CARDIAC SAFETY

Use of ECG Restitution (Beat-to-Beat QT-TQ Interval Analysis) to Assess Arrhythmogenic Risk of QTc Prolongation with Guanfacine

Anthony A. Fossa, Ph.D.,* Meijian Zhou, Ph.D.,* Antoine Robinson, M.Sc., M.B.A.,† Jaideep Purkayastha, Ph.D.,† and Patrick Martin, M.D.†

From the *iCardiac Technologies, Rochester, NY; and †Shire Pharmaceuticals, Chesterbrook, PA

Background: Guanfacine (Intuniv) is a centrally active alpha-2A adrenergic agonist for the new indication of attention-deficit/hyperactivity disorder. QTc (QTcF and QTcNi) was prolonged at both therapeutic (4 mg) and supratherapeutic (8 mg) doses of a thorough QT study even though guanfacine has had a safe clinical history of over 3 million prescriptions for the treatment of hypertension. In an attempt to understand this disparity, retrospective evaluation of the continuous ECG data utilized dynamic beat-to-beat and ECG restitution analyses was performed.

Methods: Sixty healthy subjects using 24-hour Holters were examined in a 3-arm, placebo- and positive-controlled, double-blind crossover study for effects on beat-to-beat QT, TQ, and RR intervals.

Results: ECG restitution analyses indicated that, at all time points, a disproportionate effect to increase the TQ interval (rest) occurred more in relationship to each QT interval lengthening resulting in a placebo-adjusted reduced QT/TQ ratio of 21% after 4 mg and 31% after 8 mg (both antiarrhythmic responses). Additionally, the percentage of time and magnitude of stress on the heart, as measured by the upper limits of the QT/TQ ratio, were reduced with guanfacine by 22% to 24%. In contrast to guanfacine, moxifloxacin did not show a significant improvement in any restitution parameters but reflected a trend toward proarrhythmia with an increase in the QT/TQ ratio of up to 11%.

Conclusion: These results indicate that guanfacine causes a stabilizing effect on cardiac restitution that helps reconcile the clinical evidence for a lack of arrhythmia liability despite apparent increases in typical QT/QTc prolongation measures.

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QTc/QT prolongation; restitution; beat-to-beat; arrhythmia; guanfacine; risk assessment

Guanfacine is an alpha-2A adrenergic receptor agonist, which historically has been used as an antihypertensive because of its centrally mediated action to reduce heart rate and blood pressure. Despite its use for many years in high-risk cardiovascular patients, the clinical history of over 3 million prescriptions indicates that guanfacine is safe with respect to a low incidence of reported ventricular arrhythmias and no deaths related to torsades de pointes (TdP) using the Multi-item Gamma Poisson

Shrinker analysis method¹ of the FDA-Adverse Events Reporting System (FDA-AERS).² Intuniv (Shire Pharmaceuticals, Chesterbrook, PA, USA) is an extended release formulation of guanfacine that has been demonstrated to be clinically efficacious in the treatment of attention-deficit/hyperactivity disorder, or ADHD. A thorough QT (TQT) study was completed by Shire according to International Conference on Harmonization E14 (ICH E14) guideline using the proposed therapeutic (4 mg)

Address for correspondence: Anthony A. Fossa, Ph.D., 20 Niles Rd. Mystic, CT 06355. Fax (860) 572-7681; E-mail: anthony@fossa.us

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and supratherapeutic (8 mg) total daily doses of Intuniv.

The primary endpoint of the TQT study was the mean placebo-adjusted, time-matched change in the Fridericia ($\Delta\Delta Q_{TcF}$) and individual ($\Delta\Delta Q_{TcNi}$) QT-corrected values. In the initial evaluation submitted to the US Food and Drug Administration (FDA), the study did not exclude a 10 ms increase in $\Delta\Delta Q_{Tc}$ (both correction methods), which is the criterion used by ICH E14 for identifying drugs of arrhythmogenic concern.³ The largest upper bound of the 2-sided 90% confidence interval (CI) for the mean difference between guanfacine (4 mg and 8 mg) and placebo were 9.3 and 20.7 ms, respectively.² At 24 hours, the largest mean $\Delta\Delta Q_{TcF}$ was observed after dosing 4 and 8 mg on Days 1 and 6, respectively.² The delay after guanfacine was unclear because peak concentrations occurred at approximately 3 to 6 hours and no metabolites were reported.² Guanfacine did not inhibit repolarization current in hERG transfected HEK293 cells studied at clinically relevant concentrations (30.1% at 3.6 μ M). A possible cause noted by the FDA was *"variation in autonomic tone in response to drug-induced heart rate and possibly blood pressure changes."*² Errors in interpretation of TQT data have been recognized by the FDA under similar circumstances to a lesser extent⁴ thus a further examination of the data using the dynamic beat-to-beat analysis method was suggested.²

