,444 (29.8) *	2,610 (3.6) ~	9,314 (12.9) *	40,000 (55.6) *	0 (0.0) ~	2 (0.0) ~
Hyperbolic Treatment Lights (hQTcTL)	5,651 (7.8) *	27,932 (38.8) *	20,484 (28.5) *	20,766 (28.8) *	62,624 (87.0) *	63,556 (88.3) *

Table 7: BK9 Bootstrap Comparison. Two post-treatment ECG recordings from 18 male and 18 female BK9 subjects were bootstrap resampled 1,000 times to create 72,000 simulated ECG sample recordings. For each sample, the Spearman correlation coefficient was determined between heart rate and the results of each QT correction method. The standard deviation of each method's results was also determined for each sample. For each method, the number of samples in which that method had the lowest correlation and the number of samples in which that method had the lowest standard deviation were counted. The counts were then used to find the proportion of the 72,000 samples for pairwise comparison. These analyses were performed on both light periods of each recording and the entire recording. Each column refers to the same 72,000 samples, only varying in the time-period being examined and/or the comparison being made. Percentages are rounded up to the nearest tenths place. Significance is defined as (p-value ≤ 0.05).

* results are significantly different from all other results in that column.

~ or # results are significantly different from all other results in that column, except for the other results that share the same symbol.

However, when examining only the lights on or lights off periods, the lQTcTL method has the lowest correlation more often than all other methods. The hQTcTL methods have the second-best results for the light periods. Similar results were observed during the standard deviation comparison. These comparisons were also performed using the BK9 samples, the results of which are presented in Table 7. From these BK9 data it is evident that the lQTcT method also had the lowest QTc-HR correlation more often than other methods when examining the entire 24-hour period. However, the hQTcT performance was similar. When examining either of the 12-hour light periods, the hQTcTL method had the lowest correlation most often when examining either of the 12-hour light periods, while lQTcTL performance was similar. Interestingly, the BK9 results demonstrate that the hyperbolic individual corrections produced the lowest standard deviation more often than all other methods no matter which time-period was examined. When examining the entire 24-hour period, hQTcT performed best, while hQTcTL performed best during the two 12-hour light periods. Linear correction methods appear to be better suited for NHP subjects, while hyperbolic correction methods appear to be better suited for BK9 subjects.

Tables 5 and 6 indicate how frequently each method outperformed the others, but they say nothing of the quantitative difference between the results of these methods. To address this, the correlation coefficients and standard deviations used to determine the results of these tables were used as datapoints in the box and whisker plots presented in Figures 13-16. The NHP correlation comparisons are presented in Figure 13. Figure 13A represents results from the entire recording, Figure 13B represents the results during lights-on, and Figure 13C represents the results during lights-off. As expected from Table 6, lQTcT had the lowest median and mean correlation when examining the entire 24-hour period, while lQTcTL had the lowest mean and median correlation for the 12-hour light periods. Notably, the distribution of correlation coefficients is smaller in the methods with the comparative highest or lowest medians.

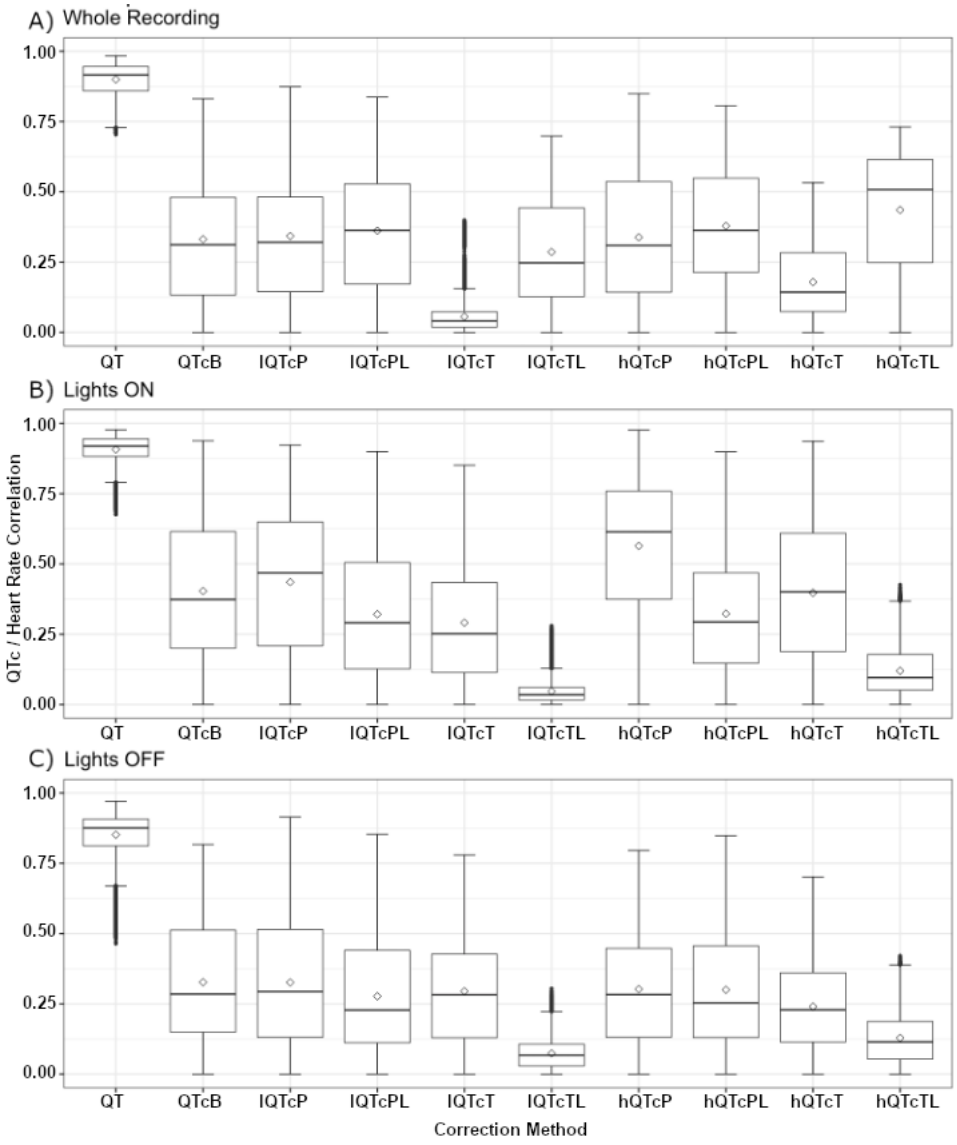

Figure 13: Box and Whisker Plots Comparing HR Correlation from NHP Bootstrap Data. Two post-treatment ECG recordings from 17 male and 17 female NHP subjects were bootstrap resampled 1,000 times to create 68,000 simulated ECG sample recordings. For each sample, the Spearman correlation coefficient was determined between heart rate and the results of each QT correction method. Each coefficient was then plotted in these box and whisker plots to compare the mean, median, and variation in QT-HR correlation results. The data presented in (A) are from the whole 24-hours, the data presented in (B) are from the 12-hour lights on, and the data presented in (C) are from the 12-hour lights off period of the recordings. The center horizontal line in a box represents the median value, while the hollow diamonds represent the mean value. The length of the box is equal to the interquartile range (IQR), while the top and bottom of the box represent the upper and lower quartile range. The whiskers represent the extremes in the distribution. If a whisker has no circles past its reach, then it represents the length between the furthest data point and the box. If there are circles past the reach of the whisker then that whisker has a length 1.5*IQR, while the circles outside its reach represent outlying data points. All methods are significantly different from all other methods in their plots except: (B) lQTcPL / hQTcPL, (C) QTcB / lQTcP. Significance defined as a P-value ≤ 0.05

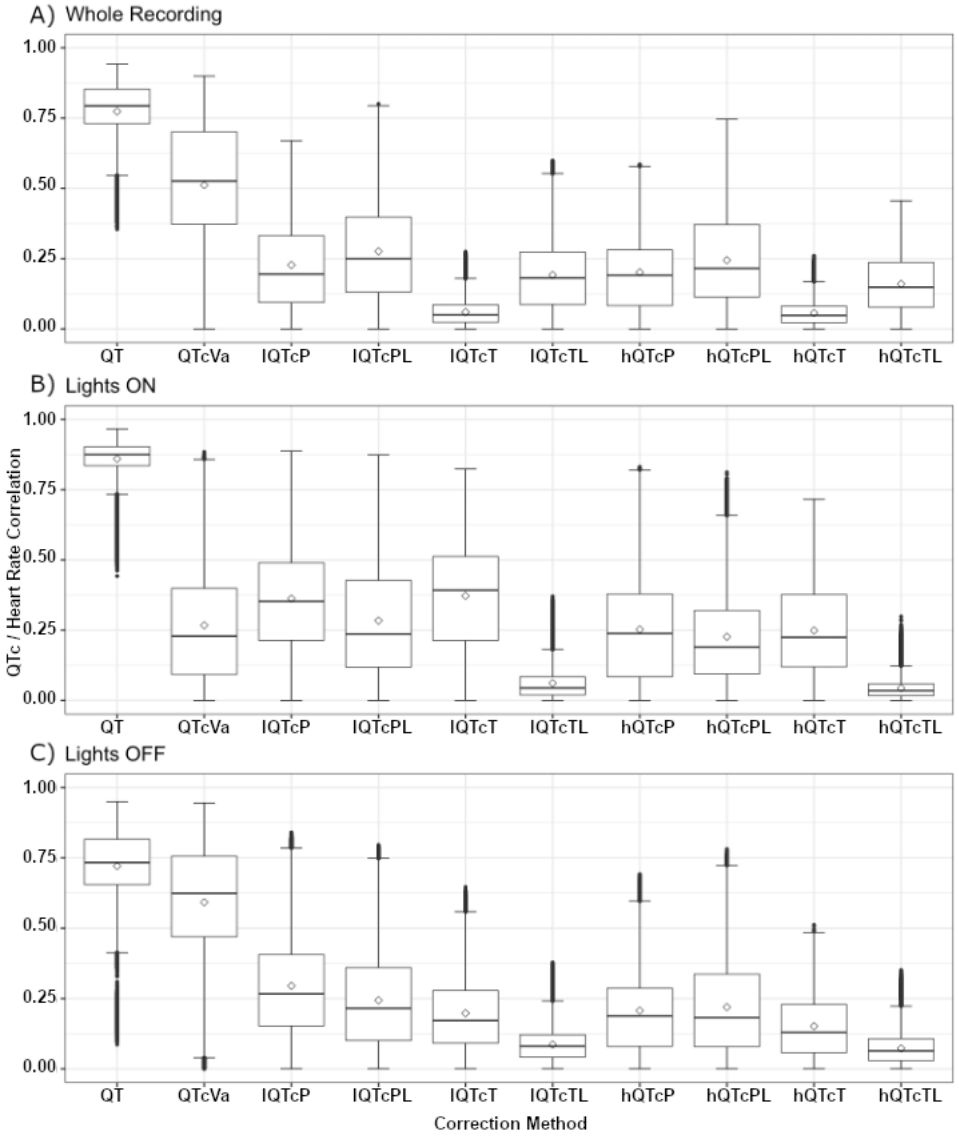

Figure 14: Box and Whisker Plots Comparing HR Correlation from BK9 Bootstrap Data. Two post-treatment ECG recordings from 18 male and 18 female BK9 subjects were bootstrap resampled 1,000 times to create 72,000 simulated ECG sample recordings. For each sample, the Spearman correlation coefficient was determined between heart rate and the results of each QT correction method. Each coefficient was then plotted in these box and whisker plots to compare the mean, median, and variation in QT-HR correlation results. The data presented in (A) are from the whole 24-hours, the data presented in (B) are from the 12-hour lights on period, and the data presented in (C) are from the 12-hour lights off period. The center horizontal line in a box represents the median value, while the hollow diamonds represent the mean value. The length of the box is equal to the interquartile range (IQR), while the top and bottom of the box represent the upper and lower quartile range. The whiskers represent the extremes in the distribution. If a whisker has no circles past its reach, then it represents the length between the furthest data point and the box. If there are circles past the reach of the whisker then that whisker has a length 1.5*IQR, while the circles outside its reach represent outlying data points. All methods are significantly different from all other methods represented in their plots. Significance defined as a P-value ≤ 0.05

The results of the BK9 correlation boxplots are presented in Figure 14, with Figures 14A, 14B, and 14C covering the 24-hour, lights-on, and lights-off periods, respectively. Like the NHP data, the QTcT methods performed better during the whole recording, while the QTcTL methods performed better during the two light periods. Linear and hyperbolic corrections had similar reductions in correlation, but the hyperbolic methods generally have lower variability. The standard deviation values from the NHP samples are presented as box and whisker plots in Figure 15, while the BK9 standard deviation results are presented in Figure 16. These figures demonstrate that standard deviation reduction is consistent between all the methods, remaining within a range of 5-10ms. This contrasts the data presented in Tables 5 and 6, which presented a clear difference between the methods. This is because Tables 5 and 6 show which methods most consistently outperform the other methods, while figures 13-16 depict the quantifiable differences between the results. This means that, while the standard deviations of each method may be comparable, some methods are consistently better than others.

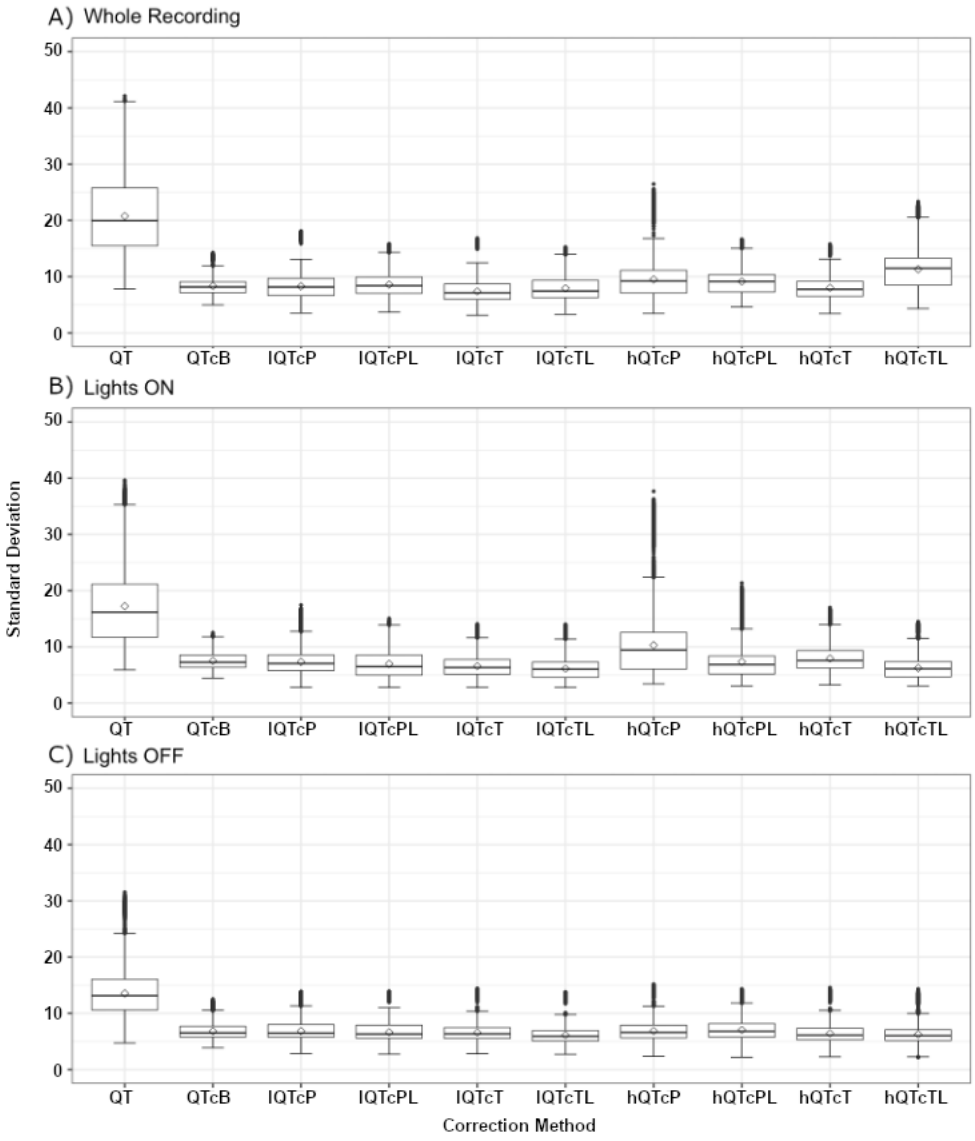

Figure 15: Box and Whisker Plots Comparing Standard Deviation of NHP Bootstrap Data. Two post-treatment ECG recordings from 17 male and 17 female NHP subjects were bootstrap resampled 1,000 times to create 68,000 simulated ECG sample recordings. For each sample, the standard deviation of the results from each QT correction method was determined. Each value was then plotted in these box and whisker plots to compare the mean, median, and variation in the results. The data presented in (A) are from the whole 24-hours, the data presented in (B) are from the 12-hour lights on period, and the data presented in (C) are from the 12-hour lights off period. The center horizontal line in a box represents the median value, while the hollow diamonds represent the mean value. The length of the box is equal to the interquartile range (IQR), while the top and bottom of the box represent the upper and lower quartile range. The whiskers represent the extremes in the distribution. If a whisker has no circles past its reach, then it represents the length between the furthest data point and the box. Circles past the whisker means that whisker has a length 1.5*IQR, while the circles outside its reach represent outlying data points. All methods are significantly different from all other methods in their plots except: (A) lQTcP / lQTcPL, and lQTcTL / hQTcT, (B) lQTcP / hQTcPL, (C) lQTcP / hQTcP. Significance defined as a P-value ≤ 0.05

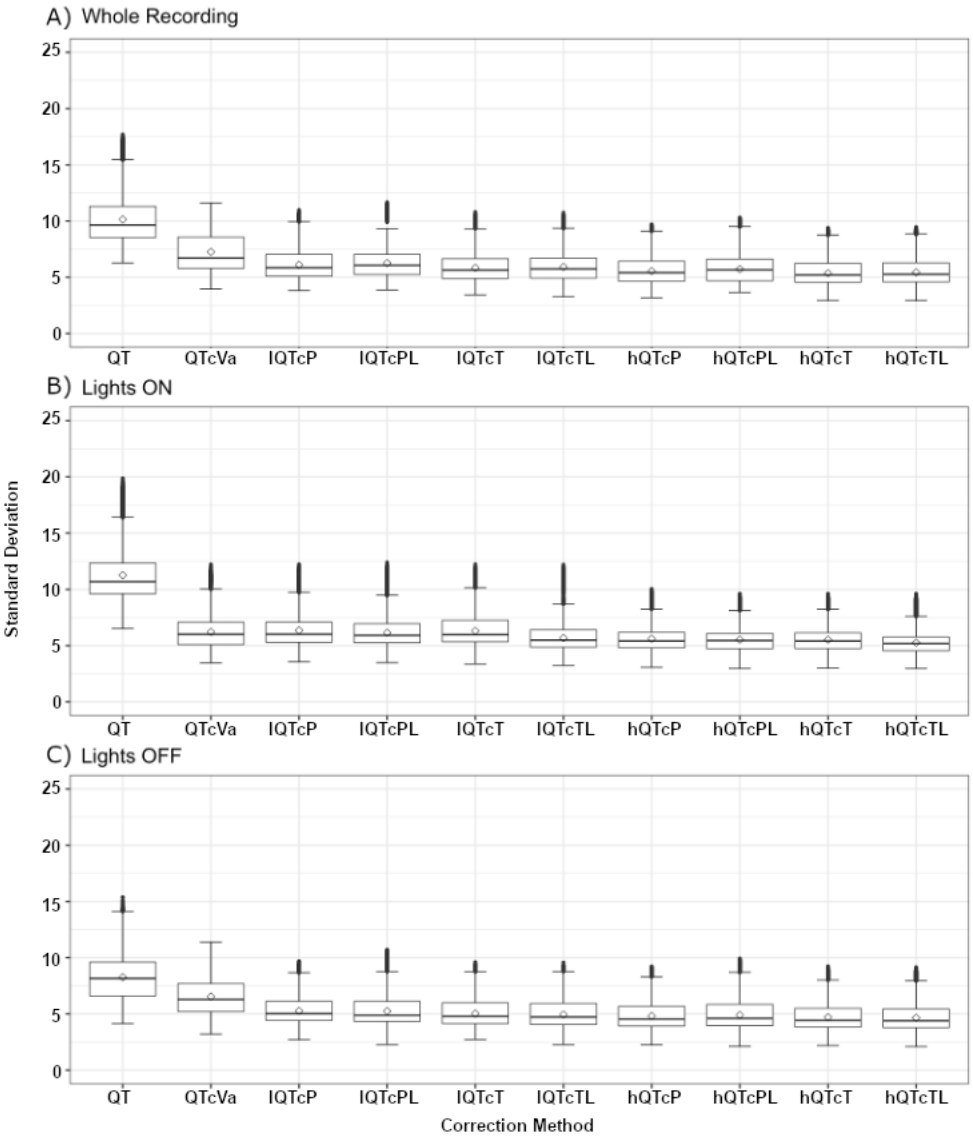

Figure 16: Box and Whisker Plots Comparing Standard Deviation of BK9 Bootstrap Data. Two post-treatment ECG recordings from 18 male and 18 female NHP subjects were bootstrap resampled 1,000 times to create 72,000 simulated ECG sample recordings. For each sample, the standard deviation of the results from each QT correction method was determined. Each value was then plotted in these box and whisker plots to compare the mean, median, and variation in the results. The data presented in (A) are from the whole 24-hour recording, the data presented in (B) are from the 12-hour lights on period of the recording, and the data presented in (C) are from the 12-hour lights off period. The center horizontal line in a box represents the median value, while the hollow diamonds represent the mean value. The length of the box is equal to the interquartile range (IQR), while the top and bottom of the box represent the upper and lower quartile range. The whiskers represent the extremes in the distribution. If a whisker has no circles past its reach, then it represents the length between the furthest data point and the box. If there are circles past the reach of the whisker then that whisker has a length 1.5*IQR, while the circles outside its reach represent outlying data points. All methods are significantly different from all other methods in their plots except: (B) hQTcPL / hQTcT. Significance defined as a P-value ≤ 0.05

Lights On vs Lights Off - Change in QT Interval

While investigating the effectiveness of QT correction methods, we observed an unexpected characteristic of BK9. During periods of light, subjects are generally more active and therefore have faster and more variable HR values; while subjects typically rest during dark periods, resulting in slower and more consistent heart rates. With what is known about the relationship between heart rate and the QT interval, it is reasonable to assume that when the HR slows during rest the QT interval will become prolonged.

