Effects of Peginesatide Injection on QTc Interval in Healthy Adults

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Abstract

A single-dose, randomized, double-blind, placebo- and positive-controlled, three-period crossover study was conducted to evaluate the effect of peginesatide injection on QT interval in healthy adults. Subjects received single doses of placebo, peginesatide injection 0.1 mg/kg intravenous, or moxifloxacin 400 mg during three treatment periods, separated by 14-day washout intervals. ECG recordings and blood samples for peginesatide and moxifloxacin plasma concentrations were collected prior to dosing and through 22 hours postdose. QT intervals were measured with a high resolution manual on-screen caliper method. The study endpoint was the mean difference between peginesatide and placebo in baseline-adjusted corrected QT interval (ddQTc). The maximum upper bound of the one-sided 95% CI was 2.2 milliseconds at 0.75 hours for Fridericia-corrected ddQTc (ddQTcF) and 2.2 milliseconds at 0.25 hours for individual corrected ddQTcI. The linear relationship between ddQTcF and peginesatide concentrations was essentially flat and not statistically significant [slope = 0.001, $P = 0.126$, 90% CI: (< -0.0005, 0.002)]. Using this model, the projected ddQTcF effect at the observed mean peak plasma concentration is estimated to be 0.9 milliseconds, 90% CI: (-2.0, 0.3 milliseconds). There were no peginesatide-related effects on heart rate, PR interval, or QRS interval. Thus, there is no anticipated cardiovascular effect of peginesatide injection 0.1 mg/kg in patients.

Peginesatide (OMONTYS®; Takeda Pharmaceuticals America, Inc., Deerfield, IL) is a novel, synthetic, dimeric peptide linked to polyethylene glycol that is designed to specifically activate the erythropoietin receptor and stimulate erythropoiesis.[1] It was approved in the United States for once-monthly administration for the treatment of anemia due to chronic kidney disease (CKD) in adult patients on dialysis in March 2012 and was subsequently voluntarily recalled in February 2013 because of new postmarketing reports regarding serious hypersensitivity reactions. The molecular size of peginesatide (44.8 ± 4.5 kDa) and preclinical data suggest it is unlikely to exert electrophysiologic cardiovascular effects in humans. In vitro and in vivo studies show that peginesatide undergoes negligible metabolism (data on file) and it is unlikely that the intact PEGylated peptide interacts with ionic channels in the cell membrane or is transported into the cell due to its size. In preclinical studies, peginesatide did not cause appreciable inhibition of the human *ether-à-go-go*-related gene potassium current at a concentration approximately 31-fold higher than the observed plasma concentration for a human dose of 0.35 mg/kg, which was the upper limit of the maximum dose for 95% of patients in Phase 3 clinical trials in dialysis patients (data on file). This suggests negligible potential for an in vivo inhibitory effect on cardiac potassium channel conduction.

This study was undertaken to confirm the cardiovascular safety of peginesatide injection with regard to the QT interval, to evaluate the relationship between peginesatide plasma levels and QT interval, and to examine the safety profile following a single dose of peginesatide injection 0.1 mg/kg intravenous (IV) in healthy subjects.

Methods

Subjects

Healthy male and female subjects between 18 and 50 years of age with a body mass index ≥ 18 and $\leq 30\,kg/m^2$ were eligible for inclusion in the study.

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Subjects must have had a resting blood pressure between 90 and 140 mmHg systolic and 50–90 mmHg diastolic, and a heart rate (HR) between 45 and 90 bpm at screening and check-in.

Subjects with ECG abnormalities, including QTcF > 450 milliseconds were excluded. Subjects with a hemoglobin (Hb) level ≥ 15 g/dL for men or ≥ 14 g/dL for women, white blood cell count $\leq 4.0 \times 10^9$/L, neutrophil count $\leq 1.8 \times 10^9$/L, or platelet count $\leq 140 \times 10^9$/L or $> 400 \times 10^9$/L at screening or check-in were also excluded. Other exclusion criteria included documented history of clinically significant arrhythmias, history of seizure or unexplained syncopal episodes, family history of Long QT Syndrome, and standard criteria used in studies in healthy volunteers.

Study Design

The study was designed in keeping with the E14 International Committee on Harmonization (ICH) Guidance for Industry on the Clinical Evaluation of QT/QTc interval prolongation.[2] It was conducted at two sites in the United States between April 2009 and August 2009, in accordance with the Declaration of Helsinki and the ICH Harmonized Tripartite Guideline for Good Clinical Practice. The study protocol was approved by the Independent Investigational Review Board (Plantation, FL). Written informed consent was obtained from each subject prior to the initiation of any study procedures.

This was a Phase 1, single-dose, randomized, double-blind, placebo- and positive-controlled, three-period crossover study. Each treatment period consisted of a 3-day confinement period, with the dosing days separated by 14-day washout intervals (Figure 1A). Adverse events were monitored from screening to 30 days after the last dose of study drug.

Subjects were randomized to one of six treatment regimen sequence groups, determined using a Williams Square adjusted for first-order carry-over effects (Figure 1B). Peginesatide injection and corresponding placebo were administered IV as a bolus injection over 30 seconds. Over-encapsulated moxifloxacin and corresponding placebo were administered orally with 240 mL water 1 hour after peginesatide or placebo injection (i.e., at Hour 1).