In recent years, Fossa and colleagues proposed a dynamic beat-to-beat analysis that has been reported in dogs⁵ and humans.⁴⁻⁷ In brief, the method is used to differentiate changes in QT interval duration due to heart rate or autonomic state from impaired repolarization. The method does not utilize any heart rate correction factors but compares the raw QT interval measurements on treatment (i.e., Day 1) to each cardiac cycle (QT-RR data points) within the same RR interval range ($\pm$ 12 ms) extracted at each nominal time point from a beat-to-beat 24-hour baseline dataset obtained from the same individual on a different day (i.e., Day -1). The baseline dataset thus represents all beats obtained under normal physiological states of hysteresis and autonomic tone that are assumed to be without risk of arrhythmia such as eating, diurnal/nocturnal sleeping, and minor activity (i.e., walking). A time-matched adjustment is made by subtracting the same individual's response on the placebo treatment day from the treatment effect at each nominal time point. If an increased percentage

of beats after drug is observed exceeding the upper QT interval reference bounds established at baseline, ECG restitution analysis^{6,7} can be used on the same beat-to-beat dataset to determine whether any changes in QT interval would be more or less likely to increase arrhythmia vulnerability.

Cardiac restitution is the ability of the heart to recover dynamically from one beat to the next.⁸ On the ECG, this can be measured by assessing the QT interval (working phase of the heart) in relationship to the previous TQ interval (resting phase of the heart). When the heart is not under stress, this ratio is less than 1, meaning the heart is resting more than it is working. However, as stress increases on the heart, for example, during exercise, the heart works more than it rests, increasing this ratio to greater than 1. Sustained periods with inadequate recovery between beats would presumably lead to increased arrhythmia vulnerability (i.e., R on T beats where TQ interval equals zero are associated with increased arrhythmia).

The primary objective of this study was to reanalyze the ECG Holter data from the guanfacine TQT study utilizing the dynamic beat-to-beat method assessing placebo-adjusted time-matched changes in QT interval ($\Delta\Delta Q_{Tbtb}$) and compare findings to $\Delta\Delta Q_{TcF}$ values obtained from the same beats. ECG restitution was employed to help clarify the discrepancy found between the known clinical safety record of guanfacine and any observed QT prolongation.

MATERIAL AND METHODS

Study Design

This study is a retrospective analysis of data obtained from a phase I, gender-stratified, placebo- and positive-controlled, double-blind, 3-period, 6-sequence, crossover trial (ClinicalTrials.gov Identifier: NCT00672984) that compared the effect of 2 doses of immediate-release guanfacine HCl (a 4-mg therapeutic dose and an 8-mg supratherapeutic dose) to the effect seen with moxifloxacin HCl (positive control) and placebo. Each subject had a baseline 24-hour ECG Holter recorded (n = 60) starting on Day -1 prior to the start of treatment periods. On Day 1 of the guanfacine treatment group, each subject received a starting oral dose of 4 mg in the fasted state in the AM. ECGs were assessed at time point measurements within 10 minutes of the 1-, 2-, 3-, 4-, 5-, 6-, 8-, 12-, 14-,

16-, 18-, 20-, and 22-hour nominal time points with a 24-hour measurement also acquired from a standard 12-lead system. Each subject also had a 24-hour Holter initiated approximately 1 hour prior to dosing. On dose escalation Days 2 and 3, guanfacine dosage was increased to 4 mg BID (AM and PM dosing) and on Days 4 and 5 again increased to 6 mg/day BID. No ECG measurements were obtained between Days 2 through 5. On Day 6, each subject received a final single dose of 8 mg in the AM and ECG time point measurements were recorded as on Day 1. For the placebo arm, each subject received placebo on Days 1 through 6; for the positive control (400 mg moxifloxacin) arms, each subject received placebo tablets except on Days 1 and 6, when those in the moxifloxacin arm received active treatment. ECGs were recorded at identical nominal time points. Informed consent was obtained from all subjects.