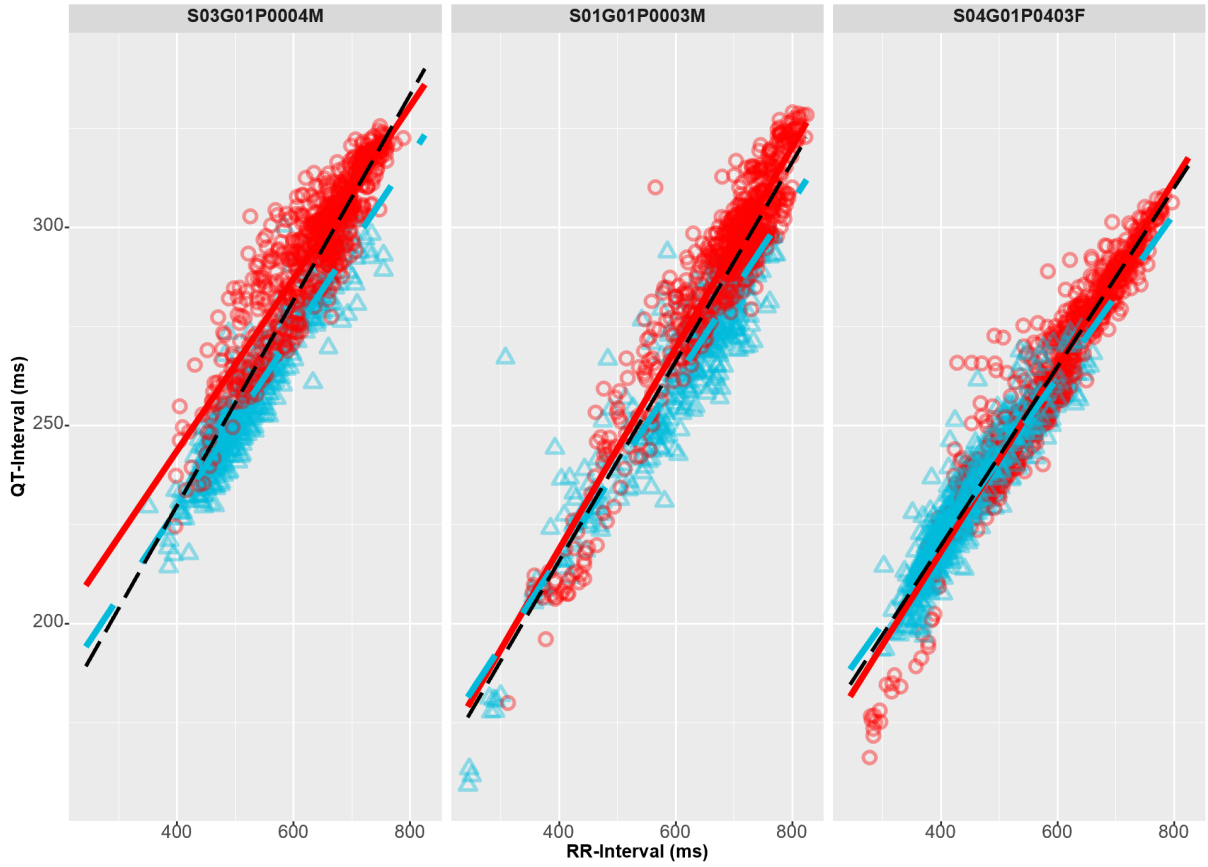

Figure 17: NHP – Differences in QT-RR Relationships Between Light Status. The QT-RR relationship plots for pre-treatment recordings of three non-human primate subjects are displayed, with their unique subject ID displayed above each plot. RR is used instead of heart rate to conform to industry standard practice for presenting primate subject data. Each plot contains the data from a subject's pre-treatment ECG recording, color and shape coded by light status. The lines represent the slope of the linear regression and have been extended for visibility. The blue long-dashed line and triangular points represent data recorded while lights were on. The red solid line with the circular points represents data recorded while the lights were off. The short-dashed black line represents the QT-RR relationship for the entire 24-hour recording. Each point represents a 1-minute mean value of QT and its corresponding 1-minute mean HR value.

The non-human primate data aligns with this assumption, as seen in Figure 17. Pre-treatment recordings of three randomly selected NHP subjects are displayed in this figure as the relationship between QT and RR. In all three subjects most data from the 12-hour period when lights were off, have slower heart rates (longer RR) and have relatively longer QT than those from the lights on period. In contrast, the data from beagle canines contradicts our assumptions, as exemplified by Figure 18.

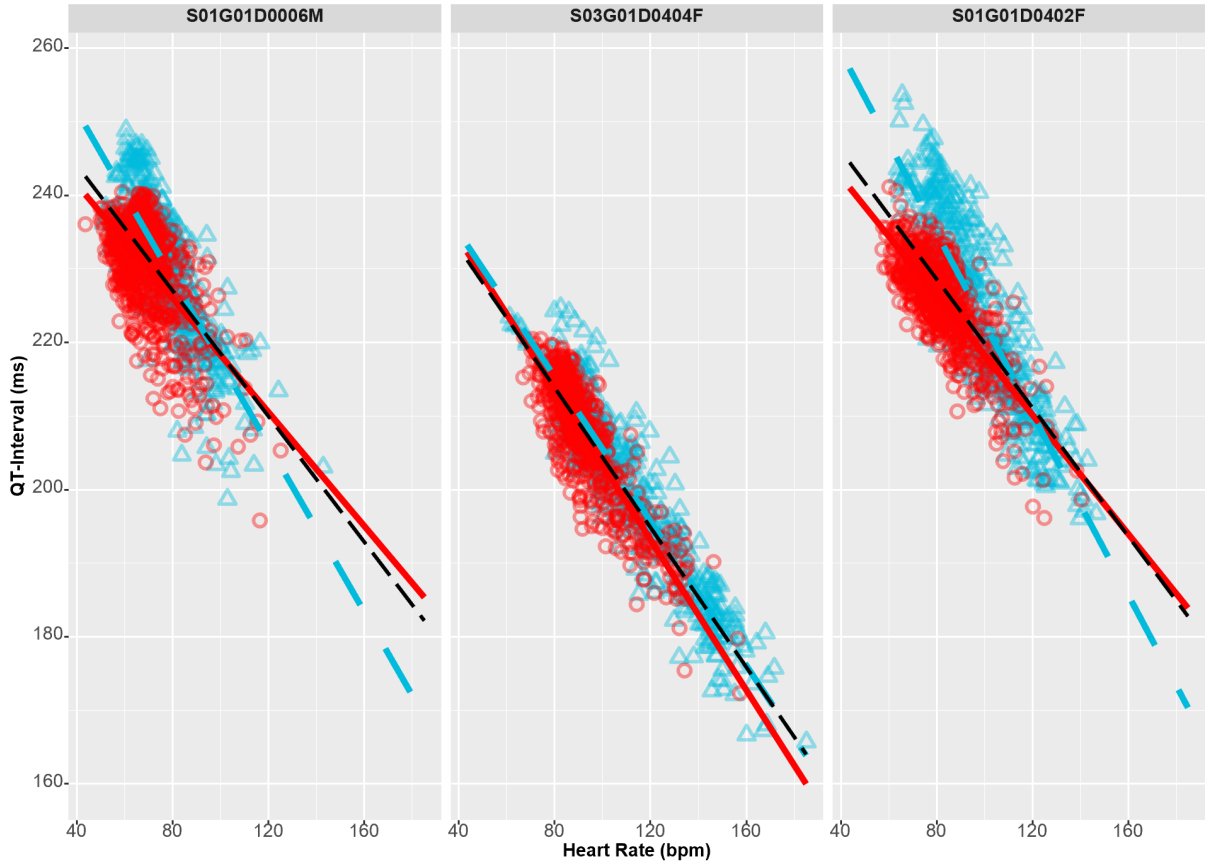

Figure 18: BK9 – Differences in QT-HR Relationships Between Light Status. The QT-HR relationship plots for pre-treatment recordings of three beagle canine (BK9) subjects are displayed, with their unique subject ID displayed above each plot. Heart rate is used instead of RR interval to conform to industry standard practice for canine subjects. Each plot contains the data from a subject's pre-treatment ECG recording, color and shape coded by light status. The lines represent the slope of the linear regression and have been extended for visibility. The blue long-dashed line and triangular points represent data recorded while lights were on. The red solid line with the circular points represents data recorded while the lights were off. The short-dashed black line represents the QT-RR relationship for the entire 24-hour recording. Each point represents a 1-minute mean value of QT and its corresponding 1-minute mean HR value.

In this figure, the pre-treatment QT-HR relationship is plotted for three randomly selected beagle canines. Heart rate was used instead of RR to match the industry standard for presenting canine data. These results show that during 12-hour periods when lights were off the subject's HR data are condensed to lower rates, but there is not clear separation between the two light periods as was seen in primates. Even though the HR is lower on average while the lights are off, the QT interval values associated with those heart rates are typically shorter than those associated with similar heart rates during periods with lights on. This means that the relationship between QT and heart rate in these beagle canines is considerably different depending on which 12-hour period is being observed and a decrease in heart rate while lights are off do not appear to result in as long of an increase in QT as it does while the lights are on.

Discussion

This study examined the effects of various QT correction methods tailored to individual subjects through a meta-analysis of preclinical toxicology studies. General correction methods, such as Bazett's and Van de Water's, are typically used to control for the relationship between QT and RR during safety studies. However, this practice is unlikely to eliminate the variability in QT interval associated with heart rate because the QT-HR relationship is variable between subjects, study days, and light cycles. Relying on a correction method that fails to take these factors into account limits the effectiveness and sensitivity of QTc analysis. Because of these concerns there is an increased interest in individual correction methods that consider the unique relationship between QT interval and heart rate present in each subject. We quantified this relationship by using linear and hyperbolic regressions of QT versus RR. Other methods of quantifying this relationship exist, such as using heart rate instead of RR interval. Variations in these approaches to quantify the rate relationship and what species-specific reference rate is used may change the effectiveness of the individual correction methods. Similarly, the subject's data used to derive these relationships dictates what sources of variation are being addressed. One approach examined in this study was the use of a subject's pre-treatment ECG recording (lQTcP, hQTcP).

This approach addresses the inter-subject variability in the rate relationship by tailoring the correction based on the specific subject's data. However, it fails to account for the intra-subject variability that occurs between study days. Both inter- and intra-subject variability are addressed by quantifying the rate relationship using the subject's ECG recording over the 24-hour period in which the QT interval being corrected occurred (lQTcT, hQTcT). This represents a correction that is derived from on-treatment data. One advantage of this correction method is that it accounts for treatment related effects on the QT-HR relationship. Neither of these correction methods account for the differences in rate relationship observed between light/dark cycles. Splitting a 24-hour recording into two 12-hour data sets based on the light status allowed for the expansion of the correction methods into pre-treatment lights (lQTcPL, hQTcPL) and post-treatment lights (lQTcTL, hQTcTL). We also compared the effectiveness of linear vs non-linear (hyperbolic) models. We pursued this comparison due to the non-linear curves seen in the QT-RR plots of BK9 subjects, which is most noticeable in the center plot of Figure 12. This curve demonstrates why BK9 data is typically presented as QT versus HR rather than QT versus RR, which is shown in Figure 18. Such curvature in the distribution of RR data points is typically why canine subjects use heart rate for their corrections. However, from these observations, we hypothesized that the hyperbolic individual corrections would provide a better fit for the BK9 rate relationship and therefore reduce the need to convert to heart rate for the corrections.

After performing each QT correction method on the bootstrap data, we found that the individual correction methods consistently outperformed the general methods (QTcB, QTcVa). As anticipated, we found that post-treatment corrections performed best when examining the whole recording, while post-treatment lights corrections performed best when examining either of the 12-hour light periods. Additionally, the linear corrections performed well for both NHP and BK9. However, the non-linear hyperbolic corrections appear to correct for heart rate in BK9 subjects more accurately.

During this study, we also observed a surprising divergent phenomenon between BK9 and NHP that warrants further analysis. In NHP subjects, there were clear distinctions between lights on and lights off periods in the distribution of the data points representing the rate relationship. Rate was generally slower and QT interval generally longer while lights were off, compared to while lights were on. However, in the BK9 subjects these differences in light periods were not as distinct. The average rate while lights were off was slower than while lights were on, but there was more overlap than what was seen in the NHP data. Strikingly, the QT intervals were generally shorter while the lights were off compared to the QT intervals associated with similar heart rates that were measured while lights were on. This implies that in BK9 subjects the relationship between QT and rate varies between the two light cycle periods more than expected, despite their near identical living conditions. In fact, it appears that the QT rate relationship in BK9 subjects during lights off is inverse to what would be intuitive. It is possible that this is due to species specific differences in autonomic signaling between light cycles or differences in their sleeping habits. Canines have a varied sleep cycle that can be comprised of multiple rest periods that can total up to 16 hours [167,168]. Such a difference in this relationship between light periods requires more attention and further study but supports the need to account for additional effects on the QT-HR relationship such as light status and sleep cycles during study design.

Limitations

This study is one step towards identifying improved QT correction methods for preclinical research, but it is not without its limitations. The data used were all sourced from the same contract research organization and their subjects were from a single vendor. Additionally, these data were recorded using jacketed telemetry, which may not be representative of data obtainable through other methods, such as implanted telemeters, though literature has shown them to be comparable [169,170]. Validation of these result should be performed by replicating them with additional data sources. Amassing larger historic pre-treatment and vehicle control datasets will allow for more of the subtle co-variates such as vendor and technology to be assessed. Bootstrap

sampling was used to increase the power of our assessments on these data. However, these simulated recordings are still derived from the original recordings used for the sampling and are no replacement for replication. It must be noted that analyses and corrections such as those described in this study require many datapoints that include a wide range of real RR values, such as from continuous telemetry measurements.

While accounting for variations between subjects and light periods proved effective, there may be other variables to consider, such as the activity, body temperature, or sex of a subject. Both male and female subjects were equally represented in this study and additional bootstrap result tables are included in the supplement that separate results by sex. A conclusive difference in correction method efficacy was not found between the sexes, but female canines appear to benefit from the non-linear methods more than their male counterparts. This could be due to a number of sex-related cardiovascular differences, two of note being the differences in mean heart rates and body temperatures [171]. More research must be done to determine if a subject's sex affects the ability of these methods to elucidate a dose response. It is also important to note that there is literature evidence that drug treatment can impact the QT-HR relationship. Accounting for this impact could improve correction methods, so the principles established here need to be extended into data from drug-treated subjects. Considering these limitations, investigating the varying rate relationship of canines more thoroughly, and expanding to include data from drug-treated subjects will allow for future studies to improve upon these conclusions.

Conclusions

As we hypothesized, using individual correction methods that accounted for ancillary sources of variability provided improved QT correction and lower variability. From the results of the bootstrap analysis, we can conclude that using an individual correction method that accounts for both inter-subject and intra-subject variability provides the best corrections in these vehicle control data. Accounting for light status can further improve these results. By using these individual methods, QTc variability was reduced and the dependence of QTc on heart rate was minimized, thereby

improving the effectiveness and consistency of QT correction. Finally, understanding the nature of the rate relationship is important, as demonstrated by the performance of the hyperbolic individual corrections for BK9. Using a method that better fits the rate relationship can improve effectiveness and reliability of correction. This increased reliability in QT correction facilitates more accurate understanding of the dose-dependent effect a drug has on the QT interval, which would mean more reliable non-clinical safety studies.

CHAPTER 4:

COMPARING THE NOVEL RATIO CORRECTION METHOD TO GENERAL AND INDIVIDUAL METHODS USING ECG DATA FROM NON-HUMAN PRIMATES TREATED WITH QT PROLONGING DRUGS

Modified from: Ether ND, Leishman DJ, Bailie MB, Lauver DA. QT Ratio: A Simple Solution to Individual QT Correction. J Pharmacol Toxicol Methods. 2022 *Under Revision.*

Abstract

QT correction methods vary in complexity and don't always consider changes in the QT-rate relationship between subjects or treatments. To address this, we utilized the linear relationships between QT and rate in non-human primates (QT-RR). After adjusting QT for a species-specific y-intercept value (100 ms), we calculated the minute-to-minute ratio between intercept-adjusted QT and rate (RR interval), a process resembling that of calculating the slope between the two points. We found that the resulting ratio increases in an exposure-dependent manner after administering QT-prolonging reference agents (dofetilide or moxifloxacin). This led us to hypothesize that this Ratio may be capable of representing a more dynamic QT-rate relationship at each timepoint. We demonstrate that this Ratio can be used as a datapoint-specific estimated slope of the QT-RR relationship to correct QT, removing substantial influence of rate. Based on this information and the ability of the Ratio method to account for minute-to-minute variations in the rate relationship, we hypothesize that using a Ratio corrected QT provides a simpler, more dynamic, and more consistent method for detecting treatment-related QT effects compared to traditional methods. We used non-human primate ECG data from subjects treated with vehicle and dofetilide (0.03, 0.10, and 0.30 mg/kg) and separated into two groups (n=4) as test cases, then we confirmed our results using data from non-human primates treated with vehicle and 80 mg/kg moxifloxacin, also separated into two groups (n=4). This allowed for robust examination of the effectiveness of the QT correction methods in multiple study scenarios, while limiting the group size to assess their usefulness in reducing the number of subjects needed for such safety studies. The ECG data from these studies were summarized into 1-minute means and used to calculate typical QT corrections: Bazett's formula and individualized linear regression corrections. We also calculated the minute-to-minute ratio between interval-adjusted QT and rate values for the novel ratio correction method. The methods were then compared based on the slope of the relationship between the resulting corrected QT values and RR intervals, the standard error of the 60-minute means of each treatment group, and power analyses performed to identify the minimal detectable

difference each method was capable of. Our results demonstrate that the Ratio method produced smaller corrected QT-rate relationship slopes than the Bazett's method, more closely matching the results of the linear regression methods. The Ratio method was also equally or more sensitive at detecting dose-dependent drug effects compared with the other QT methods and often resulted in the most consistent reduction in standard error. Additionally, the power calculations revealed that the Ratio method outperformed Bazett's in every scenario while providing similar results to the linear regression methods in most instances. These results provide evidence that the Ratio method is a capable QT correction method that may benefit the industry due to its simplicity and effectiveness. These features also imbue in the ratio method the potential for easy translatability across nonclinical species and humans.

Introduction

During the preclinical stages of drug development, it is vital to evaluate a candidate's risk of inducing arrhythmias in the clinical population. Due to the potentially fatal nature of drug-induced arrhythmia, this characteristic is a common factor that leads to discontinued development or clinical use restrictions [155]. While the risk of harm posed by drug-induced arrhythmias is significant, they are quite rare and may only affect a small subset of the clinical population [140]. The ventricular arrhythmia TdP is one example of a drug-induced arrhythmia that may occur at a rate of up to 1-in-20 cases for high-risk drugs or as low as 1-in-100,000+ in lower-risk drugs [40]. Due to the plausibility of missing such rare incidents, researchers cannot rely on electrocardiogram (ECG) arrhythmia detection to thoroughly screen drug risk. To address this, a surrogate biomarker of arrhythmia risk was identified: the prolongation of the ECG waveform QT interval (QT), which is known to correlate with arrhythmia risk [40,120]. However, the QT interval cannot be evaluated in isolation, as it is well established that heart rate (HR) influences QT via an inverse relationship. This necessitates QT correction (QTc) methods to control for heart rate and isolate drug effects on QT [72,123,131,147,148,150]. In consideration of this relationship, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has published recommended guidelines on using heart rate corrected QT prolongation as an arrhythmia risk biomarker both preclinically (S7B) and clinically (E14) [44,45]. These guidelines have led to the development of industry and regulatory protocols for preclinical *in vivo* testing as well as clinical Thorough-QT protocols. Despite the sensitivity of these preclinical evaluations, their results rarely influence the subsequent clinical Thorough QT procedures. This has led to increased interest in the standardization and integration of preclinical and clinical assessments [139]. More recently, the ICH has worked to develop updated guidelines for the clinical and nonclinical evaluation of QT/QTc interval prolongation as it relates to the proarrhythmic potential of drugs. As part of these updates, the ICH has focused on evaluating the quality of QT correction methods by assessing the ability of a selected method to reduce QT-rate

correlation, increase the sensitivity of detection, and reduce variability [113]. Through this, the ICH emphasizes the importance of choosing a QT correction method that is capable of accurately correcting the study data to obtain results that are of use for consideration during clinical study preparation.

Methods for correcting QT for rate can vary in execution, often described by their approach as either general or individual, but their goal is consistent: to reveal relevant drug-induced QT prolongation. The intention behind using these methods in safety studies is to minimize the QT-rate relationship so that any remaining QT prolongation can be more confidently associated with drug effect. General correction methods are those that utilize a general understanding of the QT-rate relationship obtained from a single, usually historical, population and apply it to all individuals in a study equally. Examples of these methods include Bazett's correction, a method with a fixed formula derived from observing data from 39 humans back in 1920 [79]. Despite the origin of this method and the criticisms it has received over the years it is still used for both human and non-human primate (NHP) subjects [113,121,123,124]. Bazett's correction, and other general methods like it which rely on formulae with fixed correlation constants, rely on the assumption that the QT-rate relationship observed in individuals will not vary drastically. Unfortunately, this assumption is far from true as research has shown that the relationship between QT and rate will vary between subjects, over time, and even between treatments [128,133,151,157,159,172]. To address the error introduced by the assumptions of general correction methods, researchers are now utilizing individual correction methods when possible [132,134,135,173]. Typically, these methods rely on an individual-specific correlation constant obtained from the slope of a linear regression performed on an individual's ECG data obtained pre-treatment or after vehicle treatment. This approach addresses the inter-subject variability in the QT-rate relationship but is not without assumptions of its own. By using a single dataset to represent all future data collected for that subject, this version of individual correction still ignores the potential for the QT-rate relationship to change between occasions or treatments.