Study ECGs were acquired from a 24-hour Holter recording. On the day of dosing in each treatment period, three 10-second ECG recordings were extracted at approximately Hour -1, -0.5, -0.25, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 10, and 22, relative to the peginesatide or placebo injection. All ECG measurements were performed at a central laboratory (eResearch Technology, Inc., Philadelphia, PA) and were interpreted by United States board-certified cardiologists. All ECG readers and technicians were blinded to study treatment, timepoints, and subject identifiers (including age and gender).

The dose of peginesatide (0.1 mg/kg) was selected based on the median dose used in subjects on
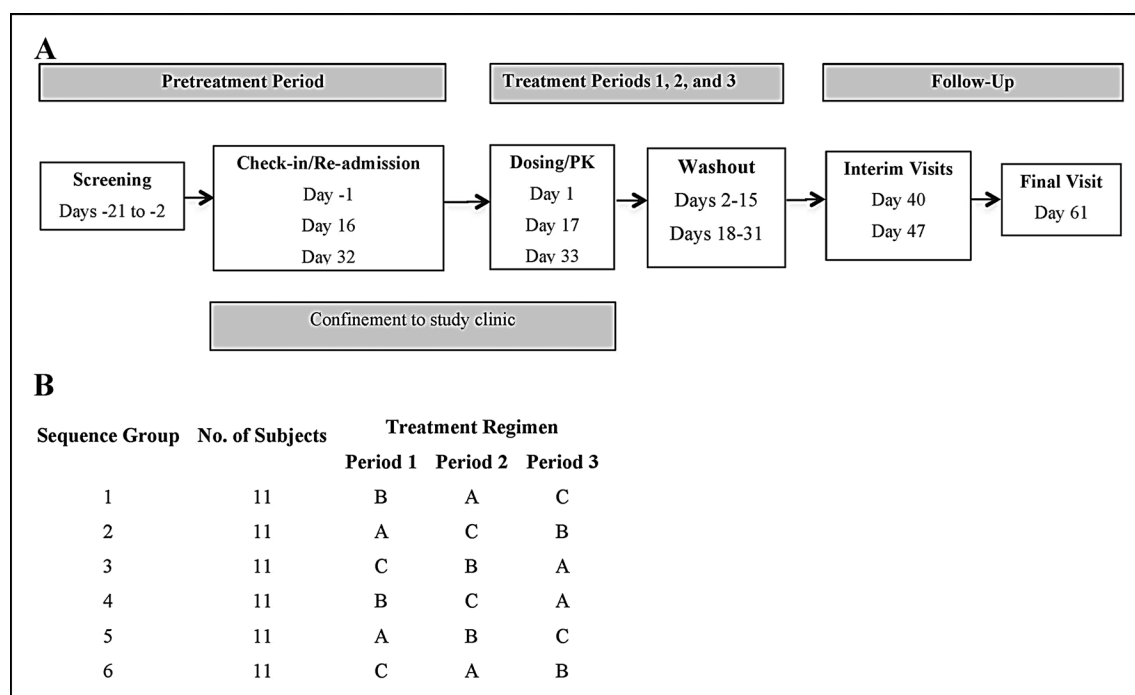
Figure 1. (A) Study flow diagram. (B) Treatment sequences. Regimen A: peginesatide placebo (0.9% sodium chloride) plus moxifloxacin placebo. Regimen B: peginesatide injection plus moxifloxacin placebo. Regimen C: peginesatide placebo plus moxifloxacin.

hemodialysis (0.09 mg/kg every 4 weeks; range 0.02–0.5 mg/kg). A 0.1 mg/kg dose was determined to be the highest dose that could be safely administered to nonanemic healthy adults, minimizing the risk of absolute Hb levels >18 g/dL or an excessive rate of rise in Hb (>1 g/dL over 2 weeks), either of which could increase the potential for cardiovascular events such as hypertension or thrombotic events. A single oral dose of moxifloxacin 400 mg is the standard positive control accepted in QT/QTc studies in healthy subjects.[3]

The endpoints for the study were the mean difference in the postdose time-matched baseline-adjusted corrected QT intervals between peginesatide and placebo (ddQTc) using three different correction methods: individual corrected QTc (QTcI), QTcF, and QT interval corrected using the Bazett formula (QTcB). The number and percentage of subjects with time-matched increases >30 and >60 milliseconds from baseline and the number and percentage of subjects with QT intervals >450, >480, and >500 milliseconds were also determined.

Statistical Analyses

QTc values were derived using three different correction methods (QTcI, QTcF, and QTcB).[4–6] Linear mixed-effect models with random intercepts and slopes were used to evaluate these three HR correction methods.[7] Baseline for the postdose QT/QTc in a period was defined as the average of the QT/QTc values at three predose time points (−0.25, −0.5, and −1 hour) in that period.