ECG Analysis

To process the data using COMPAS software,⁹ all 12-lead Holter data were converted to ISHNE format using HS2ISHNE software version 1.2.1 (AMPS LLC, New York, NY). Criteria for inclusion into analyses resulted in a maximal number of 59 of 62 subjects Holters from each arm available for analyses.

The central ECG laboratory readers at iCardiac Technologies (Rochester, NY) were blinded to treatment allocation and sequence. ECGs from each single subject were reviewed by the same ECG analyst. Highly automated analysis¹⁰ was performed using measurements of all ECG parameters of interest in all recorded beats. All low confidence beats (as determined by signal-to-noise ratio, RR, QT, T-wave morphology, and other variability in the ECG parameters) were reviewed and over-read by technologists with subsequent QC by Cardiologists in the same manner as in the conventional semi-automated ECG analysis. The beats found acceptable by manual review were included in the analysis. All data entry, analysis, and review followed iCardiac standard Quality Control processes.

Statistical Analyses

ECGs originally collected were blinded to all treatments, but for subsequent transfer of data and computer analyses, it was necessary to break blind

on data files. The descriptive statistics, including arithmetic means, 90% two-sided CI (based on t-distribution), and the number of patients, were calculated for the placebo-adjusted time-matched changes in each parameter. All other data were expressed as means, standard errors, and the number of patients. For the outlier analysis, the upper 97.5% confidence bound of QT interval was constructed based on the quantile regression technique using the R package¹¹ "quantreg." The default method (i.e., the modified version of the Barrodale and Roberts algorithm for ℓ_1 -regression) in the package was used for the calculation. The response and independent variables for the quantile regression are QT and RR, respectively, and the percentage of the beats exceeding the upper bound was calculated for each individual at each nominal time point. For all other analyses, the means and standard errors were calculated from the medians of the parameters for the selected beats from different time periods.

Restitution Parameters

To quantify effects on restitution, several parameters were used:

- *Lower 5% of TQ intervals:* It has been proposed that as the relative refractory period approaches zero, arrhythmia vulnerability may increase due to the likelihood of reentry.¹² TQ interval is the ECG equivalent to the diastolic interval and thus measuring the lower limit for 95% of the beats was utilized.
- *Percentage of beats with QT/TQ ratio greater than 1:* As the ventricle spends more time working (QT interval or action potential duration) per cycle of rest (TQ or diastolic intervals), cardiac instability may ensue, theoretically leading to increased arrhythmia vulnerability. This relationship has been associated with transition of ventricular tachycardia to fibrillation by the steepness of the restitution relationship.^{13,14} Assessment of the QT/TQ slope from normal sinus rhythm data would not take into account the density of beats occurring at any 1 point and would be further complicated by hysteresis at a particular heart rate. Therefore, the percentage of beats with a QT/TQ ratio greater than 1 reflects the relative time spent on the restitution curve where stability is not as certain.

- *Upper 98% quantile of the QT/TQ ratio:* This measure reflects the magnitude of the steepness of the restitution relationship. The 98% quantile takes into account the most extreme beats with the highest likelihood of leading to arrhythmia.

RESULTS

Baseline Values

Mean baseline values for all readable beats occurring on the 24-hour ambulatory Holter on Day -1 and during the 20-minute predose period on Day 1 are provided in Table 1. It should be noted that changes from baseline for QTb_{tb} do not use the predose Day 1 period but rather are compared to all beats from the 24-hour ambulatory Holter on Day -1.

An important part of the beat-to-beat method is to determine whether repolarization is significantly impaired beyond normal autonomic boundaries by applying statistical techniques to define the upper (or lower) 97.5% confidence boundary of QT-RR interval relationship from the normal 24-hour data (from baseline/placebo day of the study). The outlier analysis examines the percentage of beats that exceed the upper 97.5% confidence boundary of the baseline QT interval data during any period. By definition, 2.5% of beats will be present as outliers at baseline.

Effects on Heart Rate, QTcF, and QTb_{tb}

Heart rate, as measured by RR interval, was decreased on both Days 1 (4-mg therapeutic dose; Fig. 1A) and 6 (8-mg supratherapeutic dose; Fig. 1B). The mean maximal change was -12 bpm (RR interval = 212 ms) at 8 hours after 4 mg dosing and -20 bpm (419 ms) at 2 hours after dosing of 8 mg. After approximately 16 hours, heart rate began to slowly return toward normal. Heart rate remained decreased a mean of 10 bpm (163 ms) after 8 mg at 22 hours.