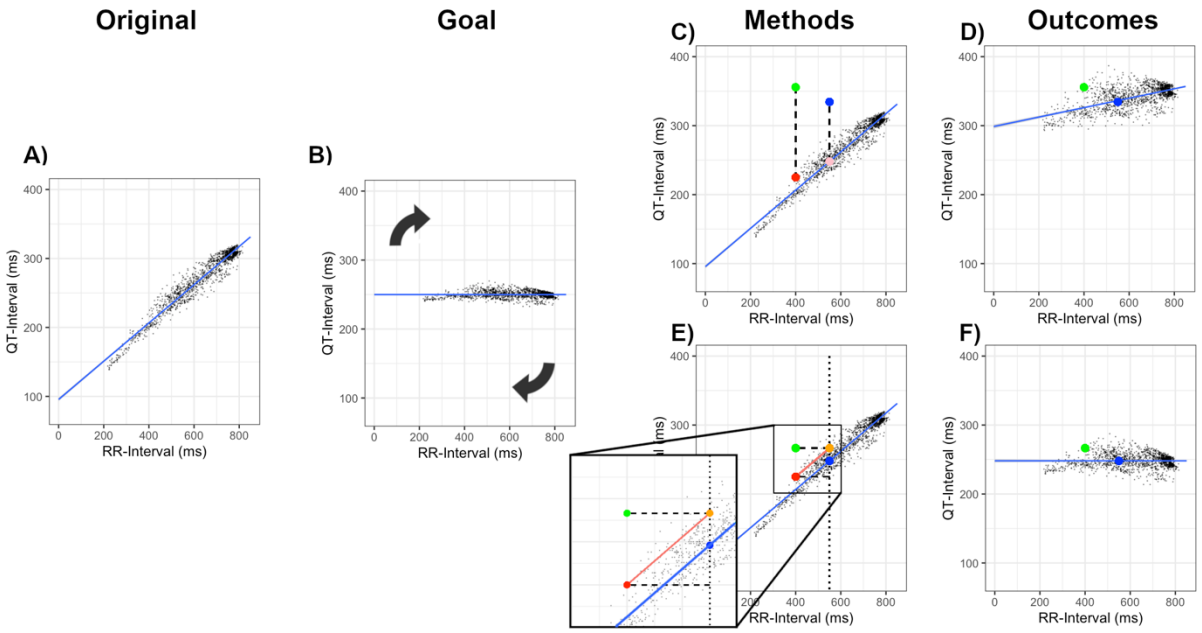

Figure 19: General vs Individual QT Correction Method Examples. These QT vs RR plots each contain data from a 24-hour ECG recoding of post-vehicle treated non-human primate (4004d). The blue line through the data is a linear regression line with a slope representing the QT-RR relationship. A) The original data that will be corrected. B) Is a visual representation of the correction goal to minimize the QT-RR relationship (slope ≅ 0), while maintaining the variation in QT that is unrelated to rate. C) exemplifies how Bazett's correction affects QT based on a fixed formula. The pink dot represents a theoretical point on the regression line at the species-specific reference rate (550 ms) used in this study. The red dot represents an actual datapoint that does not fall on the regression line. The vertical dashed lines represent the effects of Bazett's, connecting the original datapoints to their corresponding QTc results. D) the outcome of Bazett's correction on all data. E) demonstrates how an individual QT correction method works using the original slope of the data to correct the QT values to the reference rate. For clarity, an enlarged section of the plot has been embedded. The red dot is the same datapoint from (C), the blue dot is the same as the pink dot in (C). Because the blue dot is at the reference rate being corrected to, it will not change after correction, but the change to the red dot is represented. The individual correction first finds the difference between the rate of the corrected data point and the reference rate (horizontal dashed line connected to the red dot). Using this in a linear equation (y = mx + b) for x sets the reference rate as a relative axis the data is corrected to (vertical dotted line). The m in the equation is the slope of the QT-RR relationship (orange line), while the y is the QT value being corrected (red dot). Now the QTc value (orange dot) can be calculated as the intercept (b) of the dotted vertical line by a line with slope m that passes through the datapoint being corrected: QTc = QT – m*(RR-RefRR). The green dot connected to the orange dot by the dashed line represents the actual placement of the corrected datapoint. F) is a plot containing the result of all data corrected like shown in (E) with a near zero regression slope.

Our group has previously explored the benefits of using an "on-treatment" individual correction method that determines a different correlation constant for each ECG recording being corrected [174]. This approach to individual correction relies on fewer assumptions than the other methods and resulted in more consistent reduction in the QT-rate relationship and variability of QTc results.

Figure 19 contains a collection of QT vs RR plots from a single NHP subject and is intended to demonstrate the difference between the approach taken by general and individual correction methods. The subject's original uncorrected 1-minute mean ECG data from a post-vehicle treated recording is shown in Figure 19A, while the goal of correction is represented by the hypothetical transformation presented in Figure 19B. Figures 19C and 19E contain depictions of the techniques utilized by general and individual correction methods, respectively. The general Bazett's method applies a direct transformation to each datapoint based on a fixed correction coefficient that increases the magnitude of correction the further a datapoint is from the reference rate assumed in the original study (HR: 60 bpm, RR: 1000 ms). Conversely, the individual correction method uses the subject-specific slope of a linear regression as its correction coefficient. By finding the relative distance of a datapoint from a species-specific reference RR (NHP RefRR: 550 ms), the individual method can use a linear equation ($y = mx + b$) to determine QTc as an intercept (b) of the hypothetical axis at $x = 550$ ms. This is where a line with the subject-specific slope (m) passing through the relative position of the datapoint $(x, y) = (RR – RefRR, QT)$ will pass through this hypothetical axis: $QTc = QT – m*(RR – RefRR)$. Figures 19D and 19F contain the results of the general and individual corrections, respectively. The individual method results in the rotation of the data around the reference rate based on a relevant correlation coefficient, resulting in more reduction in QTc vs RR slope and less overcorrection than the general method. Not all individual correction methods work like this, some may use the relationship determined from a subject-specific baseline set of data instead of the example of "on-treatment" individual correction demonstrated in Figure 19. Individual methods, especially the "on-treatment" version, may seem ideal due to their ability to address inter- and intra-subject variability and produce QTc values with practically no relationship with rate. However, they require the collection of many datapoints containing a wide range of rates to achieve an accurate estimate of the relationship for a single ECG recording, which they assume will be representative of the relationship at each timepoint. This means that no QT correction can be performed until

computationally demanding regressions are performed on each ECG recording after the study concludes. Alternatively, the fixed formulae of general correction methods provide a simplified and easily adopted way to correct any datapoint as it is collected, despite their inability to adapt to changes in the QT-rate relationship. One potential drawback that both approaches rely on is that they pre-assign a QT-rate relationship, ignoring the possibility that a QT prolonging drug might function by altering this relationship in a concentration dependent manner. There is need for accurate correction methods that do not create an unreasonable burden of analysis on the researcher or clinician and which can dynamically respond to minute-to-minute changes in the rate relationship without adding additional assumptions to the evaluation. Ideally, this new method would be effortlessly adaptable for any species and easily updated as required. Such a method would help further our understanding of QT prolonging drug effect and may improve the reliability of preclinical risk assessments, increasing their translatability to clinical studies.

In this study, we demonstrate a new approach - referred to as the Ratio method - which may be used with any species and dataset without the requirement of additional reference data (e.g., baseline), relying only on a species-specific fixed variable selected based on the y-intercept of a population's QT-RR relationship. By subtracting this constant from a QT value, then dividing the result by the corresponding rate measurement, a relative ratio value is obtained that works as an estimated slope of the relationship for that single datapoint. Comparing these ratios can be used to assess the change in the QT-rate relationship, as well as the magnitude and direction of that change. They can also be used to obtain a corrected QT value in a manner similar to individual correction methods by utilizing the ratio value in place of the slope typically derived from linear regressions. In this way, the Ratio correction method may bridge the gap between the simplicity of general correction methods and the adaptability of individual methods. This method, like the individual methods, relies on the linear relationships between rate and QT that have been previously observed. In some species (such as the beagle canine), a near-linear relationship is observed between QT and HR but in humans and non-human primates, this near-linear

relationship is observed using QT and the RR interval (RR) [123,153]. The ratio method assumes that using a species-specific y-intercept constant is sufficient to represent a hypothetical starting point for a line that will represent the slope of the QT-rate relationship for most datapoints from most individuals. By using this fixed initial starting point the ratio method can be used as a general correction method while still considering the QT-rate relationship of the individual subject by accounting for moment-to-moment differences in the relationship at each data point by calculating the slope of the line connecting a single point to the fixed y-intercept value. This allows the ratio method to account for the variations in the rate relationship observed between QT values that share the same or similar rate values, all without the need for computationally complex regressions or large data sets. While the Ratio method may be further improved by using study-specific or subject-specific y-intercept values, doing so would remove the benefit of such a simplified method. Instead, we believe that the ability of the Ratio method to adapt to these moment-to-moment changes can compensate for the fixed nature of the intercept value. We hypothesize that using the Ratio based correction method on data from small study groups (n=4) of non-human primates administered dofetilide or moxifloxacin will produce corrected QT values that have a smaller QT-RR relationship compared to the historically accepted general correction (Bazett's) and comparable results to individual linear regression correction methods. Additionally, we believe that the Ratio method will provide more consistent error reduction and more sensitivity than a typical general correction method.

Methods

Subjects and Data

The present investigation utilized post hoc analyses of nonclinical ECG data provided by Eli Lily and Company and Charles River Laboratories. The data were collected during two different animal studies involving QT-prolonging drugs. Study A included male cynomolgus primates (NHPs) treated with 0.03, 0.10, and 0.30 mg/kg dofetilide along with vehicle control in a Latin square design (n=16). These subjects each received a single treatment per week and were

monitored 24-hours prior to and after administration of the treatment. Study B included a separate cohort of male NHPs treated with vehicle control and 80 mg/kg moxifloxacin in a cross-over design (n=12). Each subject was administered both treatments in the same week and monitored for 120-minutes prior to and 48-hours after administration of each treatment, with vehicle control being administered first and 80 mg/kg of moxifloxacin being administered several days later. This treatment design was repeated on a subsequent week, though this paper only utilizes the data from the first 24-hours post-treatments recorded on the first week. Additionally, 48-hour baseline ECG recordings were collected from each subject before the first treatment week for the subjects of Study B. These baseline recordings were used for the power calculations described in the Statistical Analysis section of the Methods, while Study A used the 24-hour pre-treatment recordings from two separate weeks for their power calculations. The 120-minutes immediately prior to treatment were used to calculate pre-treatment baseline means for determining change from baseline results. All studies were approved by the institutional animal use committee, and each subject had intravascular leads from a radiotelemetry device surgically implanted for data collection; Data Science International L21 model telemeters for dofetilide treated subjects, and Data Science International M11 model telemeters for moxifloxacin treated subjects. For each study, two groups (n=4) were selected containing schedule-matched subjects. This allowed for the standardization of the number of subjects examined between studies and enabled the testing of correction methods on smaller (n=4) studies while immediately replicating those tests on the same and different drug treatments. Telemetry data collected from each subject was provided as 1-minute means of ECG wave intervals as measured using the ECG analysis software Ponemah (Data Science International). Relationships determined based on linear regression used this 1-minute mean data. Each minute of data was then corrected by each QT correction method. Before treatment group data was averaged together, each subject had their data averaged into 1-hour means to reduce variability between subjects compared to minute-to-minute observations. Change from baseline was then calculated by subtracting the 1-hour treatment means from the

subject-specific 120-minute mean of the pre-treatment baseline data recorded prior to initial treatment administration. These change from baseline data were then used to determine the hourly group means for each treatment along with their standard error.

QT Corrections

Multiple methods of QT correction were used to compare results, including Bazett's correction ($QTcB = QT / (RR / 1000)^{1/2}$), a species-appropriate general correction traditionally used in NHPs [79,113,126,153], individualized linear regression corrections, and the Ratio correction. The individualized linear regression corrections and the Ratio method use the same formula ($QT - m*(RR-RefRR) = QTc$), which multiplies the difference between the observed RR and a species-specific reference RR (550ms) by the slope (m) of the QT-RR relationship and subtracts this value from the observed QT value. These methods differ in how they determine the slope used in this formula. The Ratio method (QTcR) calculated the slope between the datapoint being corrected (RR, QT) and a species-specific y-intercept (0, 100ms) providing a datapoint specific slope in place of m. This species-specific y-intercept is one chosen by this research team based on the baseline data of the subjects used in the two drug studies (Figure 20). Two linear regression correction methods were examined in this study that also used the individual correction formula, with the only difference between the methods being the data used for the regression of QT vs. RR. First, a subject-specific vehicle correction (QTcV) was assessed using subject-specific recorded for 24-hours post-vehicle treatment. Second, a subject-specific "on-treatment" correction (QTcT) was applied using regressions performed on the 24-hour post-treatment data that contains the QT interval measurement being corrected. Due to the nature of QTcV, it acts as an “on-treatment” correction for a subject’s vehicle treatment data but does not account for variability in the QT-rate relationship across subsequent weeks.

Statistical Analysis

Results were compared graphically and statistically using 95% confidence intervals and an alpha value of 0.05. Side-by-side graphical comparisons are used to illustrate the effectiveness of the

novel Ratio correction method to reduce the QTc-rate relationship and variability compared to the other methods. The effectiveness of each method to correct the rate (RR) effect on the QT interval was compared to all other methods as well as uncorrected data using QTc vs. RR plots. These plots contained the extrapolated linear regression lines with confidence intervals as a visualization of the QT or QTc relationship with rate (RR). Additionally, the linear equations of these regression lines are also included in the plots to better compare the ability to reduce the rate effect on QT. QTc vs Time (hours) plots were created to compare the effectiveness of each method to reduce variability, represented by standard error, and detect a treatment effect at each hour. These plots compare the change from pre-treatment baseline data (dQT) for vehicle and drug treatments with corresponding graphical ribbons representing the standard error of the means. Additionally, the change in RR from pre-treatment baseline (dRR) was plotted over time (hours) by treatment for each study group, with graphical ribbons once again representing standard error. Individual subject QTc vs. RR plots were generated using the subject's 1-minute mean data, colored by treatment. The group averaged dQT vs Time and dRR vs Time plots used the group averages of subject's change of 60-minute means from the 120-minute pre-treatment mean.

An ANOVA (QTc ~ Occasion + Subject Mean Baseline + Time) was performed for each method using the 60-minute mean change from baseline of all four subjects from a group to compare the QT or QTc values between two 24-hour baseline recordings. The resulting residual standard errors were then used to determine the minimal detectable difference (MDD) between recordings and create power curves for each method, by study group (n=4). These power curves allow for the graphical comparison of the ability of each method to detect a change in QT or QTc in an n=4 study at a range of powers from 0 to 1. The results at a power of 0.8 are highlighted in these figures.

Results

Selecting the Species-Specific y-intercept Value

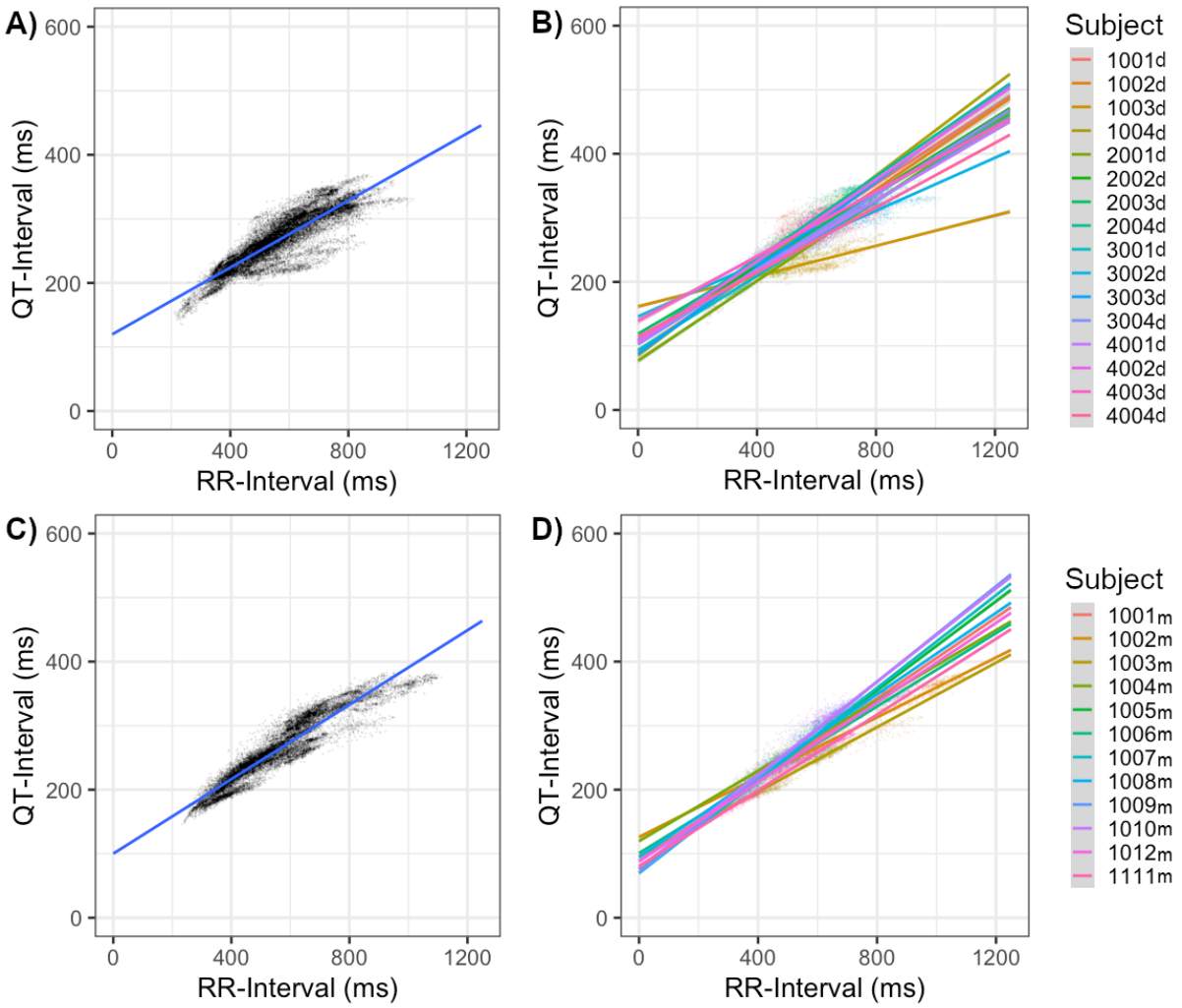

Figure 20: Selecting a Relevant y-intercept Value. These figures contain baseline pre-treatment data from each NHP subject used to determine a species-specific y-intercept value. A) contains the first 24-hour pre-treatment ECG data recorded for all 16 NHP subjects from the dofetilide study. The blue line is a linear regression through the data with a y-intercept of 120 ms. B) contains the same data used in 20A, except color coded by subject ID. The y-intercepts ranged from 77 ms to 161 ms, with a median of 108 ms, a mean of 110 ms, and a standard deviation of 22 ms. C) contains the first 24-hour baseline data recorded from the 12 NHP subjects from the moxifloxacin study. The blue line is a linear regression through the data with a y-intercept of 100ms. D) contains the same data as 20C, except the data is color coded by subject ID. The y-intercepts ranged from 70ms to 126ms, with a median of 89 ms, a mean of 91ms, and a standard deviation of 18ms. From this data, a species-specific y-intercept value of 100ms was selected as sufficiently representative of the data.

Selecting a relevant y-intercept value for use in the QTcR method was the first step of this study. This value was selected based on 24-hour pre-treatment baseline data from all NHP subjects available in the original dofetilide and moxifloxacin studies, which are presented in Figure 20. The data from the dofetilide study are collectively presented in Figure 20A along with a single regression line (blue line) that has a y-intercept value of 120 ms. Figure 20B contains the same data separated by the 16 individual subjects whose mean y-intercepts equal 110 ms. The data from the 12 subjects from the moxifloxacin study are collectively presented in Figure 20C with a y-intercept of 100 ms, and individually presented in Figure 20D with a mean y-intercept of 91 ms. Based on these results we determined that a species-specific y-intercept of 100 ms would be sufficiently relevant for use in QTcR.

Variations in Rate Relationship

This paper is intended to evaluate the effectiveness of the novel Ratio correction method compared to general and individual QT correction methods, and its ability to adapt to changes in the QT-rate relationship. To demonstrate the need for subject-specific and occasion-specific correction methods, the regression slopes of the 24-hour pre-treatment recordings of the four subjects in dofetilide group 2 are presented in Table 8. These results are organized by subject in the columns and by week in the rows.

	Subject 1004d	***Subject 2004d***	***Subject 3004d***	***Subject 4004d***	***Week Mean***	***(StdDev)***
Week 1	*0.352*	*0.321*	*0.293*	*0.252*	*0.305*	*(0.042)*
Week 2	*0.260*	*0.348*	*0.287*	*0.221*	*0.279*	*(0.053)*
Week 3	*0.281*	*0.326*	*0.313*	*0.228*	*0.287*	*(0.044)*
Week 4	*0.286*	*0.368*	*0.304*	*0.252*	*0.302*	*(0.049)*
Subject Mean (StdDev)	*0.294 (0.040)*	*0.341 (0.021)*	*0.299 (0.012)*	*0.238 (0.016)*		

Table 8: Pre-Treatment QT v RR Relationship Slopes by Subject and Week. This table contains the slopes of the QT-RR relationship for each subject of a single NHP group (n=4) by week, as determined by linear regression. The 24-hour pre-treatment recordings from each week were used to compare the slopes between subjects in a single week and between weeks in a single subject. The final column on the right contains the means and standard deviations for each week, while the final row on the bottom contains the means and standard deviation for each subject.

Data are from four of the NHPs from the dofetilide study due to the Latin square design making this comparison possible. Weekly means and standard deviations of the slopes are present in the right-most column, while the subject means and standard deviations are presented in the bottom row. Variability between subjects is widely understood to be worth accounting for, which is why individualized correction methods are commonly discussed in the literature [128,129,131,133–135]. This necessity for subject-specific correction is supported by the weekly standard deviations found between subjects that range between 13-18% of the means. However, rarely is the variability within subjects (between occasions) taken into consideration when selecting a QT correction method. Subject 1004d in this table has an inter-occasion standard deviation that is 13.6% of its mean slope. This approximates the differences between subjects and is therefore also important to consider when a QT correction method is applied.

Dofetilide (mg/kg)	***Subject 1004d***	***Subject 2004d***	***Subject 3004d***	***Subject 4004d***	***Treatment Mean (StdDev)***	
0.00	*0.356*	*0.389*	*0.338*	*0.277*	*0.34*	*(0.047)*
0.03	*0.277*	*0.384*	*0.331*	*0.259*	*0.313*	*(0.057)*
0.10	*0.259*	*0.429*	*0.386*	*0.191*	*0.316*	*(0.110)*
0.30	*0.412*	*0.417*	*0.399*	*0.219*	*0.362*	*(0.095)*
Subject Mean (StdDev)	*0.326 (0.071)*	*0.405 (0.022)*	*0.364 (0.034)*	*0.237 (0.039)*		

Table 9: Post-Dofetilide Treatment QT v RR Relationship Slopes by Subject and Dose. This table contains the slopes of the QT-RR relationship for each subject of a single NHP group (n=4) by dofetilide dose, as determined by linear regression. The 24-hour post-treatment recordings were used to compare the slopes between subjects in a single week and between treatments in a single subject. The final column on the right contains the means and standard deviations for each treatment, while the final row on the bottom contains the means and standard deviation for each subject.

Table 9 contains the linear regression slopes from the post-treatment data of the same subjects. The standard deviation between subjects treated with vehicle is similar to the baseline data of Table 8. The standard deviation increases in almost a dose dependent manner from near 14% of the mean, up to almost 35%, across dosing instances. This suggests that these treatments are affecting the QT-rate relationship and the effects may vary between subjects. In addition to increased variability between subjects during higher treatment doses, the variability of an

individual subject's QT-rate relationship increases in the presence of drug treatment compared to the baseline data. Both tables emphasize the need to account for the variations in QT-rate relationships between subjects, occasions, and treatments. Without using a QT correction method designed with these variations in mind, the method will be less likely to accurately correct the data.

QT vs RR Comparisons

To confirm the variations in slope between treatments, as well as compare the ability of each method to reduce the rate effect, the 1-minute means of QT and RR intervals were plotted against each other by subject, treatment, and correction method. Figure 21 contains the data from the four subjects in the 1st dofetilide treatment group along with the four treatments they received (0.00, 0.03, 0.10, and 0.30 mg/kg dofetilide; red, green, blue, and purple, respectively). The regression lines and their corresponding linear equations are included in each plot and color-matched to the data points. Each column represents a different subject, and each row represents the results of a different correction method, except the first row, which is uncorrected. In the first row, a difference in slopes can be seen between the treatments for each subject. Also, the y-intercept value at the end of each linear equation in this uncorrected data can be referenced to understand where the data falls in relation to the species-specific y-intercept value (100ms). The second row contains the same data as the first, except with the results of Bazett's QT correction (QTcB) replacing the raw QT values. While QTcB resulted in a reduction in slope compared to uncorrected data, there are still noticeable relationships between QTcB and RR for many of the treatment and subject combinations. In the third row, the results of QTcR are presented which demonstrate notable reductions in the slopes of the relationships compared to QTcB in 12 out of 16 of the scenarios. The fourth row contains the results of QTcV, which acts as an "on-treatment" correction for the vehicle data of each subject and a subject-specific linear regression based individual correction for the rest of the treatments.

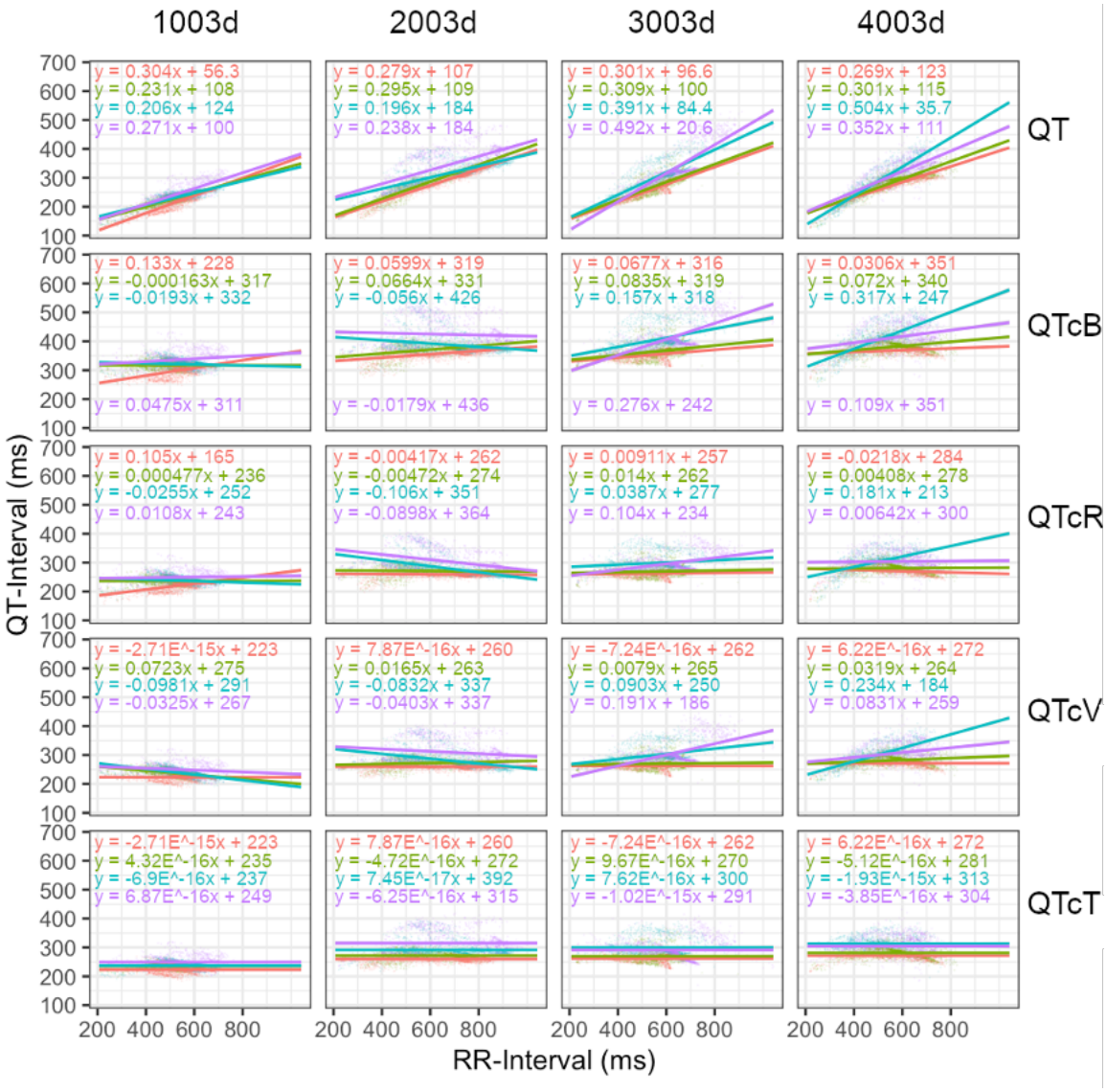

Figure 21: Correction Method QTc vs RR Comparison by Dofetilide Treated Subjects – First Group. This figure contains the 1-minute mean QT or QTc interval over the corresponding RR interval from four NHP subjects treated with dofetilide, referred to as dofetilide group 1. Subjects were age matched male NHPs dosed with vehicle control (red), 0.03 mg/kg dofetilide treatment (green), 0.10 mg/kg dofetilide (blue), and 0.30 mg/kg dofetilide (purple). Each column of graphs depicts the results of a different subject while each row represents the results of each QT correction method on the same data (except for the first row containing uncorrected QT). Data was recorded over 24-hours post-treatments. Linear regression lines have been added and are extrapolated to better visualize the slope of the relationships. The linear equations in the corner of each plot are color matched to the regression lines in the format of y = mx + b; in this formula y represents a QT value on the line, x represents an RR value, m represents the slope of the line, and b represents the y-intercept. QT: raw QT interval, QTcB: Bazett's corrected interval, QTcR: Ratio corrected interval, QTcV: post-Vehicle treatment-based linear regression corrected interval, QTcT: post-On Treatment-based linear regression corrected interval.

Due to this, like will be seen in the identical results for the QTcT correction of the vehicle data, QTcV produced near zero slopes for the vehicle treatment data. While results were close, it was outperformed by QTcR in most scenarios (9/16), even with its advantage for the vehicle treatment data. The final row contains the results of QTcT, which uses the subject and treatment-specific regression slopes for correction. Because of this, QTcT naturally results in a corrected slope near zero for each instance. These perfect results would be different if we examined a smaller time-period than was used in the regression, but they are included as-is to function as a positive control demonstrating what is possible with a hypothetically perfect representation of the QT-RR relationship. Data from the 2nd dofetilide treated group is presented similarly in Figure 22. Once again, the uncorrected QT data is presented in the first row to demonstrate the varied relationships between treatments and subjects. Each subject also has a different distribution in y-intercept values; with 1004d having a spread of values below and above 100ms, 2004d and 3004d all having values below 100ms, and 4004d having most intercept values above 100ms. In the second row the results of QTcB once again show a reduction in the relationship that could be improved in most scenarios. The third row with the QTcR results shows more consistent reductions in slope than QTcB, with all but 3 subject/treatment combinations having smaller slopes than QTcB. In the fourth row, QTcV produced near zero slopes for the vehicle treatment data again, while QTcR produced smaller slopes than QTcV for 7 out of 12 subject/drug-treatment combinations. The final row of Figure 22 contains the results of QTcT, which results in near zero slopes for each treatment.

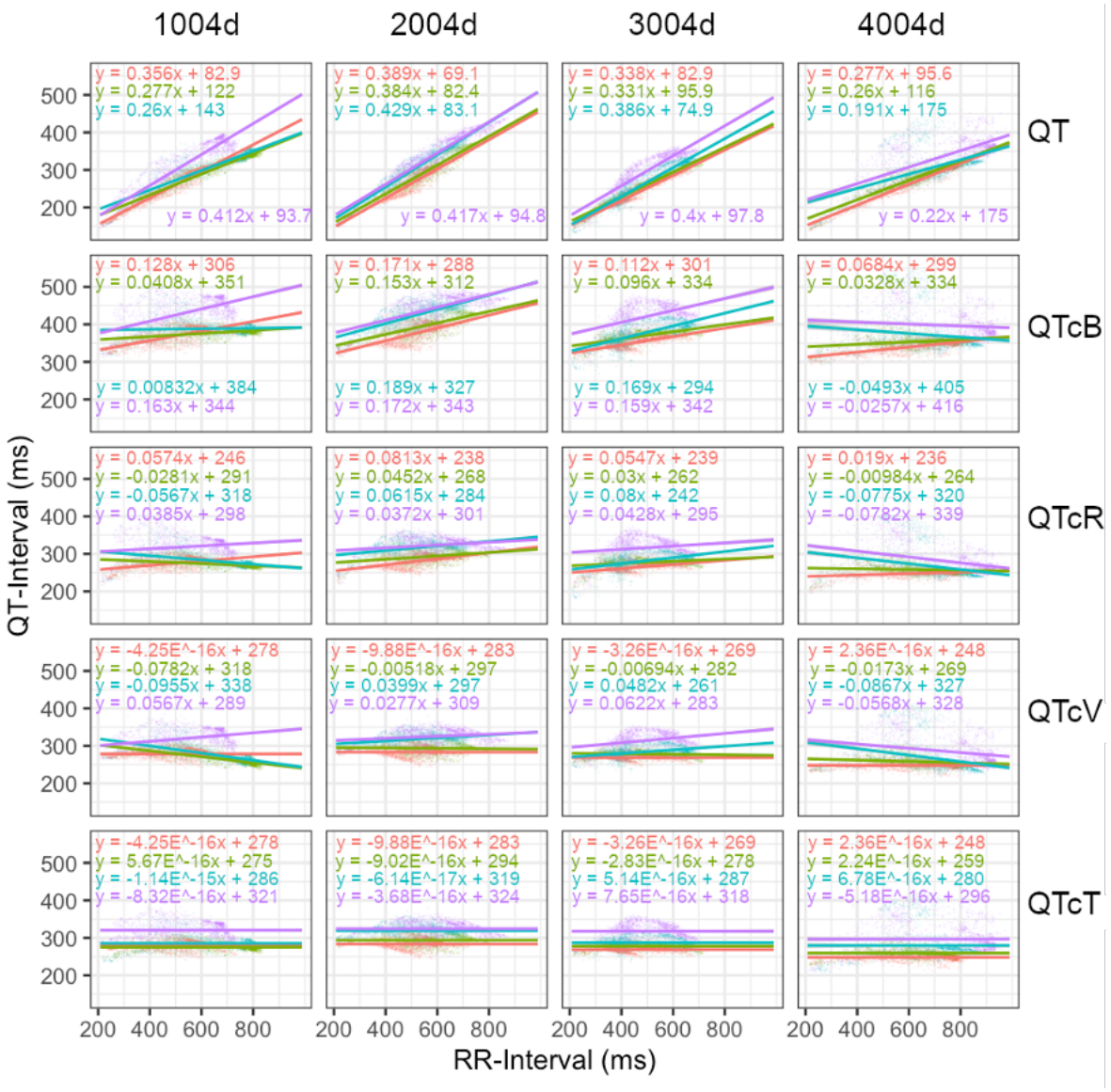

Figure 22: Correction Method QTc vs RR Comparison by Dofetilide Treated Subjects – Second Group. This figure contains the 1-minute mean QT or QTc interval over the corresponding RR interval from four NHP subjects treated with dofetilide, referred to as dofetilide group 2. Subjects were age matched male NHPs dosed with vehicle control (red), 0.03 mg/kg dofetilide treatment (green), 0.10 mg/kg dofetilide (blue), and 0.30 mg/kg dofetilide (purple). Each column of graphs depicts the results of a different subject while each row represents the results of each QT correction method on the same data (except for the first row containing uncorrected QT). Data was recorded over 24-hours post-treatments. Linear regression lines have been added and are extrapolated to better visualize the slope of the relationships. The linear equations in the corner of each plot are color matched to the regression lines in the format of y = mx + b; in this formula y represents a QT value on the line, x represents an RR value, m represents the slope of the line, and b represents the y-intercept. QT: raw QT interval, QTcB: Bazett's corrected interval, QTcR: Ratio corrected interval, QTcV: post-Vehicle treatment-based linear regression corrected interval, QTcT: post-On Treatment-based linear regression corrected interval.

In Figure 23, this process was repeated for the four subjects in the 1st moxifloxacin group which were treated with vehicle control (red) and 80 mg/kg moxifloxacin (blue). In this data, the difference in slopes between treatments is less apparent in the uncorrected QT data presented in the first row, except for subject 1004m. The second row of Figure 23 containing the QTcB results show an improvement over uncorrected data and seems to be especially effective on the vehicle treatment data of subject 1003m. The third row contains the results of QTcR, which produced smaller slopes than QTcB in every instance except the vehicle treatment data from subject 1003m. The results of the QTcV correction presented in the fourth row show a near zero slope for the QTc-RR relationship of the vehicle treatment data, as expected. However, the moxifloxacin treatment data results show that QTcV produced either very similar slope reduction as QTcR (subjects 1001m and 1003m) or noticeably less slope reduction (subjects 1002m and 1004m). Once again, the fifth row contains near perfect correction by QTcT, acting as a positive control. Finally, this presentation is repeated for Figure 24, which contains the data from the 2nd moxifloxacin treated group. The first row of this figure containing the uncorrected QT data displays the most notable information about this group compared to the previous three; the y-intercepts for all but subject 1006m are well below the 100ms used in QTcR. Based on the results seen so far, it is likely that QTcR will be least effective for this group. QTcB is represented in the second row and reduces the slope of the rate relationship, yet still not as much as any of the QTcR results in the third row. As expected, QTcV in the fourth row produces near zero slopes for all the vehicle treatments. Unsurprisingly, it also outperforms QTcR in all the moxifloxacin treatments except the one from subject 1006m, the only subject with y-intercepts near 100ms. In the fifth and final row, QTcT once again produces near zero slopes with its “on-treatment” corrections.

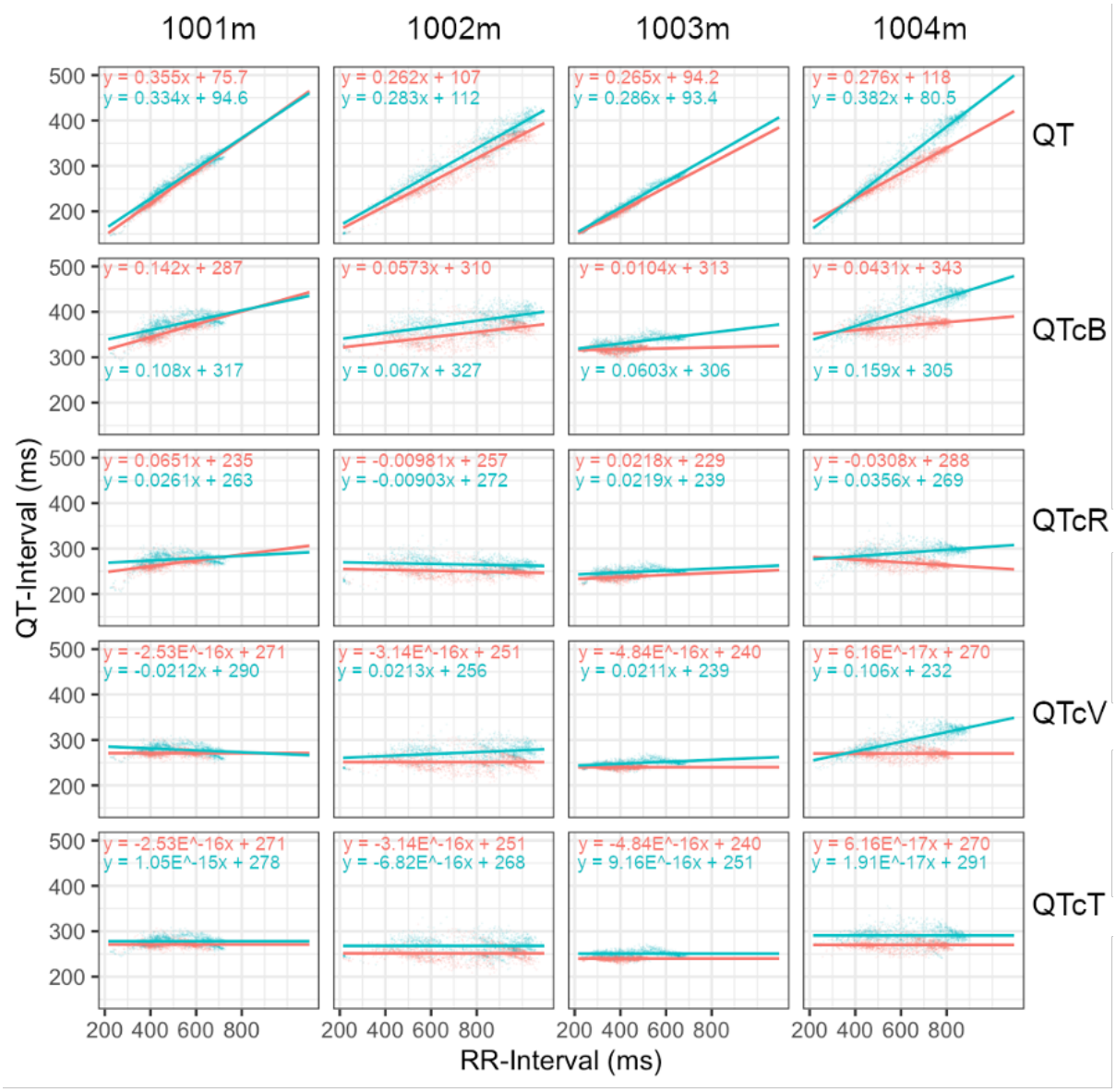

Figure 23: Correction Method QTc vs RR Comparison by Moxifloxacin Treated Subjects – First Group. This figure contains the 1-minute mean QT or QTc interval over the corresponding RR interval from four NHP subjects treated with moxifloxacin, referred to as moxifloxacin group 1. Subjects were age matched male NHPs dosed with vehicle control (red) and 80 mg/kg moxifloxacin treatment (blue). Each column of graphs depicts the results of a different subject while each row represents the results of each QT correction method on the same data (except for the first row containing uncorrected QT). Data was recorded over 24-hours post-treatments. Linear regression lines have been added and are extrapolated to better visualize the slope of the relationships. The linear equations in the corner of each plot are color matched to the regression lines in the format of y = mx + b; in this formula y represents a QT value on the line, x represents an RR value, m represents the slope of the line, and b represents the y-intercept. QT: raw QT interval, QTcB: Bazett's corrected interval, QTcR: Ratio corrected interval, QTcV: post-Vehicle treatment-based linear regression corrected interval, QTcT: post-On Treatment-based linear regression corrected interval.

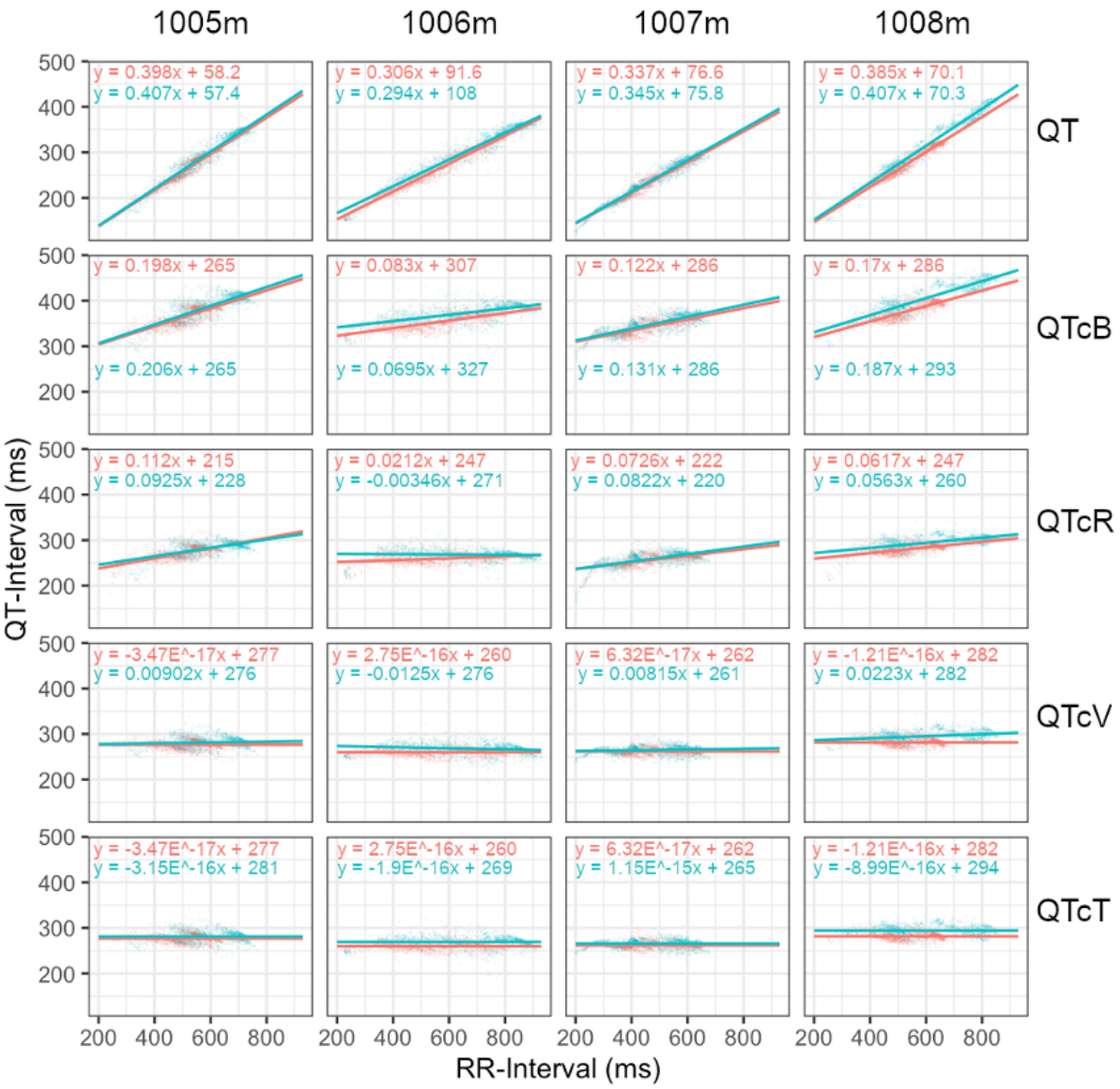

Figure 24: Correction Method QTc vs RR Comparison by Moxifloxacin Treated Subjects – Second Group. This figure contains the 1-minute mean QT or QTc interval over the corresponding RR interval from four NHP subjects treated with moxifloxacin, referred to as moxifloxacin group 2. Subjects were age matched male NHPs dosed with vehicle control (red) and 80 mg/kg moxifloxacin treatment (blue). Each column of graphs depicts the results of a different subject while each row represents the results of each QT correction method on the same data (except for the first row containing uncorrected QT). Data was recorded over 24-hours post-treatments. Linear regression lines have been added and are extrapolated to better visualize the slope of the relationships. The linear equations in the corner of each plot are color matched to the regression lines in the format of y = mx + b; in this formula y represents a QT value on the line, x represents an RR value, m represents the slope of the line, and b represents the y-intercept. QT: raw QT interval, QTcB: Bazett's corrected interval, QTcR: Ratio corrected interval, QTcV: post-Vehicle treatment-based linear regression corrected interval, QTcT: post-On Treatment-based linear regression corrected interval.