The maximum change in placebo-subtracted, time-matched mean of $\Delta\Delta$ QTb_{tb} for subjects was increased 17.92 ms at 14 hours after 8 mg dosing of guanfacine (Table 2 or Fig. 1). The 90% upper CI for the time point was 22.97 ms. No significant treatment mean increase in QTb_{tb} was observed after dosing 4 mg of guanfacine on Day 1 (Fig. 1A). The same beats assessed by QTcF showed a mean

maximum placebo- and time-adjusted treatment effect of 9.49 ms and 15.70 ms increases at 10 hours after 4 mg and at 14 hours after 8 mg, respectively.

At 8 mg of guanfacine, $\Delta\Delta$ QTb_{tb} was consistently elevated above a mean of approximately 10 ms with upper CI near 20 ms (Fig. 1B). The QTb_{tb} increased 15 ms after dosing at 1 hour and remained elevated until meal time at 6 hours. The moxifloxacin positive controls for Days 1 and 6 produced mean maximal QTb_{tb} effects of 9.72 and 8.36 ms, respectively, occurring at 4 and 5 hours with lower 90% CI on both days > 5 ms (Fig. 1A, B).

Outlier Analysis

Mean maximum placebo-adjusted changes in beats exceeding the upper 97.5% confidence bounds of QT interval for each individual's Day -1 QT-RR relationship (% outliers) are shown in Table 3 for all treatment periods. During hours 1 to 22 on Day 1, subjects treated with guanfacine had placebo-adjusted mean increases of 10 to 18% occurring initially after dosing during hours 2 to 5 (Fig. 2A). This would appear to be significant because at the same time points, the lower confidence bounds of guanfacine treatment did not overlap with the upper bounds of placebo. The same analyses on Day 6 data after 8 mg of guanfacine indicated that the number of outlier beats increased to 64%, again at 2 hours. There was little overlap in confidence bounds at each nominal time point compared to placebo response (Fig. 2B). This effect seems to coincide well with the known pharmacokinetic profile after dosing with guanfacine.

Moxifloxacin on Day 1, as a positive control, produced between 4% and 11% of outlier beats from 1 to 5 hours after dosing (Fig. 2A). However, there was 1 additional period at 20 hours after dosing where 19.28% of beats were beyond the upper 97.5% confidence bounds. On Day 6, a similar response was observed with moxifloxacin (Fig. 2B).

Restitution Analysis

Since QT prolongation was observed, an analysis of beat-to-beat ECG restitution was performed. This is particularly relevant because profound autonomic change was apparent due to the

Table 1. Mean Baseline Values for all Readable Beats Occurring on Day –1 and During the 20-Minute Predose Period on Day 1

Parameter	Mean Baseline Values					
	Day –1 24-Hour Ambulatory	SEM	N	Day 1 Predose*	SEM	N
Median RR						
Placebo	834.52	10.44	62	920.97	13.38	62
Guanfacine	868.10	10.29	62	941.58	11.76	62
Moxifloxacin	842.94	10.79	67	912.66	13.70	67
Median QT						
Placebo	379.32	2.39	62	393.39	2.75	62
Guanfacine	384.03	2.60	62	392.73	2.62	62
Moxifloxacin	380.97	2.34	67	390.43	2.72	67
Median QTcF						
Placebo	404.27	1.86	62	405.03	2.05	62
Guanfacine	403.66	2.07	62	401.24	1.97	62
Moxifloxacin	404.63	1.85	67	403.52	1.97	67
Median TQ						
Placebo	458.60	9.02	62	527.98	11.69	62
Guanfacine	486.95	8.96	62	549.35	10.18	62
Moxifloxacin	465.73	9.56	67	522.46	11.92	67
Median QT/TQ ratio						
Placebo	0.85	0.02	62	0.76	0.02	62
Guanfacine	0.81	0.01	62	0.73	0.01	62
Moxifloxacin	0.84	0.02	67	0.77	0.02	67
Lower 5% of TQ interval						
Placebo	284.48	5.17	62	384.68	8.08	62
Guanfacine	301.65	5.76	62	405.98	7.87	62
Moxifloxacin	285.04	5.40	67	380.96	8.92	67
% QT/TQ ratio > 1						
Placebo	25.48	2.30	62	11.33	2.25	62
Guanfacine	20.02	1.85	62	5.90	1.09	62
Moxifloxacin	25.36	2.18	67	12.37	2.14	67
Upper 98% of QT/TQ ratio						
Placebo	1.40	0.02	62	1.14	0.02	62
Guanfacine	1.34	0.02	62	1.07	0.02	62
Moxifloxacin	1.41	0.02	67	1.16	0.02	67

*Values used for determination of change from baseline (delta).
SEM = standard error of the mean.

centrally mediated decreased heart rate and suppressed sympathetic tone with guanfacine, which affects the dynamicity of the QT-RR interval relationship.