Exposure Dependent Change from Baseline vs. Time Comparisons

As recommended by the ICH, confirming the ability of each method to reduce the relationship between QTc and rate is an important step to ensuring that an appropriate correction method is being used. However, a perfect correction (as defined by a lack of slope in the QT-RR relationship) is useless if the method completely obfuscates the drug effect. To make sure these correction methods can still detect drug effects, treatment response was examined by examining the hourly mean change in QT or QTc from the subject- and week-specific 120-minute mean pre-treatment baseline (dQT), averaged by treatment and group. These results were plotted over the 24-hours post-treatment, with graphical ribbons representing the group standard error of the mean at each hour. Figure 25 contains these results for both dofetilide-treated NHP groups. Each plot is labeled with which correction method was used, except the top plot, which is uncorrected. The plots in the first column (Figure 25A) represent the results using the data from the 1st dofetilide treated group (subjects 1003d, 2003d, 3003d, and 4003d), while the plots in the second column (Figure 25B) represent the 2nd dofetilide treated group (subjects 1004d, 2004d, 3004d, and 4004d). Once again, the treatments are color-coded with red representing vehicle control, green representing 0.03 mg/kg dofetilide, blue representing 0.10 mg/kg dofetilide, and purple representing 0.30 mg/kg dofetilide. The top plots showing the results of uncorrected QT act as negative controls. Without correction, the only significant result (represented by a lack of overlap between their standard error ribbon with that of vehicle control) for the lowest dose of dofetilide can be seen near hours 1, 2, and 3 for both groups. This is around when peak plasma concentration of dofetilide is likely occurring [175].

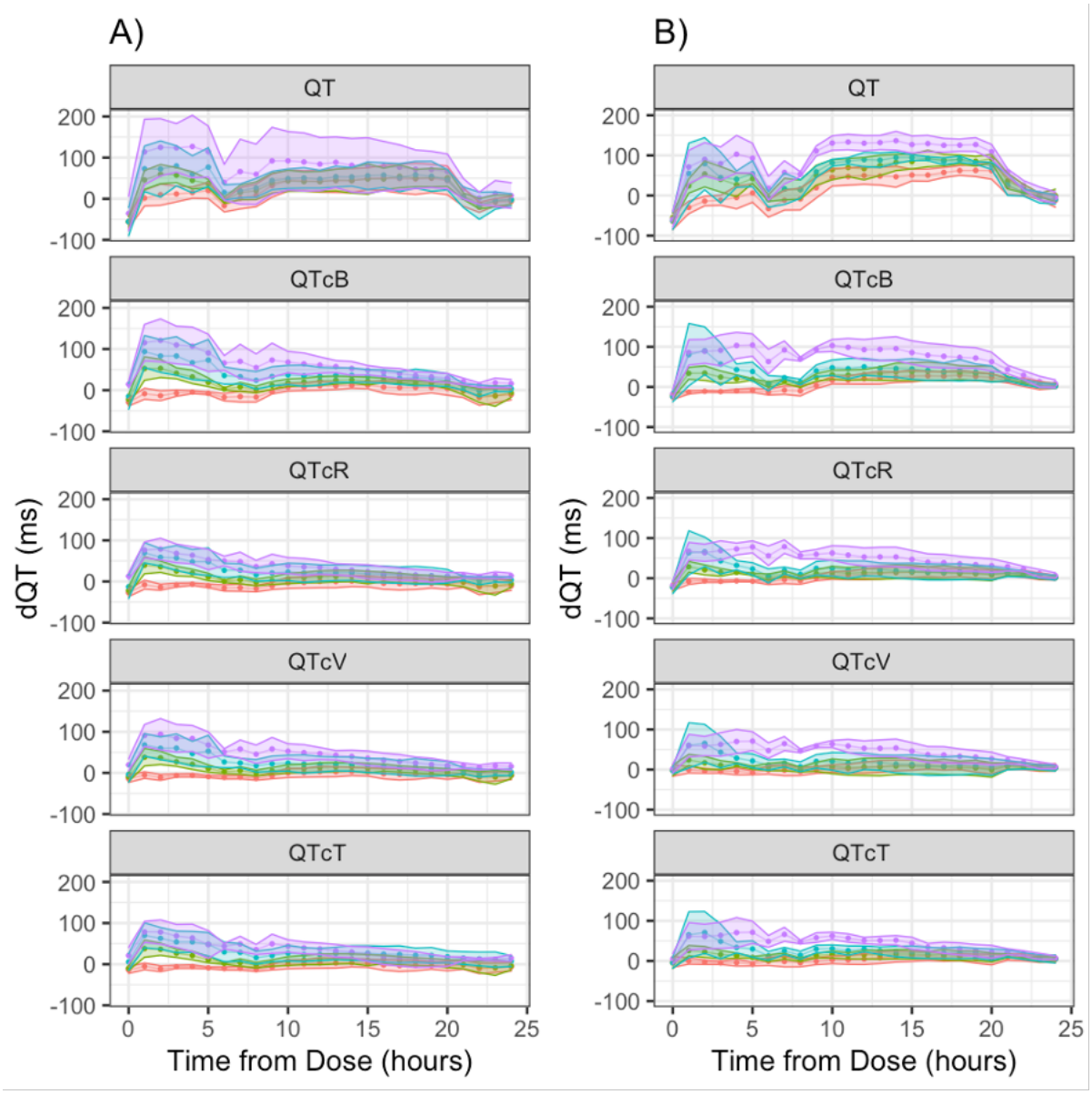

Figure 25: Method Comparison of QTc vs Time – Dofetilide Treated NHPs. These figures contain the 1-hour group means for the change in QT or QTc interval from baseline (dQT) over 24-hours from two (n=4) NHP groups treated with dofetilide. A) This column contains the results from the first dofetilide group. B) This column contains the results from the second dofetilide group. All subjects were age matched male NHPs dosed with both vehicle control (red), 0.03 mg/kg dofetilide treatment (green), 0.10 mg/kg dofetilide (blue), and 0.30 mg/kg dofetilide (purple). Data points and standard error of the mean ribbons are color-coded by treatment. The y-axis represents 60-minute mean change from a subject and week specific pre-treatment 120-minute mean value of either QT or QTc labeled at the top of the plot.

The error around the 0.10 mg/kg dose group is too large in both uncorrected QT plots to determine much significance, except for during the second hour in the 2nd dofetilide group. Meanwhile, the drug effect of the highest dofetilide treatment can be detected for hours 1 through 5 in both groups

without correction. The error from this treatment is too high in the first dose group to detect much else, but the second dose group remains significant for most of the day. These two uncorrected QT plots demonstrate a major difficulty encountered when trying to evaluate drug-induced QT prolongation: the variability of rate can often obscure the results through high error. Even in the 2nd group depicted in Figure 25B, though the error in this group may appear small enough to evaluate the drug-effect, there is no way to be certain that the effect being shown isn't the result of the drug acting on rate. The benefits of QT correction are demonstrated in the subsequent rows, with QTcB increasing the separation between dose results and reducing some of the error. However, this error is further reduced by QTcR, which maintains consistent error reduction throughout the whole treatment period for both groups. The individual correction methods (QTcV and QTcT) result in even more error reduction than QTcR past hour 5, but they are not able to reduce the error as much as QTcR in the first 5 hours containing the highest drug effect. The individual corrections also did not provide as much separation between the treatment results, causing QTcV to lose significance at hours 2 and 3 for the low-dose treatment in the 2nd group. Each plot demonstrated QT prolongation near 40-50ms at the Tmax of the 0.03mg/kg dose of dofetilide as expected, with the exceptions of the QTcV and QTcT methods in Figure 25B [175].

To make sure that exposure-dependent rate changes are not the cause of the QT prolongation detected, Figure 26 contains plots depicting the treatment effects on rate. These plots show the change in hourly mean RR from the pre-treatment baseline mean RR, over the course of the 24-hour post-treatment observations. Like the previous figure, data is color-coded by treatment and contains the data from both dofetilide study groups (n=4). Figure 26A has the data from the 1st dofetilide group, which shows no significant treatment effects on rate. Similarly, Figure 26B shows no significant treatment-related effects on rate for the 2nd dofetilide group, except for the high dose of dofetilide at hour 16.

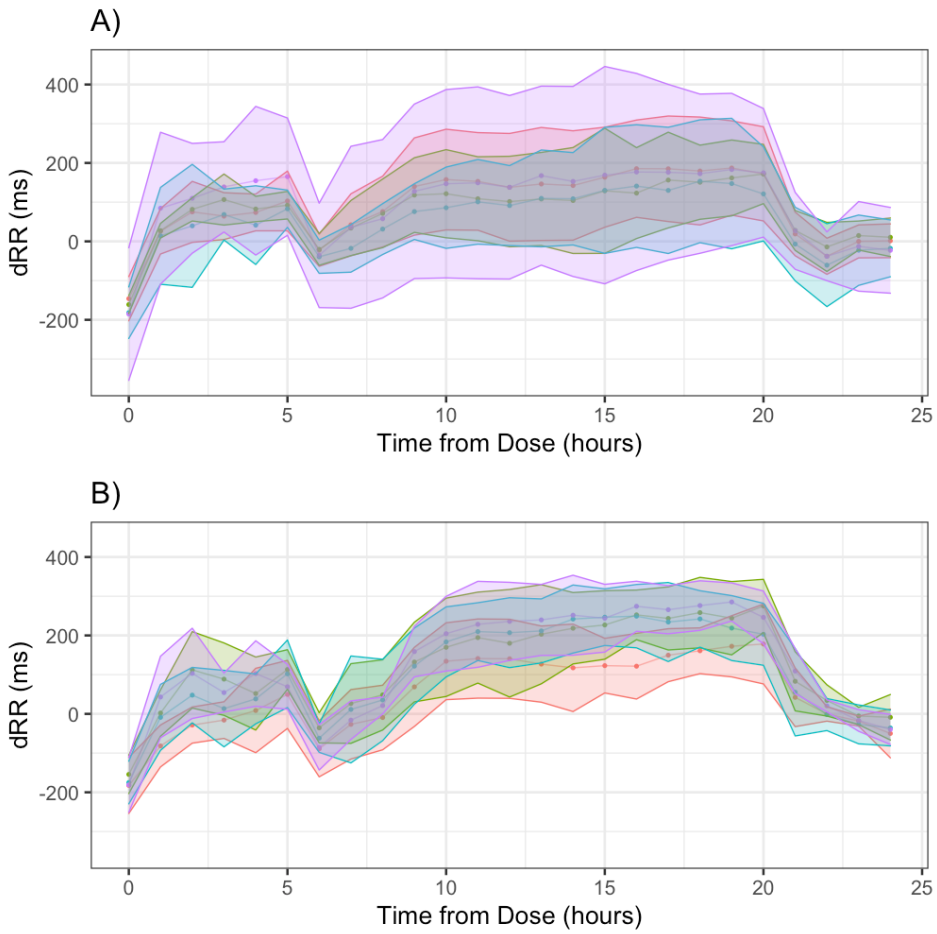

Figure 26: RR vs Time – Dofetilide Treated NHPs. These figures contain the 1-hour group means for the change in RR interval from baseline (dRR) over 24-hours from two (n=4) NHP groups treated with dofetilide. A) This plot contains the results from the first dofetilide group. B) This plot contains the results from the second dofetilide group. All subjects were age matched male NHPs dosed with both vehicle control (red), 0.03 mg/kg dofetilide treatment (green), 0.10 mg/kg dofetilide (blue), and 0.30 mg/kg dofetilide (purple). Data points and standard error of the mean ribbons are color coded by treatment. The y-axis represents 60-minute mean change from a subject and week specific pre-treatment 120-minute mean value of the RR interval.

The plots in Figure 27 are similar to those presented in Figure 25, except they contain the data from the two moxifloxacin treated NHP groups (n=4). Data points and standard error of the mean ribbons are color-coded so that red represents vehicle control and blue represents 80 mg/kg moxifloxacin. The column of plots in Figure 27A contain data from the 1st moxifloxacin group (subjects 1001m, 1002m, 1003m, and 1004m), while the column of plots in Figure 27B contain data from the 2nd moxifloxacin group (subjects 1005m, 1006m, 1007m, and 1008m). Data is

presented as hourly mean difference in QT from the 120-minute pre-treatment baseline mean QT (dQT) that has then been averaged by group.

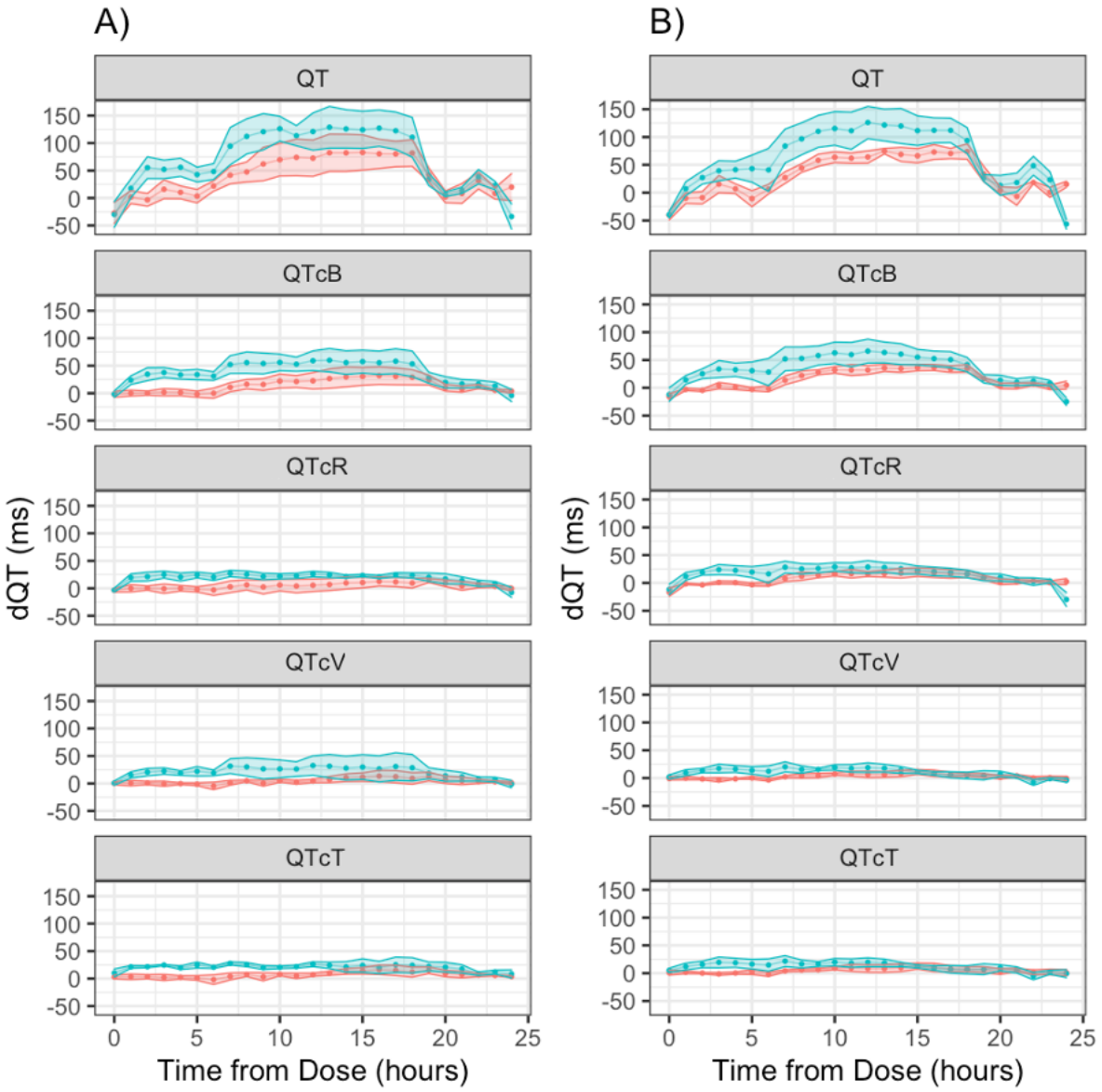

Figure 27: Method Comparison of QTc vs Time – Moxifloxacin Treated NHPs. These figures contain the 1-hour group means for the change in QT or QTc interval from baseline over 24-hours from two (n=4) NHP groups treated with moxifloxacin. A) This column contains the results from the first moxifloxacin group. B) This column contains the results from the second moxifloxacin group. All subjects were age matched male NHPs dosed with both vehicle control (red) or 80 mg/kg moxifloxacin (blue). Data points and standard error of the mean ribbons are color coded by treatment. The y-axis represents 60-minute mean change from a subject and week specific pre-treatment 120-minute mean value of either QT interval or the corrected QT labeled at the top of the plot. QT: raw QT interval, QTcB: Bazett's corrected interval, QTcR: Ratio corrected interval, QTcV: post-Vehicle treatment-based linear regression corrected interval, QTcT: post-On Treatment-based linear regression corrected interval.

Uncorrected QT, presented in the first plot of both Figures 27A and 27B, represents a worst-case scenario in which a treatment effect can be seen, but the significance of that effect cannot be accurately assessed, but sporadic significant treatment effect may be detected throughout the day. However, without correction a clearer understanding of this significance cannot be determined and the risk of the effect being the result of rate changes remains. Using the general correction QTcB increases the reliability of detecting drug-effect as it produced results that maintain dose separation while reducing error for both groups. Further improvements are provided by QTcR which had more error reduction than QTcB in both study groups. Like the results for the dofetilide groups, QTcR provided consistent error reduction throughout the study. Both linear regression methods, QTcV and QTcT, achieve similar or better error reduction when compared to QTcR (except for the QTcV results for the moxifloxacin treatment in Figure 27A). Though, their effectiveness at error reduction appears to fluctuate throughout the study period, unlike QTcR, showing potential bias towards higher plasma concentration or time periods while environmental lights were on. Each correction method provided similar significant separation of drug effect, with some variability in the later hours, though it is difficult to say which scenario is more accurate. The effect of moxifloxacin on the QT interval has been shown to reach a maximum QT prolongation around 28ms for NHPs treated with 90mg/kg, which reached maximum plasma concentration around hour 4, which is similar to the results from both study groups [176].

Evaluating the dQT vs Time plots in Figure 27 would be incomplete without also examining the effect treatment may have on rate. Figure 28 contains the change in hourly mean RR (dRR) from 120-minute pre-treatment baseline mean RRs, averaged by treatment and group, plotted over 24-hours. Both plots in this figure show distinct visual differences in dRR between the two treatments, however this difference is not consistently significant. Figure 28A, which contains the data from the 1st moxifloxacin group, only shows a significant difference at hour 3. Meanwhile, the data from the 2nd moxifloxacin group depicted in Figure 28B shows significant differences between

hours 7 and 12, at hour 22, and just barely at hour 5. These plots suggest that moxifloxacin may influence rate which, if not properly corrected for, may exaggerate measured QT prolongation.

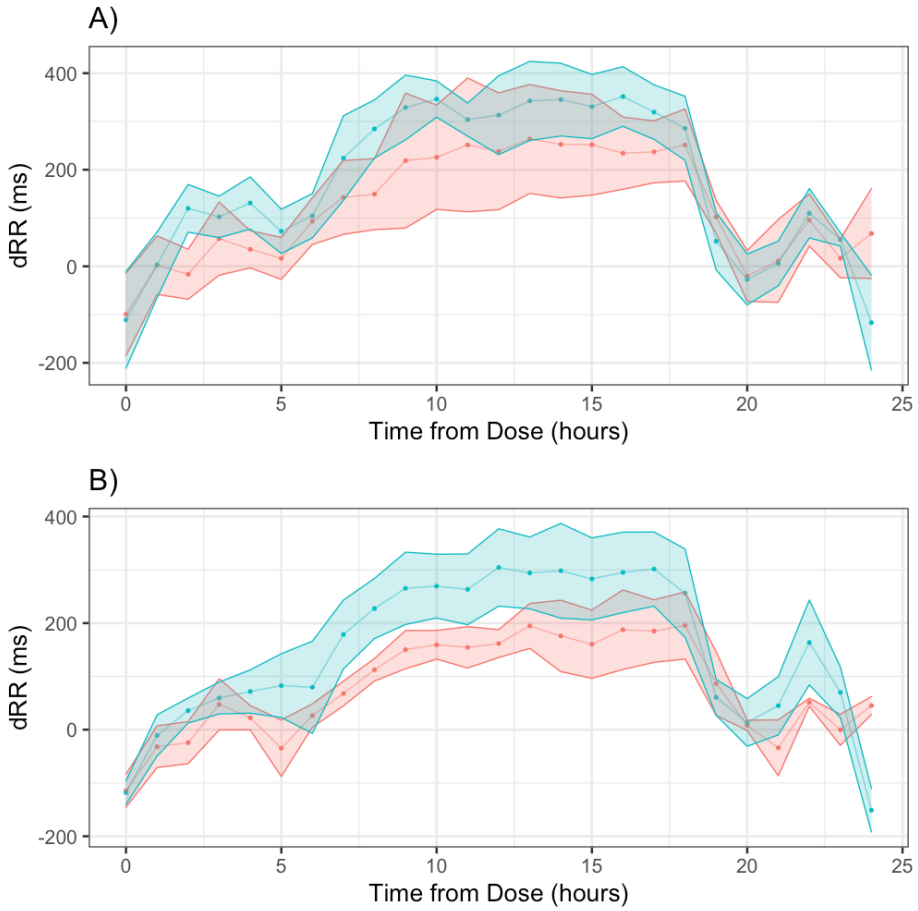

Figure 28: RR vs Time – Moxifloxacin Treated NHPs. These figures contain the 1-hour group means for the change in RR interval from baseline (dRR) over 24-hours from two (n=4) NHP groups treated with moxifloxacin. A) This plot contains the results from the first moxifloxacin group. B) This plot contains the results from the second moxifloxacin group. All subjects were age matched male NHPs dosed with both vehicle control (red) and 80 mg/kg moxifloxacin (blue). Data points and standard error of the mean ribbons are color coded by treatment. The y-axis represents 60-minute mean change from a subject and week specific pre-treatment 120-minute mean value of the RR interval.

Power Curve Comparisons

To compare the sensitivity of each QT correction method to identify treatment effect, the minimal detectable difference at a range of power levels was calculated using the residual standard error of an ANOVA comparing 24-hour pre-treatment baseline data for dofetilide groups 1 and 2 (Figures 29A and 29B, respectively) or 24-hour pre-study baseline data for the moxifloxacin

groups (Figures 29D and 29E, respectively). These plots contain each method as a color-coded curve that demonstrates the minimal detectable difference of QT (or QTc) on the x-axis at the corresponding power shown on the y-axis. Blue represents QTcT, purple represent QTcV, green represents QTcR, yellow represents QTcB, and red represents the uncorrected QT results. A horizontal dark blue dashed line was added to represent a power of 0.8, and the color-coded vertical lines dotted lines represent the minimal detectable difference of each method at that power. Each plot uses data from subjects that were not treated with a QT prolonging drug to compare how small of a theoretical effect can be found in four subjects based on the residual error present after QT correction. The data in Figure 29A are from the subjects in the 1st dofetilide group (1003d, 2003d, 3003d, and 4003d). In this plot QTcT resulted in a MDD of 10.8ms, followed by QTcV with a MDD of 13.8ms, then QTcR with a MDD of 14.5ms, and QTcB with a MDD of 19.5ms. Using uncorrected QT data for this group results in a MDD of 43.8ms for the worst-case scenario. Figure 29B contains data from the 2nd dofetilide group (subjects 1004d, 2004d, 3004d, and 4004d). This plot shows that uncorrected QT produced a MDD of 61.8ms, with QTcB again falling in last place of the correction methods with a MDD of 24.5ms. The remaining correction methods (QTcT, QTcV, and QTcR) all produced similar results (10.7ms, 11.0ms, and 11.3ms, respectively). The results from the 1st moxifloxacin group (1001m, 1002m, 1003m, and 1004m) shown in Figure 29C put QTcR in first place with a MDD of 9.9ms, just ahead of the MDD of 10.2ms for QTcT. QTcV lagged in these results with a MDD of 11.9ms but was well ahead of QTcB which had a MDD of 22.7ms. Of course, uncorrected QT acting as a negative control continues to do the worst with a MDD of 63.1ms. Subjects from the 2nd moxifloxacin group (1005m, 1006m, 1007m, and 1008m) were the ones with the substantially lower y-intercepts shown previously (Figure 24), so it is no surprise that QTcR did not perform as well Figure 29D using their data. QTcV has the lowest MDD in this plot (7.8ms), with QTcT in second (9.5ms). As expected, QTcR falls behind the two individual regression methods with a MDD of 15.6ms. Though it still outperformed QTcB and uncorrected QT which had MDD values of 29.6ms and

65.6ms respectively. In most of the plots of Figure 29, QTcR competes with the individualized linear regression methods, despite using a fixed variable like that of a general method. However, it does fall short when used for a group of subjects with y-intercepts substantially lower than the fixed value chosen.