Restitution changes showed consistent reductions with all parameters of cardiac stress at all time points after both dosing treatments of guanfacine. The time course for each parameter is displayed in Figures 3 and 4 in comparison to the positive control, moxifloxacin. The mean maximum placebo-adjusted change in TQ interval increased 176.40 ms after 4 mg and 367.19 ms 8 mg (Fig. 3A) of guanfacine. The QT/TQ ratio decreased

0.18 (21%) from a mean baseline of 0.84 after 4 mg of guanfacine and 0.28 (31%) from a mean baseline of 0.89 after 8 mg (Fig. 3B). The lower bounds of the TQ interval, where the highest risk of beats leading to arrhythmogenic reentry are most likely to occur because of the shortest diastolic periods, showed a mean increase of 162.97 ms after 4 mg and 351.25 ms after 8 mg of guanfacine (Fig. 4A). The time spent under stress where the heart is working more than resting is indicated by the percentage of beats with a QT/TQ ratio greater than 1. This was reduced by guanfacine 22.61% at 4 mg and 24.15% at 8 mg (Fig. 4B). Finally, the magnitude

Figure 1. Mean change-from-baseline QT beat-to-beat (QTbtb) or Fridericia-corrected QT interval (QTcF; ms) and heart rate (HR; beats/min) for all subjects after 4 mg (Panel A) or 8 mg (Panel B) of guanfacine. QTbtb—dashed blue line with squares; QTcF—solid red line with circles; Heart rate—dashed black line. Shaded area between 3 and 6 hours represents time period of maximum plasma concentrations of guanfacine.

of peak stress, as indicated by the upper 2% of QT/TQ ratio values for all beats, was decreased by 0.31 (21% from baseline) at 4 mg and 0.40 (30% from baseline) at 8 mg (Fig. 4C). It should be noted that for all restitution parameters, during the 22-hour posttreatment time course for both guanfacine doses on Days 1 and 6, delta-delta values did not

exceed a change from zero in a direction for which arrhythmia liability would be increased.

In contrast to guanfacine, moxifloxacin produced restitution changes in all parameters that indicated increased cardiac stress, particularly between 1 and 6 hours after dosing. Mean placebo-adjusted TQ interval was reduced up to 48.87 ms on Day 1 and

Table 2. Maximum Mean, Paired, Placebo-Subtracted $\Delta\Delta\text{QT}_{\text{btb}}$, and $\Delta\Delta\text{QT}_{\text{cF}}$ Endpoints for Each Treatment

$\Delta\Delta\text{QT}_{\text{btb}}$	Hour	Mean (ms)	N	Upper (Lower) 90% CI 2-Sided (ms)
Guanfacine 4 mg	10	4.96	57	7.97 (1.96)
Guanfacine 8 mg	14	17.92	49	22.97 (12.86)
Moxifloxacin (Day 1)	4	9.72	58	11.80 (7.63)
Moxifloxacin (Day 6)	5	8.36	58	11.83 (4.90)
$\Delta\Delta\text{QT}_{\text{cF}}$				
Guanfacine 4 mg	10	9.49	57	12.35 (6.63)
Guanfacine 8 mg	14	15.70	56	19.67 (11.73)
Moxifloxacin (Day 1)	4	10.05	59	11.90 (8.20)
Moxifloxacin (Day 6)	5	9.43	58	12.78 (6.09)

Table 3. Mean Maximum, Paired, Placebo-Subtracted Outlier Analyses* for Each Treatment

Delta-delta Outlier Response	Hour	% Beats Exceeding Upper 97.5% Day –1 Confidence Bounds	N	Upper (Lower) 90% CI 2-Sided (%)
Guanfacine 4 mg	20	25.96	52	36.52 (15.39)
Guanfacine 8 mg	2	64.47	36	76.79 (52.14)
Moxifloxacin (Day 1)	20	19.28	48	27.77 (10.79)
Moxifloxacin (Day 6)	3	10.47	60	17.22 (3.71)

*Percentage of beats with QT intervals exceeding upper 97.5% confidence bounds for QT intervals from each individual Day –1 24-hour ambulatory QT-RR relationship.
CI = confidence interval.