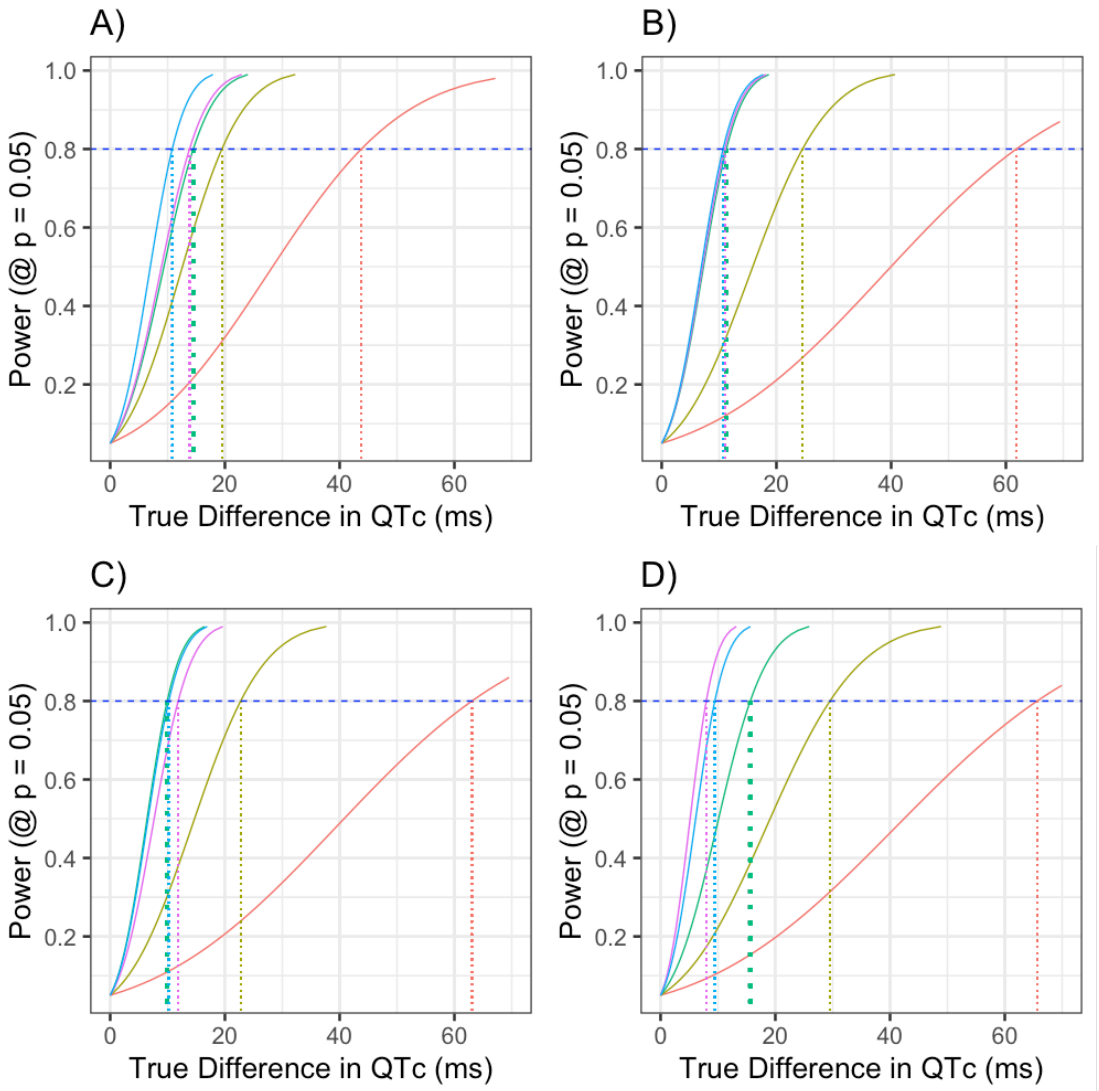

Figure 29: Correction Method Power Curves. Power curves comparing the ability of each method to detect a difference in QTc at a range of power values from 0.01 to 0.99. (A) curves for the first dofetilide group. (B) power curves for the second dofetilide group. (C) power curves for the first moxifloxacin group. (D) power curves for the second moxifloxacin group. Horizontal dashed lines highlight the results at the typical power of 0.80. Vertical lines represent the QTc difference that the corresponding method can detect at a power of 0.80. Each method is color-coded: blue represents the results of on-treatment linear correction (QTcT), purple represents vehicle treatment linear correction (QTcV), green represents the novel correction method (QTcR), yellow represents Bazett's correction (QTcB), and red represents the results of using uncorrected QT.

Discussion

We have developed the new Ratio QT correction method to address the need for a simpler QT correction method that provides improved correction of rate effect on QT, decreased variability of results, increased sensitivity to detect drug effect, and more dynamic response to changes in the QT-RR relationship. While drastically simpler in execution compared to individualized corrections based on linear regressions, our Ratio method does not rely on the assumptions made by traditional correction methods, which ignore variation in the rate relationship due to the subject, treatment, and/or temporal differences. Instead, relying on a fixed variable selected to approximate the y-intercept of the QT-RR relationship. Beyond these practical improvements offered by our method, we aimed to demonstrate the improvements in correction, variability, and sensitivity provided compared to other methods when applied to data from non-human primates treated with QT prolonging drugs (dofetilide or moxifloxacin). As expected, the general correction method (QTcB) resulted in greater variability and was consistently less effective at reducing the influence of rate on corrected QT compared to the other methods. This is unsurprising as it does not account for individual and treatment-related changes in the rate relationship and corrects based on a heart rate of 60 beats-per-minute instead of a rate more appropriate for the species. The Ratio method was more consistently effective in rate correction and error reduction than the general method (QTcB) and often matched those of the individualized linear regression method using a subject's vehicle treatment data (QTcV), with few exceptions. This novel method also produced similar results to the "on-treatment" method that used linear regression on post-treatment data (QTcT). In the time course profiles, the general method demonstrated greater variability (wider standard error ribbons) than the other methods and estimated a higher magnitude of treatment effect. The increased effect may be due to the general method correcting to an outlying rate, accentuating the magnitude of the estimated change. In the dofetilide treated groups, QTcR generally matched the shape of the three linear regression methods, though it provided more obvious separation between the treatment groups and improved reductions to the

error. For the moxifloxacin treated NHP groups, the QTcR again matched the shape and magnitude of the linear regression methods with similar error reduction. Most illuminating is the comparison of the power curves produced by each method. Using these, we were able to compare the sensitivity of each method to detect the treatment effect using a relatively small study group (n=4). With a study of this size, it is typically difficult to detect significant but small drug effects resulting in QT prolongation. While QTcT consistently resulted in one of the lowest MDD values, QTcV and QTcR weren't far behind in the dofetilide treated groups. QTcR was able to outperform QTcT in the 1st moxifloxacin treated group, while QTcV outperformed it in the 2nd. Meanwhile, QTcB consistently underperformed compared to the minimum results of the other methods. Our data suggest that, as expected, using a correction method performed better statistically than no correction at all, but the ability to correct for rate effects, reduce error, and increase specificity varied between methods. Accounting for the variability between subjects improves these results as shown by the Ratio and linear regression methods consistently outperforming the general Bazett's correction. The ability of linear regression models to improve correction is expected due to their characterizations of the QT-rate relationship being corrected, and the benefits of this have been previously demonstrated [129,132,134,174]. In this study, we have examined the ability of the Ratio method of QT correction to provide similar, and in some cases better, results to those of the linear regression-based methods. This is made possible without the need for a computationally intensive regression or the assumptions that go along with them.

Limitations

After testing these methods with four (n=4) NHP groups treated with dofetilide or moxifloxacin, the QTcR method appears to be a promising new method that can be used to correct QT during drug treatments. However, this was a *post-hoc* analysis and may not be able to control for variables as well as a prospective study. Ad hoc studies designed to compare the effectiveness of these methods on multiple species and drug treatments are still necessary. It is also important to note that any correction method relying on an accurate characterization of the relationship

between QT and rate will require a wide range of values present in the subject's data to be effective. Subjects that exhibit abnormally narrow ranges of rates at night or in general may negatively affect study results and should be considered for replacement. Additionally, there may be further improvements that can be made by updating our correction methods. For example, species-specific reference rates (RefRR) and y-intercepts used as part of the correction formulae are not intended to act as the definitive variables, as they were determined based on a limited sample population. As the results of this study have shown, when the data being corrected has a y-intercept value substantially different from the fixed value selected, the QTcR method may under or over correct the data. This may be improved by using a study specific value selected based on the baseline recordings of the subjects included on a study. In our experience, the results of the QTcR method can be further improved by using individual- and/or treatment-specific y-intercepts. However, these improvements to the accuracy would also greatly increase the complexity of the model and contradicts the goal of developing a simple, accurate, and flexible correction method. Similar to a general correction method like Bazett's, QTcR relies on a mathematical constant determined based on the characterization of a population and the ability of that characterization to represent the rate relationship of the subject or treatment being corrected will determine the effectiveness of the correction. Though, unlike other general correction methods, QTcR can dynamically adapt to moment-to-moment changes in the rate relationship, which may be how it performed so well in this study despite this limitation.

Future Directions

This study aimed to demonstrate the utility of a new QT correction method by comparing results directly to those of other methods. Ideally, all the methods would be compared against a characterizing set of data to compare their accuracy in predicting that characteristic. One possibility is to compare their ability to produce results that accurately align with pharmacokinetic time courses. If the concentration-dependent effect of treatment on QTc is due to a concentration-dependent effect on the slope of the QT-RR relationship, then it is necessary that the rate

correction method can be applied in very narrow time windows, which is impractical for regression-based methods. The Ratio method is more instantaneous and dynamic and may be a promising method for integration with concentration-QTc analysis. In addition to testing the Ratio method's ability to correct QT, we will explore the capability of using the Ratio alone for detecting significant drug effects without correcting them back to a QT value. All future research must also aim to evaluate the abilities of the Ratio method in additional species and in the presence of heart rate modulating drugs.

Conclusion

While there are already industry preferred methods of QT correction, we believe that the comparisons presented in this study demonstrates that the QT Ratio method consistently provides improved QT correction and increased sensitivity of detecting treatment effects compared to traditional correction methods and similar to the computationally advanced QTcT method, which has yet to be adopted. It can provide these benefits without relying on pre-treatment baseline data or overarching characterizations of the treatment data like regression-based methods. Additionally, the ability of the Ratio method to respond to moment-to-moment changes in the QT-rate relationship by estimating the slope of the relationship at each datapoint makes it an excellent option to address the hysteresis of drug effect for implementation with plasma concentration-based corrections. These factors, combined with its simplicity, ease of use, and cross-species potential, support the new Ratio method as a promising alternative for future preclinical cardiovascular safety studies. One that furthers our understanding of QT correction methods along the path towards more translatable preclinical study results.

CHAPTER 5:

SUMMARY, NOVEL FINDINGS, LIMITATIONS, FUTURE DIRECTIONS, AND FINAL PERSPECTIVES

Summary

It has not been long since physicians and their patients had to worry about non-cardiovascular pharmaceuticals unexpectedly inducing arrhythmias like *torsade de pointes* [55]. This polymorphic ventricular tachycardia could devolve into ventricular fibrillation, increasing the risk of sudden cardiac death [62]. In the first part of chapter 1 I presented a historical overview of drug induced TdP risk and use terfenadine as an example of how our understanding of these drugs have evolved. I detailed the electrophysiological mechanisms of TdP risk, most commonly stemming from inhibition of K^+ current through hERG-encoded ion channel I_{Kr} [110]. This disruption to ion channels essential to cardiomyocyte repolarization prolongs their action potential duration, which in turn disrupts and prolongs the repolarization period of the ventricles [5]. Disruptions to cardiac conduction such as this are identified through changes to the ECG, specifically prolongation of the QT interval [177]. This QT-prolongation has been found to be a prerequisite to most observed TdP events [10].

In the second part of chapter 1 I discussed the evolution of safety pharmacology to assess the TdP risk of novel pharmaceutical compounds based on their effect on QT. This process began in 1997 when the CPMP released Note CPMP/986/96; 'Points to Consider: The Assessment of the Potential for QT Interval Prolongation by Non-cardiovascular Medicinal Products' [43]. These suggestions were replaced in 2005 with the ICH clinical E14 and preclinical S7B guidelines, which outlined methods for assessing TdP risk during drug-development based on QT-prolongation and hERG inhibition [44,45]. A vital aspect of these assessments is that researchers control for the influence heart rate has on QT by using QT correction methods to isolate potential drug effect. These correction methods vary based on the populations used to derive them and the assumptions they make about the variability in the QT-rate relationship [124]. General correction methods are simple formulae dependent on fixed correlation constants derived from historical, usually human, populations [178]. Such methods rely on the assumption that these populations sufficiently represent the QT-rate relationship of the individual, and that this relationship will not

vary between subjects, occasions, or treatments. Alternatively, individual QT correction methods typically rely on a regression to determine the relationship between QT and rate present in an individual's ECG recording [129]. Such methods are designed to address the variability between subjects and can even be designed to address variability between occasions and/or treatments. However, the individual correction does rely on the assumption that pre-determining this relationship will be appropriate for each timepoint and won't interfere with the detection of drug effect on the relationship directly.

After introducing these QT correction methods, I concluded the first chapter by discussing the safety assessments proposed by the ICH and adopted by the FDA [137]. Specifically, how these safety assessments could still improve. Though they were successful at preventing the approval of any drugs with unacceptable TdP risk, their high sensitivity and suspected low specificity has been criticized for potentially preventing safe and useful pharmaceuticals from reaching the market. In addition to these criticisms, pharmaceutical companies called for the integration of preclinical and clinical studies [139]. They believed that preclinical results were not being utilized efficiently enough, forcing them to proceed with expensive and lengthy clinical studies even if there is no preclinical evidence of risk. In response to criticism and requests for clarification, the ICH released the E14/S7B Q&As in 2022, which fell short of fully integrating the two realms of safety pharmacology [113]. However, these Q&As did provide much needed clarification on the best practices in terms of selecting QT correction methods. They recommended individual correction methods to address the variability in the QT-rate relationship and described methods to justify the QT correction method selected for a study. Suggestions included demonstrating the reduced correlation between QTc and rate, improvements to variability of results, and increased sensitivity in drug effect. The remainder of this dissertation was dedicated to using these suggestions to identify potential improvements to QT correction methods to improve their reliability, reduce the number of subjects needed, and increase the likelihood of further integrating preclinical and clinical safety assessments. I sought to achieve these improvements and further

our understanding of QT corrections through three specific aims. The first aim was intended to compare the assumptions inherent to QT correction methods and how they affect detection of relevant drug-induced QT prolongation. This was explored in the second chapter, which provided simplified demonstrations of how various correction methods function, then compared them in the presence of three simulated drug treatments that acted on the heart rate, QT, or the QT-rate relationship directly in a linear manner. The second aim was to evaluate factors contributing to variability in the QT-rate relationship. In the third chapter I tested the effectiveness of general and individual correction methods by applying them to thousands of computationally bootstrap generated ECG recordings based on real non-human primate and beagle canine data. The third aim was designed to create and evaluate a novel QT correction method using drug-treatment data. This was done using ECG data from safety studies performed on non-human primates treated with dofetilide or moxifloxacin.

Novel Findings

My studies demonstrate the importance of selecting an appropriate QT correction method that accounts for variability in the QT-rate relationship. Through this research I have furthered our understanding of the assumptions inherent to various methods and demonstrated ways they may be improved. Additionally, I am the first to present a novel QT correction method that merges the benefits of general correction with the adaptability of individual correction. The findings in this dissertation were as follows:

In Chapter 2: Understanding QT Correction Methods we found

1. Individual QT correction methods can detect drug effect directly applied to QT and controlling for the effects of heart rate modulating drugs, but struggle to reduce the variability associated with drug effects on the QT-rate relationship.
2. The novel Ratio method of QT correction can control for the effects of heart rate modifying drugs and detect drug effects directly applied to QT, with additional variability due to over-

estimating some drug effect. However, this method was able to perfectly control for the variability of drug effect on the QT-rate relationship.

3. The general Bazett's QT correction method misidentified heart rate change as relevant drug-induced QT prolongation and was unable to minimize the variability of any treatment.

Taken together, these results indicate that dynamic correction methods like the individual or Ratio methods will provide more reliable results. Additionally, they support the importance of understanding the assumptions made by each method. We propose that both individual methods that assume the slope of the QT-rate relationship before correction may be used in parallel with the Ratio method that instead assumes to y-intercept of the relationship. This would allow for a better understanding of drug-effect, provide a reliable dynamic method for reference during the study, and allow better trust in the results.

In Chapter 3: Identifying Uncontrolled Factors that Influence QT Correction we found

1. On-treatment individual QT correction is more effective than an individual method utilizing baseline data.
2. The QT-rate relationship varies between environmental light status, and accounting for this change may improve QT correction methods.
3. The QT-RR relationship in beagle canines is less linear than in non-human primates, which can benefit from the use of logarithmic regression instead of linear.

Taken together, this data suggests that QT correction method improvement is highly dependent on how many sources of QT-rate relationship variability are accounted for. We propose that individual correction methods can be improved if they utilize on-treatment data and account for differences stemming from environmental light status.

In Chapter 4: Comparing the Novel Ratio Correction Method to General and Individual Methods Using ECG Data from Non-Human Primates Treated with QT Prolonging Drugs we found

1. The QT-rate relationship can vary between occasions (intra-subject) as much as it varies between subjects (inter-subject), which is further confounded by drug treatment.

2. The general Bazett's correction underperformed in all regards compared to the individual or Ratio methods.
3. The individual correction methods provided the most reduction in the relationship between QTc and RR, though the Ratio method produced similar results.
4. The Ratio method produced the most consistent reduction in variability of all the methods, providing the lowest variability during peak drug effect, while the individual methods resulted in the lowest variability later in the ECG recording.
5. All methods could detect relevant drug effect in all drug treatments, including the novel Ratio method and the rarely used on-treatment individual correction.
6. The Ratio method provided sensitivity on par or better than individual correction methods in all but one study group, likely due to the abnormally low y-intercept values common in that group.

Taken together, this data supports the benefits of controlling for intra-subject variability in the QT-rate relationship. They also suggest that the novel Ratio correction method can provide similar results to individual correction methods without the need for computationally demanding regressions performed on each subject *post-hoc*. It is our opinion that on-treatment individual correction is better than an individual correction method limited to a single ECG recording. Additionally, the Ratio method warrants further testing and should eventually be used in parallel with other correction methods.

Limitations

Some limitations in the methods used must be addressed, most importantly the nature of this analysis. All research was done *post-hoc*, long after the data had been collected. This severely limits my ability to plan for and control environmental factors that may influence the results. These factors include the temperature of the housing room, the time-of-day treatment is administered, how often someone enters the room, what endpoints are collected, and how long before treatment subjects are observed. Having greater control over these variables would have made it feasible

to characterize more factors that affect the corrected QT results. It is my hope that this research encourages others to consider how some of these variables might affect their results. Applying some of the methods and results presented here during *ad-hoc* development of safety pharmacology studies would help greatly improve our understanding of QT correction methods.

Additionally, the limited nature of the data available for these studies must be addressed. I attempted to supplement this limitation using computationally simulated data and bootstrap extrapolated variations of ECG recordings. These practices have clear benefits in exploring the abilities of QT correction methods, but they are no replacement for a more diverse and robust set of original data. In chapter 3 I used data from both non-human primates and canines, with male and female subjects equally represented. However, that data was limited to vehicle control treatments. When the investigation was expanded to include drug treatment data, only male non-human primate subject data was readily available. While we saw limited differences between species and sex in the study presented in chapter 3, this must still be confirmed with drug treatment data. This data was also limited to two known QT-prolonging drugs, which prevents completely blind analysis and the testing of method effectiveness for heart rate modulating drugs.

While the basis of this research was to explore the limitations of various QT correction methods, some vital limitations warrant emphasis. For individual correction methods utilizing regression to characterize the QT-rate relationship, the number and variety of datapoints is critical. Should not a long enough period be used in determining the relationship, outliers may disproportionately affect the determined relationship. Similarly, if to narrow a range of heart rates is presented in the data used, the resulting relationship estimate may not appropriately represent the data during typical or unexpected changes to rate. While the studies included in this dissertation attempted to account for these limitations, the influence of these limitations must always be kept in mind. The Ratio method presents a different set of limitations that must be considered due to its reliance on a “species specific y-intercept” value. This was predetermined based on medians and means of y-intercept values from many subjects. However, there will always be outliers for which the 100

ms selected will not accurately reflect their individual QT-rate relationship. This is an expected limitation because of our attempt to create a method that benefited from the ease provided by general methods while providing the dynamic responsiveness to QT-rate relationship variability like an individual method. Overcoming this limitation by determining individual-specific y-intercept values is possible but would remove the many benefits provided by not relying on such complex *post-hoc* assessments. Though using a more individualized version of the Ratio method would still benefit from not assuming a single relationship slope prior to correction, maintaining the dynamic moment-to-moment response to environmental and drug effects on the QT-rate relationship.

Ultimately, the goal of improving these QT correction methods is to improve the translatability of preclinical investigations to clinical observations. We tried to investigate the use of clinically reported adverse events as a way of characterizing clinical drug risk, which could be used to validate preclinical results [179]. Unfortunately, there were too many confounding factors involved with the clinical determination of TdP drug risk and no industry standard agreed upon for validating the results of our attempt. This means that the best way to support benefit to translatability that improvements to correction methods provide is to compare them directly to clinically collected safety study data. Such data was not available for use in this study but should be included in future investigations.

Future Directions

To continue these investigations into improvements to QT correction methods, the methods used in these studies must be expanded and applied to a larger variety of data. Most immediately pressing is the confirmation of these results using data from the other preclinical animal species most associated with these safety pharmacology studies, beagle canines. Their less linear relationship between QT and RR requires the use of their more linear QT and heart rate relationship. This could provide unique challenges to correction methods investigated. Using a wider variety of drug treatments is also a necessary part of the future of this research. Correction

methods must be able to differentiate between risk-relevant QT prolongation and relatively safe prolongation. This requires testing with heart rate modulating drugs that do not affect QT, QT prolonging drugs in the presence of heart rate modulation, and QT prolonging drugs that aren't associated with TdP risk. Eventually this work must be extended to include clinical human data as well. Future studies should include equal representation of the sexes to confirm consistent efficacy of the individual and Ratio methods.