30.76 ms on Day 6 (Fig. 3A). The mean QT/TQ ratio was also increased 0.06 to 0.08 on Days 1 and 6 between 1 and 2 hours after dosing, respectively (Fig. 3B). The remaining parameters also showed impaired restitution as reflected in a decrease of 24% to 42% in the mean lower 5% of TQ interval, the percentage of beats with QT/TQ ratio greater than 1 being increased up to 12%, and the upper 98% boundary for QT/TQ ratio increased up to 0.11 (Fig. 4).

DISCUSSION

In the previous TQT study, both QT_{cF} and QT_{cNi} were primary endpoints. Increases in QT_{c} were observed at later time points, between 12 and 24 hours postdose, not seemingly related to peak plasma concentrations of approximately 8 and 22 ng/mL that occurred approximately 3 to 6 hours after dosing 4 and 8 mg of guanfacine, respectively.² Since profound heart rate changes occurred throughout the study and it is known that alterations in autonomic state can potentially impact the interpretation of the corrected QT interval, QT_{btb} and ECG restitution analyses were employed in order to further characterize observed

changes in QT_{c} .⁴ These methods have been reported to differentiate changes in QT interval duration due to heart rate or autonomic state from impaired repolarization, and do not rely upon any heart rate correction factors.^{5,7}

QT_{btb} and QT_{cF} group mean analyses showed increases in QT interval duration exceeding ICH E14 criteria for a positive study after supratherapeutic concentrations achieved on Day 6 with a single 8 mg dose of guanfacine treatment. These data are further supported by the increase in outlier beats compared to Day –1 normal QT -RR interval boundaries, independent of heart rate. The magnitude of change with QT_{btb} was generally consistent with QT_{cF} at time points when heart rate changes were moderate on Day 1 after 4 mg of guanfacine. The QT_{btb} changes and percentage of outlier effects coincided with plasma concentrations/pharmacokinetics of guanfacine, whereas there was discordance with QT_{cF} and QT_{cNi} .² The disparity of the two measures is consistent with the well-known occurrence of undercorrection using QT_{cF} or QT_{cNi} formulas during bradycardic periods that occurred at maximal drug concentrations. Figure 5 shows a plot of the continuous beat-to-beat QT -RR intervals or “clouds” from a representative subject.

Figure 2. Mean percentage of beats on treatment (A) Day 1 and (B) Day 6 exceeding the upper 97.5% confidence bounds of QT interval for each individual's Day -1 baseline QT-RR relationship. Placebo—solid blue line; guanfacine—solid red with filled circles; moxifloxacin—solid black line with filled triangles. Dotted lines of same color represent upper and lower 90% confidence intervals. Shaded area between 3 and 6 hours represents time period of maximum plasma concentrations of guanfacine.

Beats at peak drug effect showed bradycardia with all QT intervals that were clearly beyond the upper 97.5% QT interval reference bounds. QT interval determinations at this time point indicated an appropriate increase in placebo-subtracted $\Delta\Delta\text{QT}_{\text{bfb}}$ of 14 ms but the same beats

corrected with QT_{cF} or QT_{cNi} indicated little or no change (0 and -6 ms, respectively) from baseline.

Since QT prolongation (via ICH E14) was observed, the correlation of these measures with arrhythmogenicity is another question that needs

Figure 3. Mean placebo-adjusted change from baseline in TQ interval (A) and QT/TQ ratio (B) for all subjects after 4 mg or 8 mg of guanfacine and moxifloxacin on Days 1 and 6. Guanfacine 4 mg—blue filled circles; guanfacine 8 mg—red filled circles; moxifloxacin on Day 1—black hollow triangle; moxifloxacin Day 6—black filled triangle. Dotted lines of same color represent upper and lower 90% confidence intervals. Shaded area between 3 and 6 hours represents time period of maximum plasma concentrations of guanfacine.

to be evaluated. Information exists as a result of over 20 years of commercial use of guanfacine for hypertension, often in people with high risk of arrhythmia, as well as 5 years of market data for Intuniv inpatients with ADHD. Since its marketing approval for hypertension, approximately 2.7 million patients have been exposed to guanfacine in the United States and over 1900 adverse events (AE) reports were submitted to FOI FDA-AERS database through 2008. Over 1500 publications including guanfacine as a keyword were identified

through MedLine and EMBASE database searches. A comprehensive review of the published data and the AEs reported to the FOI FDA-AERS database did not provide evidence of, or individual case reports pointing to an association of guanfacine with TdP. In addition, a review of the clinical data from the guanfacine- Intuniv program indicated no evidence of pro-arrhythmic effects

Arrhythmia liability is thought to be originated from either spatial¹⁵ or temporal electrical heterogeneities on the heart.^{16,17} Guanfacine

Figure 4. Mean placebo-adjusted change from baseline in lower 5% of TQ intervals (A), percentage of QT/TQ ratios > 1 (B), and the upper 2% of QT/TQ ratios (C) for all subjects after 4 mg or 8 mg of guanfacine and moxifloxacin on Days 1 and 6. Guanfacine 4 mg—blue filled circles; guanfacine 8 mg—red filled circles; moxifloxacin on Day 1—black hollow triangle; moxifloxacin Day 6—black filled triangle. Dotted lines of same color represent upper and lower 90% confidence intervals. Shaded area between 3 and 6 hours represents time period of maximum plasma concentrations of guanfacine.

Figure 5. Changes from baseline in QT beat-to-beat (QTbtb), Fridericia-corrected QT interval (QTcF), and individual-corrected QT interval (QTcNi) values for the same beats at time-matched placebo and 8 mg guanfacine treatment periods in comparison to the 24-hour normal boundary and the Fridericia correction line derived at baseline from a single-subject beat-to-beat QT-RR interval relationship.

reduces sympathetic influences to the heart leading to bradycardia and changes in the QT-RR dynamics. This reduction in sympathetic outflow limits the ability of the heart to accelerate rapidly and reduces heterogeneity¹⁸ thus mitigating the events reported to be associated with triggering TdP during drug-induced QT prolongation¹⁹ or LQT1/LQT2 syndromes.^{20,21} No morphology changes have been observed to date, indicating potentially minimal spatial considerations.² Consequently, an assessment of temporal arrhythmia liability was undertaken using quantifiable measures of dynamicity from the ECG beat-to-beat restitution.

Figure 6 shows an example of the restitution relationship as a function of heart rate in a representative subject during each treatment at 5 hours compared to the 24-hour baseline. At rest during supine measurement, the placebo shows a slight reduction in QT/TQ ratio from 0.72 to 0.67. Also note how very few beats exceed a QT/TQ ratio of 1 which results in a substantial lowering of the upper 98% quantile from a baseline 1.22 to 0.98. At approximately peak drug concentrations of both drugs producing maximum

QT prolongation, 8 mg of guanfacine shows a reduction in the QT/TQ ratio from 0.70 to 0.46 compared to an increase with moxifloxacin from 0.66 to 0.72.

Restitution analyses indicated that at all time points, a disproportionate effect to increase the TQ interval (rest) occurred more in relationship to each QT interval lengthening, resulting in a reduced QT/TQ ratio (an antiarrhythmic response). Additionally, the percentage of time and magnitude of stress on the heart, as measured by the upper limits of the QT/TQ ratio, were reduced with guanfacine. Unlike moxifloxacin, guanfacine causes a stabilizing effect on cardiac restitution that reconcile the clinical evidence for a lack of arrhythmia liability despite apparent increases in typical QT/QTc prolongation measures. These data help support the resulting Intuniv patient prescribing information label²² wording "An apparent increase in mean QTc was observed at both doses. However guanfacine does not appear to interfere with repolarization of the form associated with proarrhythmic drugs. The finding has no known clinical relevance."

Figure 6. Changes from the 24-hour normal baseline beat-to-beat QT/TQ interval ratios vs. RR interval values in a single subject at time-matched placebo, 8 mg guanfacine, and 400 mg moxifloxacin treatment periods.

CONCLUSIONS

Beat-to-beat and ECG restitution analyses may be more appropriate methodologies for risk assessment when QTc interval is affected by changes in autonomic state or heart rate. These results indicate that guanfacine causes a stabilizing effect on cardiac restitution that helps reconcile the clinical evidence for a lack of arrhythmia liability despite apparent increases in typical QT/QTc prolongation measures.