The research presented in this dissertation is intended to be an introductory foundation to these considerations and our novel correction method. In the future we intend to work with the pharmaceutical industry and regulators to encourage the adoption of correction methods that better address the intra- and inter-subject variability of the QT-rate relationship. We will pursue this through informational publications and media, instructional presentations at conferences, and continued communication with contacts in the industry. Achieving widespread understanding of the considerations explored in this research and the adoption of some of these methods would speed up the optimization of QT correction methods exponentially.

Once these QT correction methods are more thoroughly tested, their integration with pharmacokinetic and pharmacodynamic models can be further explored. For example, incorporating plasma concentration timepoints into models utilizing these correction methods may be able to better isolate drug effect on QT. Improvements in this space require a better understanding of the hysteresis between rate change and QT change as well as between plasma concentration and QT change. A moment-to-moment dynamic correction method like the Ratio method might provide unique benefits to such studies, which warrants future investigation.

Ideal Outcomes

The motivation to optimize QT correction methods stems from the hypothesis that it is possible to develop one which bridges the gap between physician and researcher. I believe a method that works across species and individuals on a moment-to-moment basis is possible given enough understanding of the underlying mechanisms of the QT-rate relationship and continued

improvements in our ability to correct for that relationship. Such an ideal method would be useful in any context, whether it be a researcher performing a pre-clinical cardiovascular safety study or a physician monitoring a patient taking a QT prolonging drug. The QT Ratio method is one step towards that goal. Currently, this novel method is designed to work with population-specific reference variables out of convenience of adoption. This approach makes it easy for a researcher or physician to correct a single observation of QT with simple algebra. However, the Ratio method can be further improved through the use of individual-specific variables. By individualizing the method, a physician could correct a patient's QT value in real-time to a heart rate and y-intercept point appropriate to them. Such an improvement would require updates to ECG monitoring devices and electronic medical records to analyze and record an average heart rate and intercept value for a patient. These values could automatically be refreshed and recorded to the patient's electronic medical records as more ECG data is collected. If these systems can be updated for this functionality, an individualized correction method could be used in real-time by physicians as easily as a general method, as they will have access to the most recent estimate of these variables along with a history of their association with developing pathologies. In the interim, the novel Ratio method using population-specific values is still capable of QT correction similar to an individual method. Its simplicity and moment-to-moment determination of the QT-rate relationship offered by this method allow it to be adapted for any species or subject with further investigations. By pursuing a method capable of this, a standardized correction method may be possible for most scenarios. This may improve translatability by limiting the differences in assumptions relied upon between studies and species.

Final Perspectives

The safety assessments encouraged in the E14 and S7B guidelines provided by the ICH and adopted by the FDA have been very successful at preventing drugs with unacceptable risk of TdP from entering the market [139]. However, the high sensitivity and questionable specificity of these assessments, along with their limited integration between preclinical and clinical investigations,

have prompted pharmaceutical companies to question how much time, money, and safe drug candidates have been unnecessarily lost [146]. Addressing these concerns requires increased trust in the results obtained during preclinical safety pharmacology studies. In this dissertation I have explored one avenue of improving the reliability of these results by optimizing the QT correction methods integral to these assessments. I have explored a variety of factors that can negatively impact the reliability of corrected QT values. These factors include how a drug is influencing QT prolongation, the inter-subject variability in the QT-rate relationship as well as its variability between occasion, treatment, and environmental light status. Most importantly, this research emphasizes the need to understand the assumption being made by the QT correction method selected and how those assumptions can confound results. The industry has already begun adopting individual QT correction methods to address inter-subject variability, but my results indicate that taking this one step further by utilizing on-treatment individual correction may be ideal. I also introduce the novel Ratio method of QT correction, which is designed to provide an alternative to the computationally demanding individual corrections while providing similar effectiveness thanks to its dynamic response to changes in the QT-rate relationship. Overall, this work has increased the understanding of how the assumptions and techniques of QT correction methods may affect study results, provided new options for QT correction, and acted as a necessary step towards optimizing QT correction to increase the chance of preclinical and clinical study integration.

LITERATURE CITED

LITERATURE CITED

1. Stecker EC, Reinier K, Marijon E, Narayanan K, Teodorescu C, Uy-Evanado A, et al. Public health burden of sudden cardiac death in the United States. Circ Arrhythm Electrophysiol. 2014;7: 212–217. doi:10.1161/CIRCEP.113.001034

2. Kumar A, Avishay DM, Jones CR, Shaikh JD, Kaur R, Aljadah M, et al. Sudden cardiac death: epidemiology, pathogenesis and management. Rev Cardiovasc Med. 2021;22: 147–158. doi:10.31083/j.rcm.2021.01.207

3. European Heart Rhythm Association, Heart Rhythm Society, Zipes DP, Camm AJ, Borggrefe M, Buxton AE, et al. ACC/AHA/ESC 2006 guidelines for management of patients with ventricular arrhythmias and the prevention of sudden cardiac death: a report of the American College of Cardiology/American Heart Association Task Force and the European Society of Cardiology Committee for Practice Guidelines (Writing Committee to Develop Guidelines for Management of Patients With Ventricular Arrhythmias and the Prevention of Sudden Cardiac Death). J Am Coll Cardiol. 2006;48: e247-346. doi:10.1016/j.jacc.2006.07.010

4. Markwerth P, Bajanowski T, Tzimas I, Dettmeyer R. Sudden cardiac death-update. Int J Legal Med. 2021;135: 483–495. doi:10.1007/s00414-020-02481-z

5. Kuriachan VP, Sumner GL, Mitchell LB. Sudden cardiac death. Curr Probl Cardiol. 2015;40: 133–200. doi:10.1016/j.cpcardiol.2015.01.002

6. Macwilliam JA. Some applications of physiology to medicine: ii.-ventricular fibrillation and sudden death. Br Med J. 1923;2: 215–219.

7. Dessertenne F. [Ventricular tachycardia with 2 variable opposing foci]. Arch Mal Coeur Vaiss. 1966;59: 263–272.

8. Krikler DM, Curry PV. Torsade De Pointes, an atypical ventricular tachycardia. Br Heart J. 1976;38: 117–120.

9. Lazzara R. Twisting of the points. J Am Coll Cardiol. 1997;29: 843–845. doi:10.1016/s0735-1097(96)00589-x

10. Neal Kay G, Vance J. P, Joaquin G. A, Richard W. H, Albert L. W. Torsade de pointes: The long-short initiating sequence and other clinical features: observations in 32 patients. J Am Coll Cardiol. 1983;2: 806–817. doi:10.1016/S0735-1097(83)80226-5

11. Napolitano C, Priori SG, Schwartz PJ. Torsade de pointes. Mechanisms and management. Drugs. 1994;47: 51–65. doi:10.2165/00003495-199447010-00004

12. Vos MA, van Opstal JM, Leunissen JD, Verduyn SC. Electrophysiologic parameters and predisposing factors in the generation of drug-induced Torsade de Pointes arrhythmias. Pharmacol Ther. 2001;92: 109–122.

13. Roden DM, Thompson KA, Hoffman BF, Woosley RL. Clinical features and basic mechanisms of quinidine-induced arrhythmias. J Am Coll Cardiol. 1986;8: 73A-78A. doi:10.1016/s0735-1097(86)80032-8

14. Roden DM, Woosley RL, Primm RK. Incidence and clinical features of the quinidine-associated long QT syndrome: implications for patient care. Am Heart J. 1986;111: 1088–1093. doi:10.1016/0002-8703(86)90010-4

15. Bauman JL, Bauernfeind RA, Hoff JV, Strasberg B, Swiryn S, Rosen KM. Torsade de pointes due to quinidine: observations in 31 patients. Am Heart J. 1984;107: 425–430. doi:10.1016/0002-8703(84)90081-4

16. Jenzer HR, Hagemeijer F. Quinidine syncope: torsade de pointes with low quinidine plasma concentrations. Eur J Cardiol. 1976;4: 447–451.

17. Nguyen PT, Scheinman MM, Seger J. Polymorphous ventricular tachycardia: clinical characterization, therapy, and the QT interval. Circulation. 1986;74: 340–349. doi:10.1161/01.cir.74.2.340

18. Fowler NO, McCall D, Chou TC, Holmes JC, Hanenson IB. Electrocardiographic changes and cardiac arrhythmias in patients receiving psychotropic drugs. Am J Cardiol. 1976;37: 223–230. doi:10.1016/0002-9149(76)90316-7

19. Guelon D, Bedock B, Chartier C, Haberer JP. QT prolongation and recurrent “torsades de pointes” during erythromycin lactobionate infusion. Am J Cardiol. 1986;58: 666. doi:10.1016/0002-9149(86)90306-1

20. Kilani F, Marsepoil T. Accès de torsades de pointes induits par une injection intraveineuse de lactobionate d’érythromycine. Annales Françaises d’Anesthésie et de Réanimation. 1988;7: 270–271. doi:10.1016/S0750-7658(88)80126-6

21. Kelly HG, Fay JE, Laverty SG. Thioridazine hydrochloride (mellaril): its effect on the electrocardiogram and a report of two fatalities with electrocardiographic abnormalities. Can Med Assoc J. 1963;89: 546–554.

22. Schoonmaker FW, Osteen RT, Greenfield JC. Thioridazine (mellaril)-induced ventricular tachycardia controlled with an artificial pacemaker. Ann Intern Med. 1966;65: 1076–1078. doi:10.7326/0003-4819-65-5-1076

23. Schoenenberger RA, Haefeli WE, Weiss P, Ritz RF. Association of intravenous erythromycin and potentially fatal ventricular tachycardia with Q-T prolongation (torsades de pointes). BMJ. 1990;300: 1375–1376. doi:10.1136/bmj.300.6736.1375

24. Kuo CS, Munakata K, Reddy CP, Surawicz B. Characteristics and possible mechanism of ventricular arrhythmia dependent on the dispersion of action potential durations. Circulation. 1983;67: 1356–1367. doi:10.1161/01.cir.67.6.1356

25. Dickhuth HH, Bluemner E, Auchschwelk W, Zehnder M, Irmer M, Meinertz T. The relationship between heart rate and QT interval during atrial stimulation. Pacing Clin Electrophysiol. 1991;14: 793–799. doi:10.1111/j.1540-8159.1991.tb04109.x

26. Guth BD. Preclinical cardiovascular risk assessment in modern drug development. Toxicol Sci. 2007;97: 4–20. doi:10.1093/toxsci/kfm026

27. Buss J, Neuss H, Bilgin Y, Schlepper M. Malignant ventricular tachyarrhythmias in association with propafenone treatment. Eur Heart J. 1985;6: 424–428. doi:10.1093/oxfordjournals.eurheartj.a061881

28. Lathers CM, Lipka LJ. Chlorpromazine: cardiac arrhythmogenicity in the cat. Life Sci. 1986;38: 521–538. doi:10.1016/0024-3205(86)90031-7

29. Hoffman BF, Dangman KH. The role of antiarrhythmic drugs in sudden cardiac death. J Am Coll Cardiol. 1986;8: 104A-109A. doi:10.1016/s0735-1097(86)80036-5

30. Kavanagh KM, Wyse DG. Ventricular arrhythmias. CMAJ. 1988;138: 903–913.

31. Faber TS, Zehender M, Just H. Drug-induced torsade de pointes. Incidence, management and prevention. Drug Saf. 1994;11: 463–476. doi:10.2165/00002018-199411060-00007

32. Singh BN, Hollenberg NK, Poole-Wilson PA, Robertson JI. Diuretic-induced potassium and magnesium deficiency: relation to drug-induced QT prolongation, cardiac arrhythmias and sudden death. J Hypertens. 1992;10: 301–316. doi:10.1097/00004872-199204000-00001

33. Adamantidis MM, Kerram P, Dupuis BA. In vitro electrophysiological detection of iatrogenic arrhythmogenicity. Fundam Clin Pharmacol. 1994;8: 391–407. doi:10.1111/j.1472-8206.1994.tb00818.x

34. Roden DM. Taking the “idio” out of “idiosyncratic”: predicting torsades de pointes. Pacing Clin Electrophysiol. 1998;21: 1029–1034. doi:10.1111/j.1540-8159.1998.tb00148.x

35. Nolan PE. Pharmacokinetics and Pharmacodynamics of Intravenous Agents for Ventricular Arrhythmias. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 1997.

36. Oberg KC, Bauman JL. QT Interval Prolongation and Torsades de Pointes Due to Erythromycin Lactobionate. Pharmacotherapy: The Journal of Human Pharmacology and Drug Therapy. 1995.

37. Paris DG, Parente TF, Bruschetta HR, Guzman E, Niarchos AP. Torsades de pointes induced by erythromycin and terfenadine. Am J Emerg Med. 1994;12: 636–638. doi:10.1016/0735-6757(94)90029-9

38. Kwang Kon Koh, Min Seon Rim, Jin Yoon, Sam Soo Kim. Torsade de pointes induced by terfenadine in a patient with long QT syndrome. J Electrocardiol. 1994;27: 343–346. doi:10.1016/S0022-0736(05)80274-2

39. Feenstra J, Grobbee DE, Remme WJ, Stricker BH. Drug-induced heart failure. J Am Coll Cardiol. 1999;33: 1152–1162. doi:10.1016/s0735-1097(99)00006-6

40. Malik M, Camm AJ. Evaluation of drug-induced QT interval prolongation: implications for drug approval and labelling. Drug Saf. 2001;24: 323–351. doi:10.2165/00002018-200124050-00001

41. De Ponti F, Poluzzi E, Cavalli A, Recanatini M, Montanaro N. Safety of non-antiarrhythmic drugs that prolong the QT interval or induce torsade de pointes: an overview. Drug Saf. 2002;25: 263–286. doi:10.2165/00002018-200225040-00004

42. Lester RM, Paglialunga S, Johnson IA. QT assessment in early drug development: the long and the short of it. Int J Mol Sci. 2019;20. doi:10.3390/ijms20061324

43. Crumb W, Cavero I. QT interval prolongation by non-cardiovascular drugs: issues and solutions for novel drug development. Pharm Sci Technol Today. 1999;2: 270–280.

44. Anonymous. ICH S7B: The non-clinical evaluation of the potential for delayed ventricular repolarization (QT interval prolongation) by human pharmaceuticals. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 2005.

45. Anonymous. ICH E14: The clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non- antiarrhythmic drugs. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 2005.

46. Barlow JL, Beitman RE, Tsai TH. Terfenadine, safety and tolerance in controlled clinical trials. Arzneimittelforschung. 1982;32: 1215–1217.

47. Woodward JK, Munro NL. Terfenadine, the first non-sedating antihistamine. Arzneimittelforschung. 1982;32: 1154–1156.

48. Carter CA, Wojciechowski NJ, Hayes JM, Skoutakis VA, Rickman LA. Terfenadine, a nonsedating antihistamine. Drug Intell Clin Pharm. 1985;19: 812–817. doi:10.1177/106002808501901103

49. Albengres E, Le Louët H, Tillement JP. Systemic antifungal agents. Drug interactions of clinical significance. Drug Saf. 1998;18: 83–97. doi:10.2165/00002018-199818020-00001

50. Simons FER, editor. Potential Cardiac Toxicity of H1-Antihistamines. Histamine and H1-Antihistamines in Allergic Disease. CRC Press; 2002. pp. 405–436. doi:10.3109/9780203910375-17

51. Woosley RL. Cardiac actions of antihistamines. Annu Rev Pharmacol Toxicol. 1996;36: 233–252. doi:10.1146/annurev.pa.36.040196.001313

52. Benton RE, Honig PK, Zamani K, Cantilena LR, Woosley RL. Grapefruit juice alters terfenadine pharmacokinetics, resulting in prolongation of repolarization on the electrocardiogram. Clin Pharmacol Ther. 1996;59: 383–388. doi:10.1016/S0009-9236(96)90105-8

53. Zhou S, Yung Chan S, Cher Goh B, Chan E, Duan W, Huang M, et al. Mechanism-based inhibition of cytochrome P450 3A4 by therapeutic drugs. Clin Pharmacokinet. 2005;44: 279–304. doi:10.2165/00003088-200544030-00005

54. Hakim A, Stahl A. The FDA and terfenadine. West J Med. 1994;161: 619–620.

55. Thompson D. Use of terfenadine and contraindicated drugs. JAMA. 1996;275: 1339. doi:10.1001/jama.1996.03530410053033

56. Brown AM. Drugs, hERG and sudden death. Cell Calcium. 2004;35: 543–547. doi:10.1016/j.ceca.2004.01.008

57. FDA announces plan to halt marketing of terfenadine. Am J Health Syst Pharm. 1997;54: 342–342. doi:10.1093/ajhp/54.4.342

58. Ashworth L. Is my antihistamine safe? Home Care Provid. 1997;2: 117–120. doi:10.1016/s1084-628x(97)90134-9

59. Authier S, Pugsley MK, Troncy E, Curtis MJ. Arrhythmogenic liability screening in cardiovascular safety pharmacology: commonality between non-clinical safety pharmacology and clinical thorough QT (TQT) studies. J Pharmacol Toxicol Methods. 2010;62: 83–88. doi:10.1016/j.vascn.2010.06.005

60. Thomas SHL, Behr ER. Pharmacological treatment of acquired QT prolongation and torsades de pointes. Br J Clin Pharmacol. 2016;81: 420–427. doi:10.1111/bcp.12726

61. Etchegoyen CV, Keller GA, Mrad S, Cheng S, Di Girolamo G. Drug-induced QT Interval Prolongation in the Intensive Care Unit. Curr Clin Pharmacol. 2017;12: 210–222. doi:10.2174/1574884713666180223123947

62. Wolbrette DL. Drugs that cause Torsades de pointes and increase the risk of sudden cardiac death. Curr Cardiol Rep. 2004;6: 379–384. doi:10.1007/s11886-004-0041-8

63. ECGpedia.org. Rhythm torsade.png. In: File:Rhythm torsade.png - ECGpedia [Internet]. 10 Apr 2010 [cited 31 May 2022]. Available: https://en.ecgpedia.org/index.php?title=File:Rhythm_torsade.png

64. Cranefield PF, Aronson RS. Torsade de pointes and other pause-induced ventricular tachycardias: the short-long-short sequence and early afterdepolarizations. Pacing Clin Electrophysiol. 1988;11: 670–678. doi:10.1111/j.1540-8159.1988.tb06016.x

65. Taran LM, Szilagyi N. The duration of the electrical systole, Q-T, in acute rheumatic carditis in children. Am Heart J. 1947;33: 14–26. doi:10.1016/0002-8703(47)90421-3

66. Kramer DB, Zimetbaum PJ. Long-QT syndrome. Cardiol Rev. 2011;19: 217–225. doi:10.1097/CRD.0b013e3182203504

67. De Ponti F, Poluzzi E, Montanaro N. Organising evidence on QT prolongation and occurrence of Torsades de Pointes with non-antiarrhythmic drugs: a call for consensus. Eur J Clin Pharmacol. 2001;57: 185–209.

68. Townsend C, Brown BS. Predicting drug-induced QT prolongation and torsades de pointes: a review of preclinical endpoint measures. Curr Protoc Pharmacol. 2013;Chapter 10: Unit 10.16. doi:10.1002/0471141755.ph1016s61

69. Roden DM, Woosley RL. QT prolongation and arrhythmia suppression. Am Heart J. 1985;109: 411–415. doi:10.1016/0002-8703(85)90627-1

70. CFCF. 2027 Phases of the Cardiac Cycle.jpg. In: File:2027 Phases of the Cardiac Cycle.jpg - Wikimedia Commons [Internet]. 14 Dec 2013 [cited 3 Jun 2022]. Available: https://commons.wikimedia.org/wiki/File:2027_Phases_of_the_Cardiac_Cycle.jpg

71. Vik T, Pollard C, Sager P. Early clinical development: evaluation of drug-induced torsades de pointes risk. Pharmacol Ther. 2008;119: 210–214. doi:10.1016/j.pharmthera.2008.05.006

72. Bednar MM, Harrigan EP, Anziano RJ, Camm AJ, Ruskin JN. The QT interval. Prog Cardiovasc Dis. 2001;43: 1–45.

73. Vandael E, Vandenberk B, Vandenberghe J, Willems R, Foulon V. Risk factors for QTc-prolongation: systematic review of the evidence. Int J Clin Pharm. 2017;39: 16–25. doi:10.1007/s11096-016-0414-2

74. Gowda RM, Khan IA, Wilbur SL, Vasavada BC, Sacchi TJ. Torsade de pointes: the clinical considerations. Int J Cardiol. 2004;96: 1–6. doi:10.1016/j.ijcard.2003.04.055

75. Alders M, Bikker H, Christiaans I. Long QT Syndrome. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJ, Stephens K, et al., editors. GeneReviews®. Seattle (WA): University of Washington, Seattle; 1993.

76. Waldo AL. The cardiac conduction system. Arch Intern Med. 1975;135: 411. doi:10.1001/archinte.1975.00330030061007

77. Franz MR, Bargheer K, Costard-Jäckle A, Miller DC, Lichtlen PR. Human ventricular repolarization and T wave genesis. Prog Cardiovasc Dis. 1991;33: 369–384. doi:10.1016/0033-0620(91)90003-5

78. Fridericia LS. The Duration of Systole in the Electrocardiogram of Normal Subjects and of Patients with Heart Disease. Acta Med Scand. 1920;53: 469–486.

79. Bazett HC. An Analysis of the Time-Relations of Electrocardiograms. Heart. 1920;7: 353–370.

80. Fu EY, Clemo HF, Ellenbogen KA. Acquired QT prolongation: mechanisms and implications. Cardiol Rev. 1998;6: 319–324. doi:10.1097/00045415-199811000-00007

81. Yap YG, Camm AJ. Drug induced QT prolongation and torsades de pointes. Heart. 2003;89: 1363–1372. doi:10.1136/heart.89.11.1363

82. Bandorski D, Hoeltgen R, Becker N, Padberg W, Bogossian H, Wiedenroth C, et al. Follow-Up (Measurement) of Corrected QT Interval in Adult Patients before and after Lung Transplantation. Biomed Res Int. 2017;2017: 4519796. doi:10.1155/2017/4519796

83. Antzelevitch C, Sicouri S, Litovsky SH, Lukas A, Krishnan SC, Di Diego JM, et al. Heterogeneity within the ventricular wall. Electrophysiology and pharmacology of epicardial, endocardial, and M cells. Circ Res. 1991;69: 1427–1449. doi:10.1161/01.res.69.6.1427

84. Silvia3. Action potential ventr myocyte.gif. In: File:Action potential ventr myocyte.gif - Wikimedia Commons [Internet]. 2 Jun 2010 [cited 2 Jun 2022]. Available: https://commons.wikimedia.org/wiki/File:Action_potential_ventr_myocyte.gif

85. Trenor B, Cardona K, Saiz J, Noble D, Giles W. Cardiac action potential repolarization revisited: early repolarization shows all-or-none behaviour. J Physiol (Lond). 2017;595: 6599–6612. doi:10.1113/JP273651

86. Nattel S. Sudden cardio arrest: when normal ECG variants turn lethal. Nat Med. 2010;16: 646–647. doi:10.1038/nm0610-646

87. Magyar J, Banyasz T, Szentandrassy N, Kistamas K, Nanasi PP, Satin J. Role of gap junction channel in the development of beat-to-beat action potential repolarization variability and arrhythmias. Curr Pharm Des. 2015;21: 1042–1052. doi:10.2174/1381612820666141029102443

88. Shih HT. Anatomy of the action potential in the heart. Tex Heart Inst J. 1994;21: 30–41.

89. Kass RS, Tsien RW. Control of action potential duration by calcium ions in cardiac Purkinje fibers. J Gen Physiol. 1976;67: 599–617. doi:10.1085/jgp.67.5.599

90. Paavola J, Väänänen H, Larsson K, Penttinen K, Toivonen L, Kontula K, et al. Slowed depolarization and irregular repolarization in catecholaminergic polymorphic ventricular tachycardia: a study from cellular Ca2+ transients and action potentials to clinical monophasic action potentials and electrocardiography. Europace. 2016;18: 1599–1607. doi:10.1093/europace/euv380

91. Hlaing T, DiMino T, Kowey PR, Yan G-X. ECG repolarization waves: their genesis and clinical implications. Ann Noninvasive Electrocardiol. 2005;10: 211–223. doi:10.1111/j.1542-474X.2005.05588.x

92. Mangold KE, Brumback BD, Angsutararux P, Voelker TL, Zhu W, Kang PW, et al. Mechanisms and models of cardiac sodium channel inactivation. Channels (Austin). 2017;11: 517–533. doi:10.1080/19336950.2017.1369637

93. Li G-R, Du X-L, Siow YL, O K, Tse H-F, Lau C-P. Calcium-activated transient outward chloride current and phase 1 repolarization of swine ventricular action potential. Cardiovasc Res. 2003;58: 89–98. doi:10.1016/s0008-6363(02)00859-3

94. Kurata Y, Tsumoto K, Hayashi K, Hisatome I, Kuda Y, Tanida M. Multiple Dynamical Mechanisms of Phase-2 Early Afterdepolarizations in a Human Ventricular Myocyte Model: Involvement of Spontaneous SR Ca2+Release. Front Physiol. 2019;10: 1545. doi:10.3389/fphys.2019.01545

95. Sanguinetti MC, Jurkiewicz NK. Two components of cardiac delayed rectifier K+ current. Differential sensitivity to block by class III antiarrhythmic agents. J Gen Physiol. 1990;96: 195–215. doi:10.1085/jgp.96.1.195

96. Ferreiro M, Petrosky AD, Escobar AL. Intracellular Ca2+ release underlies the development of phase 2 in mouse ventricular action potentials. Am J Physiol Heart Circ Physiol. 2012;302: H1160-72. doi:10.1152/ajpheart.00524.2011

97. So PP-S, Hu X-D, Backx PH, Puglisi JL, Dorian P. Blockade of IKs by HMR 1556 increases the reverse rate-dependence of refractoriness prolongation by dofetilide in isolated rabbit ventricles. Br J Pharmacol. 2006;148: 255–263. doi:10.1038/sj.bjp.0706721

98. Jost N, Virág L, Bitay M, Takács J, Lengyel C, Biliczki P, et al. Restricting excessive cardiac action potential and QT prolongation: a vital role for IKs in human ventricular muscle. Circulation. 2005;112: 1392–1399. doi:10.1161/CIRCULATIONAHA.105.550111

99. Dhamoon AS, Jalife J. The inward rectifier current (IK1) controls cardiac excitability and is involved in arrhythmogenesis. Heart Rhythm. 2005;2: 316–324. doi:10.1016/j.hrthm.2004.11.012

100. Tamargo J, Caballero R, Gómez R, Valenzuela C, Delpón E. Pharmacology of cardiac potassium channels. Cardiovasc Res. 2004;62: 9–33. doi:10.1016/j.cardiores.2003.12.026

101. Fink M, Noble D, Virag L, Varro A, Giles WR. Contributions of HERG K+ current to repolarization of the human ventricular action potential. Prog Biophys Mol Biol. 2008;96: 357–376. doi:10.1016/j.pbiomolbio.2007.07.011

102. Perry MD, Ng C-A, Mann SA, Sadrieh A, Imtiaz M, Hill AP, et al. Getting to the heart of hERG K(+) channel gating. J Physiol (Lond). 2015;593: 2575–2585. doi:10.1113/JP270095

103. el-Sherif N, Turitto G. The long QT syndrome and torsade de pointes. Pacing Clin Electrophysiol. 1999;22: 91–110. doi:10.1111/j.1540-8159.1999.tb00305.x

104. Vandenberg JI, Walker BD, Campbell TJ. HERG K+ channels: friend and foe. Trends Pharmacol Sci. 2001;22: 240–246.

105. Chiang CE, Roden DM. The long QT syndromes: genetic basis and clinical implications. J Am Coll Cardiol. 2000;36: 1–12. doi:10.1016/S0735-1097(00)00716-6

106. Mitcheson JS, Chen J, Lin M, Culberson C, Sanguinetti MC. A structural basis for drug-induced long QT syndrome. Proc Natl Acad Sci USA. 2000;97: 12329–12333. doi:10.1073/pnas.210244497

107. Weiss JN, Garfinkel A, Karagueuzian HS, Chen P-S, Qu Z. Early afterdepolarizations and cardiac arrhythmias. Heart Rhythm. 2010;7: 1891–1899. doi:10.1016/j.hrthm.2010.09.017

108. Salama G, Bett GCL. Sex differences in the mechanisms underlying long QT syndrome. Am J Physiol Heart Circ Physiol. 2014;307: H640-8. doi:10.1152/ajpheart.00864.2013

109. Belardinelli L, Shryock JC, Wu L, Song Y. Use of preclinical assays to predict risk of drug-induced torsades de pointes. Heart Rhythm. 2005;2: S16-22. doi:10.1016/j.hrthm.2004.10.032

110. De Bruin ML, Pettersson M, Meyboom RHB, Hoes AW, Leufkens HGM. Anti-HERG activity and the risk of drug-induced arrhythmias and sudden death. Eur Heart J. 2005;26: 590–597. doi:10.1093/eurheartj/ehi092

111. Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell. 1995;81: 299–307. doi:10.1016/0092-8674(95)90340-2

112. Holzgrefe H, Ferber G, Champeroux P, Gill M, Honda M, Greiter-Wilke A, et al. Preclinical QT safety assessment: cross-species comparisons and human translation from an industry consortium. J Pharmacol Toxicol Methods. 2014;69: 61–101. doi:10.1016/j.vascn.2013.05.004

113. Anonymous. ICH E14/S7B Implementation Working Group:Clinical and Nonclinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential Questions and Answers. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use; 2022 Feb.

114. Kramer J, Obejero-Paz CA, Myatt G, Kuryshev YA, Bruening-Wright A, Verducci JS, et al. MICE models: superior to the HERG model in predicting Torsade de Pointes. Sci Rep. 2013;3: 2100. doi:10.1038/srep02100

115. Vicente J, Zusterzeel R, Johannesen L, Mason J, Sager P, Patel V, et al. Mechanistic Model-Informed Proarrhythmic Risk Assessment of Drugs: Review of the “CiPA” Initiative and Design of a Prospective Clinical Validation Study. Clin Pharmacol Ther. 2018;103: 54–66. doi:10.1002/cpt.896

116. Colatsky T, Fermini B, Gintant G, Pierson JB, Sager P, Sekino Y, et al. The Comprehensive in Vitro Proarrhythmia Assay (CiPA) initiative - Update on progress. J Pharmacol Toxicol Methods. 2016;81: 15–20. doi:10.1016/j.vascn.2016.06.002

117. Botstein P. Is QT interval prolongation harmful? A regulatory perspective. Am J Cardiol. 1993;72: 50B-52B. doi:10.1016/0002-9149(93)90041-a

118. E14 Clinical Evaluation of QT/QTc Interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs | FDA. Oct 2012 [cited 17 Jun 2020]. Available: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/e14-clinical-evaluation-qtqtc-interval-prolongation-and-proarrhythmic-potential-non-antiarrhythmic-0

119. Lazzara R. Antiarrhythmic drugs and torsade de pointes. Eur Heart J. 1993;14 Suppl H: 88–92. doi:10.1093/eurheartj/14.suppl_h.88

120. Cavero I, Mestre M, Guillon JM, Crumb W. Drugs that prolong QT interval as an unwanted effect: assessing their likelihood of inducing hazardous cardiac dysrhythmias. Expert Opin Pharmacother. 2000;1: 947–973. doi:10.1517/14656566.1.5.947

121. Dahlberg P, Diamant U-B, Gilljam T, Rydberg A, Bergfeldt L. QT correction using Bazett's formula remains preferable in long QT syndrome type 1 and 2. Ann Noninvasive Electrocardiol. 2021;26: e12804. doi:10.1111/anec.12804

122. Mayeda I. On the time relation between the systolic duration of the heart and the pulse rate. Ada Scholae Med Univ Imp Kioto. 1934;17: 53–55.

123. Hodges MS, Salerno D, Erlinen D. Bazett's QT correction reviewed: evidence that a linear QT correction for heart rate is better. J Am Coll Cardiol. 1983;1: 694.

124. Sagie A, Larson MG, Goldberg RJ, Bengtson JR, Levy D. An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study). Am J Cardiol. 1992;70: 797–801. doi:10.1016/0002-9149(92)90562-d

125. Yamaoka A, Koie H, Sato T, Kanayama K, Taira M. Standard electrocardiographic data of young Japanese monkeys (Macaca fusucata). J Am Assoc Lab Anim Sci. 2013;52: 491–494.

126. Nakayama S, Koie H, Kato-Tateishi M, Pai C, Ito-Fujishiro Y, Kanayama K, et al. Establishment of a new formula for QT interval correction using a large colony of cynomolgus monkeys. Exp Anim. 2020;69: 18–25. doi:10.1538/expanim.19-0009

127. Van de Water A, Verheyen J, Xhonneux R, Reneman RS. An improved method to correct the QT interval of the electrocardiogram for changes in heart rate. J Pharmacol Methods. 1989;22: 207–217. doi:10.1016/0160-5402(89)90015-6

128. Extramiana F, Maison-Blanche P, Badilini F, Beaufils P, Leenhardt A. Individual QT-R-R relationship: average stability over time does not rule out an individual residual variability: implication for the assessment of drug effect on the QT interval. Ann Noninvasive Electrocardiol. 2005;10: 169–178. doi:10.1111/j.1542-474X.2005.05615.x

129. Couderc J-P, Xiaojuan X, Zareba W, Moss AJ. Assessment of the stability of the individual-based correction of QT interval for heart rate. Ann Noninvasive Electrocardiol. 2005;10: 25–34. doi:10.1111/j.1542-474X.2005.00593.x

130. Batchvarov V, Malik M. Individual patterns of QT/RR relationship. Card Electrophysiol Rev. 2002;6: 282–288. doi:10.1023/a:1016393328485

131. Malik M, Färbom P, Batchvarov V, Hnatkova K, Camm AJ. Relation between QT and RR intervals is highly individual among healthy subjects: implications for heart rate correction of the QT interval. Heart. 2002;87: 220–228. doi:10.1136/heart.87.3.220

132. Malik M, Garnett C, Hnatkova K, Vicente J, Johannesen L, Stockbridge N. Implications of Individual QT/RR Profiles-Part 1: Inaccuracies and Problems of Population-Specific QT/Heart Rate Corrections. Drug Saf. 2019;42: 401–414. doi:10.1007/s40264-018-0736-1

133. Batchvarov VN, Ghuran A, Smetana P, Hnatkova K, Harries M, Dilaveris P, et al. QT-RR relationship in healthy subjects exhibits substantial intersubject variability and high intrasubject stability. Am J Physiol Heart Circ Physiol. 2002;282: H2356-63. doi:10.1152/ajpheart.00860.2001

134. Koga T, Kuwano K, Kito G, Kanefuji K. Evaluation of QT interval using a linear model in individual cynomolgus monkeys. J Pharmacol Toxicol Methods. 2007;55: 248–253. doi:10.1016/j.vascn.2006.10.001

135. Panicker GK, Kadam P, Chakraborty S, Kothari S, Turner JR, Karnad DR. Individual-Specific QT Interval Correction for Drugs With Substantial Heart Rate Effect Using Holter ECGs Extracted Over a Wide Range of Heart Rates. J Clin Pharmacol. 2018. doi:10.1002/jcph.1258

136. Hii JT, Wyse DG, Gillis AM, Duff HJ, Solylo MA, Mitchell LB. Precordial QT interval dispersion as a marker of torsade de pointes. Disparate effects of class Ia antiarrhythmic drugs and amiodarone. Circulation. 1992;86: 1376–1382. doi:10.1161/01.cir.86.5.1376

137. Cavero I, Crumb W. The use of electrocardiograms in clinical trials: a public discussion of the proposed ICH E14 regulatory guidance. Expert Opin Drug Saf. 2005;4: 795–799. doi:10.1517/14740338.4.4.795

138. ICH Official web site : ICH. [cited 5 Jun 2022]. Available: https://www.ich.org/page/efficacy-guidelines

139. Vargas HM, Rolf MG, Wisialowski TA, Achanzar W, Bahinski A, Bass A, et al. Time for a Fully Integrated Nonclinical-Clinical Risk Assessment to Streamline QT Prolongation Liability Determinations: A Pharma Industry Perspective. Clin Pharmacol Ther. 2021;109: 310–318. doi:10.1002/cpt.2029

140. Sager PT. Key clinical considerations for demonstrating the utility of preclinical models to predict clinical drug-induced torsades de pointes. Br J Pharmacol. 2008;154: 1544–1549. doi:10.1038/bjp.2008.222

141. Hammond TG, Pollard CE. Use of in vitro methods to predict QT prolongation. Toxicol Appl Pharmacol. 2005;207: 446–450. doi:10.1016/j.taap.2005.03.022

142. Dubois VFS, Smania G, Yu H, Graf R, Chain ASY, Danhof M, et al. Translating QT interval prolongation from conscious dogs to humans. Br J Clin Pharmacol. 2017;83: 349–362. doi:10.1111/bcp.13123

143. Pollard CE, Skinner M, Lazic SE, Prior HM, Conlon KM, Valentin J-P, et al. An Analysis of the Relationship Between Preclinical and Clinical QT Interval-Related Data. Toxicol Sci. 2017;159: 94–101. doi:10.1093/toxsci/kfx125

144. Koshman YE, Wilsey AS, Bird BM, Endemann AL, Sadilek S, Treadway J, et al. Drug-induced QT prolongation: Concordance of preclinical anesthetized canine model in relation to published clinical observations for ten CiPA drugs. J Pharmacol Toxicol Methods. 2020;103: 106871. doi:10.1016/j.vascn.2020.106871

145. Gintant G. An evaluation of hERG current assay performance: Translating preclinical safety studies to clinical QT prolongation. Pharmacol Ther. 2011;129: 109–119. doi:10.1016/j.pharmthera.2010.08.008

146. Strauss DG, Wu WW, Li Z, Koerner J, Garnett C. Translational models and tools to reduce clinical trials and improve regulatory decision making for qtc and proarrhythmia risk (ICH E14/S7B updates). Clin Pharmacol Ther. 2021;109: 319–333. doi:10.1002/cpt.2137

147. Garnett CE, Zhu H, Malik M, Fossa AA, Zhang J, Badilini F, et al. Methodologies to characterize the QT/corrected QT interval in the presence of drug-induced heart rate changes or other autonomic effects. Am Heart J. 2012;163: 912–930. doi:10.1016/j.ahj.2012.02.023

148. Vandenberk B, Vandael E, Robyns T, Vandenberghe J, Garweg C, Foulon V, et al. Which QT correction formulae to use for QT monitoring? J Am Heart Assoc. 2016;5. doi:10.1161/JAHA.116.003264

149. Malik M, Hnatkova K, Batchvarov V. Differences between study-specific and subject-specific heart rate corrections of the QT interval in investigations of drug induced QTc prolongation. Pacing Clin Electrophysiol. 2004;27: 791–800. doi:10.1111/j.1540-8159.2004.00530.x

150. Tattersall ML, Dymond M, Hammond T, Valentin J-P. Correction of QT values to allow for increases in heart rate in conscious Beagle dogs in toxicology assessment. J Pharmacol Toxicol Methods. 2006;53: 11–19. doi:10.1016/j.vascn.2005.02.005

151. King A, Bailie M, Olivier NB. Magnitude of error introduced by application of heart rate correction formulas to the canine QT interval. Ann Noninvasive Electrocardiol. 2006;11: 289–298. doi:10.1111/j.1542-474X.2006.00120.x

152. Miyazaki H, Tagawa M. Rate-correction technique for QT interval in long-term telemetry ECG recording in beagle dogs. Exp Anim. 2002;51: 465–475. doi:10.1538/expanim.51.465

153. Soloviev MV, Hamlin RL, Barrett RM, Chengelis CP, Schaefer GJ. Different species require different correction factors for the QT interval. Cardiovasc Toxicol. 2006;6: 145–157. doi:10.1385/CT:6:2:145

154. Glassman AH, Bigger JT. Antipsychotic drugs: prolonged QTc interval, torsade de pointes, and sudden death. Am J Psychiatry. 2001;158: 1774–1782. doi:10.1176/appi.ajp.158.11.1774

155. Roden DM. Drug-induced prolongation of the QT interval. N Engl J Med. 2004;350: 1013–1022. doi:10.1056/NEJMra032426

156. Champeroux P, Thireau J, Judé S, Laigot-Barbé C, Maurin A, Sola ML, et al. Short-term variability in QT interval and ventricular arrhythmias induced by dofetilide are dependent on high-frequency autonomic oscillations. Br J Pharmacol. 2015;172: 2878–2891. doi:10.1111/bph.13093

157. Sasaki H, Shimizu N, Suganami H, Yamamoto K. QT PRODACT: inter-facility variability in electrocardiographic and hemodynamic parameters in conscious dogs and monkeys. J Pharmacol Sci. 2005;99: 513–522. doi:10.1254/jphs.qt-b6

158. Tang JKK, Bennett MT, Rabkin SW. Assessment of QT interval in ventricular paced rhythm: Derivation of a novel formula. J Electrocardiol. 2019;57: 55–62. doi:10.1016/j.jelectrocard.2019.05.017

159. Baumert M, Czippelova B, Porta A, Javorka M. Decoupling of QT interval variability from heart rate variability with ageing. Physiol Meas. 2013;34: 1435–1448. doi:10.1088/0967-3334/34/11/1435

160. Smetana P, Batchvarov V, Hnatkova K, Camm AJ, Malik M. Circadian rhythm of the corrected QT interval: impact of different heart rate correction models. Pacing Clin Electrophysiol. 2003;26: 383–386. doi:10.1046/j.1460-9592.2003.00054.x

161. Smith AH, Norris KJ, Roden DM, Kannankeril PJ. Autonomic tone attenuates drug-induced QT prolongation. J Cardiovasc Electrophysiol. 2007;18: 960–964. doi:10.1111/j.1540-8167.2007.00901.x

162. Kim YK, Hwang GS, Shin WJ, Bang JY, Cho SK, Han SM. Effect of propranolol on the relationship between QT interval and vagal modulation of heart rate variability in cirrhotic patients awaiting liver transplantation. Transplant Proc. 2011;43: 1654–1659. doi:10.1016/j.transproceed.2011.02.017

163. Pearce GL, Frisbie DD. Statistical evaluation of biomedical studies. Osteoarthr Cartil. 2010;18 Suppl 3: S117-22. doi:10.1016/j.joca.2010.04.014

164. Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). Biometrika. 1965;52: 591-611. doi:10.2307/2333709

165. Efron B, Tibshirani R. Bootstrap methods for standard errors, confidence intervals, and other measures of statistical accuracy. Stat Sci. 1986;1: 54–75. doi:10.1214/ss/1177013815

166. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020 [cited 11 Mar 2021]. Available: https://www.r-project.org/index.html

167. Bódizs R, Kis A, Gácsi M, Topál J. Sleep in the dog: comparative, behavioral and translational relevance. Curr Opin Behav Sci. 2020;33: 25–33. doi:10.1016/j.cobeha.2019.12.006

168. Adams GJ, Johnson KG. Sleep-wake cycles and other night-time behaviours of the domestic dog Canis familiaris. Appl Anim Behav Sci. 1993;36: 233–248. doi:10.1016/0168-1591(93)90013-F

169. Derakhchan K, Chui RW, Stevens D, Gu W, Vargas HM. Detection of QTc interval prolongation using jacket telemetry in conscious non-human primates: comparison with implanted telemetry. Br J Pharmacol. 2014;171: 509–522. doi:10.1111/bph.12484

170. Chui RW, Fosdick A, Conner R, Jiang J, Bruenner BA, Vargas HM. Assessment of two external telemetry systems (PhysioJacket and JET) in beagle dogs with telemetry implants. J Pharmacol Toxicol Methods. 2009;60: 58–68. doi:10.1016/j.vascn.2009.04.196

171. Miyazaki H, Yoshida M, Samura K, Matsumoto H, Ikemoto F, Tagawa M. Ranges of diurnal variation and the pattern of body temperature, blood pressure and heart rate in laboratory beagle dogs. Exp Anim. 2002;51: 95–98. doi:10.1538/expanim.51.95

172. Atterson PR, Voss K, Kopp G. Occurrence and variance of ECG waveform abnormalities in canines and non-human primates: What is considered normal? J Pharmacol Toxicol Methods. 2010;62: e27. doi:10.1016/j.vascn.2010.11.091

173. Andršová I, Hnatkova K, Šišáková M, Toman O, Smetana P, Huster KM, et al. Influence of heart rate correction formulas on QTc interval stability. Sci Rep. 2021;11: 14269. doi:10.1038/s41598-021-93774-9

174. Ether ND, Jantre SR, Sharma DB, Leishman DJ, Bailie MB, Lauver DA. Improving corrected QT; Why individual correction is not enough. J Pharmacol Toxicol Methods. 2022;113: 107126. doi:10.1016/j.vascn.2021.107126

175. Haushalter TM, Friedrichs GS, Reynolds DL, Barecki-Roach M, Pastino G, Hayes R, et al. The cardiovascular and pharmacokinetic profile of dofetilide in conscious telemetered beagle dogs and cynomolgus monkeys. Br J Pharmacol. 2008;154: 1457–1464. doi:10.1038/bjp.2008.275

176. Watson KJ, Gorczyca WP, Umland J, Zhang Y, Chen X, Sun SZ, et al. Pharmacokinetic-pharmacodynamic modelling of the effect of Moxifloxacin on QTc prolongation in telemetered cynomolgus monkeys. J Pharmacol Toxicol Methods. 2011;63: 304–313. doi:10.1016/j.vascn.2011.03.002

177. Woosley RL, Sale M. QT interval: a measure of drug action. Am J Cardiol. 1993;72: 36B-43B. doi:10.1016/0002-9149(93)90039-f

178. Diemberger I, Raschi E, Trifirò G. Balancing the need for personalization of QT correction and generalization of study results: going beyond thorough QT studies. Clin Drug Investig. 2017;37: 985–988. doi:10.1007/s40261-017-0563-7

179. Ether N, Leishman D, Bailie M, Lauver DA. Relationship of clinical adverse event reports to models of arrhythmia risk. J Pharmacol Toxicol Methods. 2019; 106622. doi:10.1016/j.vascn.2019.106622