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REFERENCES

- DuMouchel W, Pregibon D. Proceedings of the Seventh ACM SIGKDD International Conference on Knowledge Discovery and Data Mining, San Diego, CA, August 26–29, 2001. New York: ACM Press; 2001. Empirical Bayes Screening for Multi-Item Associations; pp. 67–76.
- Freedom of information files. Center for Drug Evaluation and Research, application number 22–037, Medical Reviews. Available at http://www.accessdata.fda.gov/drugsatfda_docs/nda/2009/022037_intuniv_toc.cfm
- E14 Clinical evaluation of QT/QTc interval prolongation and proarrhythmic potential for non-antiarrhythmic drugs. Guidance to industry. Fed Regist 2005;70:61134–61135.
- Garnett CE, Zhu H, Malik MM, 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.
- Fossa AA, Wisialowski T, Magnano A, et al. Dynamic beat-to-beat modeling of the QT-RR interval relationship: Analysis of QT prolongation during alterations of autonomic state versus human ether a-go-go-related gene inhibition. J Pharmacol Exp Ther 2005;312: 1–11.
- Fossa AA, Wisialowski T, Crimin K, et al. Analyses of dynamic beat-to-beat QT-TQ interval (ECG restitution) changes in humans under normal sinus rhythm and prior to an event of torsades de pointes during QT prolongation caused by sotalol. Ann Noninvasive Electrocardiol 2007;12:338–348.
- Fossa AA, Zhou M. Assessing QT prolongation and electrocardiography restitution using a beat-to-beat method. Cardiol J 2010;17:230–243.
- Qu Z, Weiss JN. Dynamics and cardiac arrhythmias J Cardiovasc Electrophysiol 2006;17:1042–1049.
- Couderc J-P, Zareba W, Moss AJ, et al. Identification of sotalol-induced changes in repolarization with T wave area-based repolarization duration parameters. J Electrocardiol 2003;36:115–120.
- Couderc JP, Garnett C, Li M, et al. Highly automated QT measurement techniques in 7 thorough QT studies implemented under ICH E14 guidelines. Ann Noninvasive Electrocardiol 2011;16(1):13–24.
- R Development Core Team (2010). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>
- Karma A. Electrical alternans and spiral wave break-up in cardiac tissue. Chaos 1994;4:461–472.
- Karagueuzian HS, Chen P-S. Graded response and restitution hypotheses of ventricular vulnerability to fibrillation: Insights into the mechanism of initiation of fibrillation. J Electrocardiol 1999;32:S87–S91.
- Riccio ML, Koller ML, Gilmour Jr. RF. Electrical restitution and spatiotemporal organization during ventricular fibrillation. Circ Res 1999;84:955–963.
- Verrier RL, Antzelevitch C. Autonomic aspects of arrhythmogenesis: The enduring and the new. Curr Opin Cardiol 2003;19:2–11.
- Kleiger RE, Miller JP, Bigger JT Jr., et al. Decreased heart rate variability and its association with increased mortality after acute myocardial infarction. Am J Cardiol 1987;59:256–262.
- Satomi K, Shimizu W, Takaki H, et al. Response of beat-by-beat QT variability to sympathetic stimulation in the

- LQT1 form of congenital long QT syndrome. *Heart Rhythm* 2005;2:149–154.
18. Ng AG, Mantravadi R, Walker WH, et al. Sympathetic nerve stimulation produces spatial heterogeneities of action potential restitution. *Heart Rhythm* 2009;6: 696–706.
19. Locati EH, Maison-blanc P, Dejode P, et al. Spontaneous sequences of onset of Torsade de Pointes in patients with acquired prolonged repolarization: Quantitative analysis of Holter recording. *J Am Coll Cardiol* 1995;25: 1564–1575.
20. Schwartz PJ, Locati EH, Moss AJ, et al. Left cardiac sympathetic denervation in the therapy of congenital long QT syndrome – A worldwide report. *Circulation* 1991;84:503–511.
21. Schwartz PJ, Priori SG, Spazzolini C, et al. Genotype-phenotype correlation in the long-QT syndrome gene-specific triggers for life-threatening arrhythmias. *Circulation* 2001;103:89–95.
22. Intuniv® guanfacine, Extended-release tablets, highlights of prescribing information. Available at: http://pi.shirecontent.com/PI/PDFs/Intuniv_USA_ENG.pdf