Assessment of the peginesatide effect on QT/QTc prolongation was performed using a linear mixed-effect model with repeated measures for the baseline-adjusted QTc (dQTc) values. The fixed factors in the model were sequence, period, treatment, scheduled time points, the interaction between treatment and the scheduled time points, and the interaction between period and scheduled time points. Subject within sequence was the random factor. One-sided 95% upper confidence intervals (CIs) were constructed for the difference between peginesatide and placebo (ddQTc) and "no QT prolongation effect" of peginesatide was declared if the upper CI at each time point was no higher than 10 milliseconds.

The study's ability to detect a small QTc effect was validated by the effect of moxifloxacin on QT/QTc. Assay sensitivity was confirmed if the lower bound of the one-sided 95% CI of the ddQTc was greater than 5 milliseconds for at least one time point between Hours 2 and 5 (i.e., 1 and 4 hours post-moxifloxacin dosing). The Bonferroni adjustment was employed to account for multiple comparisons.[8]

Pharmacokinetic parameters were derived using non-compartmental methods using peginesatide and moxifloxacin concentration–time data and actual sampling times relative to dose and summarized using descriptive statistics.

The relationship between ddQTc and peginesatide concentration was characterized using a linear mixed-effect model with random intercept and slope. The mean ddQTc at relevant concentration levels (i.e., mean C_{max} under therapeutic dose) was estimated using the observed mean C_{max} and the population slope estimated from the linear mixed-effect model.[9,10]

The incidence of treatment-emergent adverse events (TEAEs), serious TEAEs, and TEAEs leading to study discontinuation were summarized by regimen using descriptive statistics.

Bioanalytical Methods

Blood samples for the measurement of peginesatide plasma concentrations were collected prior to dosing and immediately after each of the ECG extractions. Samples for the measurement of moxifloxacin plasma concentrations were collected after the peginesatide sample at Hour 1 (prior to moxifloxacin dose), 2, 3, 4, 5, 6, 7, 10, and 22. All samples were centrifuged and the plasma was stored at −20°C until analysis. Concentrations below the lower limit of quantitation (LLOQ) were set to 0 for pharmacokinetic analyses.

Peginesatide concentrations were measured using a validated enzyme-linked immunoabsorbent assay. Human plasma containing antigen (peginesatide) and anti-peginesatide antibody were pre-incubated for 30 minutes. The pre-incubated solution was added onto a microplate previously coated with the antigen and the microplate was incubated at room temperature for approximately 1 hour. Subsequently, anti-rabbit immunoglobulin antibody conjugated with alkaline phosphatase was added and the microplate was incubated at room temperature for 1 hour. Finally, p-nitrophenyl phosphate (pNPP) substrate solution was added and the microplate was incubated at room temperature for 36–46 minutes, followed by the addition of base to stop the reaction. The absorbance at 450 nm was measured using a microplate reader. A standard curve was fit to a four-parameter logistic equation, from which the concentrations were calculated. The standard curve range was 25–750 ng/mL with additional anchor points included. The LLOQ was determined to be 25 ng/mL. The intra-day precision and accuracy ranged from 0.7% to 22.8% and 78.7% to 117.8%, respectively. The inter-day precision and accuracy ranged from 7.2% to 16.0% and 90.5% to 102.9%, respectively.

Moxifloxacin concentrations were measured using a validated high performance liquid chromatography (HPLC)/tandem mass spectroscopy (MS/MS) assay. Human plasma containing moxifloxacin was fortified with internal standard, d4-moxifloxacin, and extracted using an Oasis HLB SPE cartridge. The final eluent was evaporated to dryness and then reconstituted in 300 μL of 0.1% formic acid. An aliquot, 25 μL, of the final extract was injected onto an HPLC with MS/MS detection. The

HPLC column was a 50 mm × 2.1 mm, 3 μm, BDS Hypersil C_{18} column. The HPLC separation was achieved using a flow rate of 0.300 mL/min and gradient with a mobile phase A of 5.0 mM ammonium formate and mobile phase B of 1.0% formic acid in acetonitrile. Initial conditions were 10% mobile phase B to 20% mobile phase B over 1 minute and then an increase to 40% mobile phase B over the next 1.5 minutes. The column was then returned to initial conditions for reinjection at 5 minutes. The Mass Spec used electrospray in the positive ion mode with MRM for moxifloxacin ions at 402.1 and 384.1 and for d4-moxifloxacin ions at 406.3 and 388.2 m/z. The standard curve range was 25.0 to 5000 ng/mL; the LLOQ was determined to be 25.0 ng/mL. The intra-day precision and accuracy (% difference from theoretical) ranged from 3.89% to 4.83% and −0.957% to 3.44%, respectively.

Results

Subject Population

Sixty-five subjects were enrolled in the study, 4 subjects prematurely discontinued (2 due to TEAEs, 1 due to a positive alcohol test, and 1 with voluntarily withdrawal), and 61 subjects completed all 3 regimens. Sixty-two subjects received placebo, 64 received peginesatide, and 62 received moxifloxacin. The mean age of all subjects was 35.6 years (range 19–50 years); 19 subjects (29.2%) were men and 46 (70.8%) were women. A majority (70.8%) of the subjects were white and 44.6% were Hispanic or Latino.
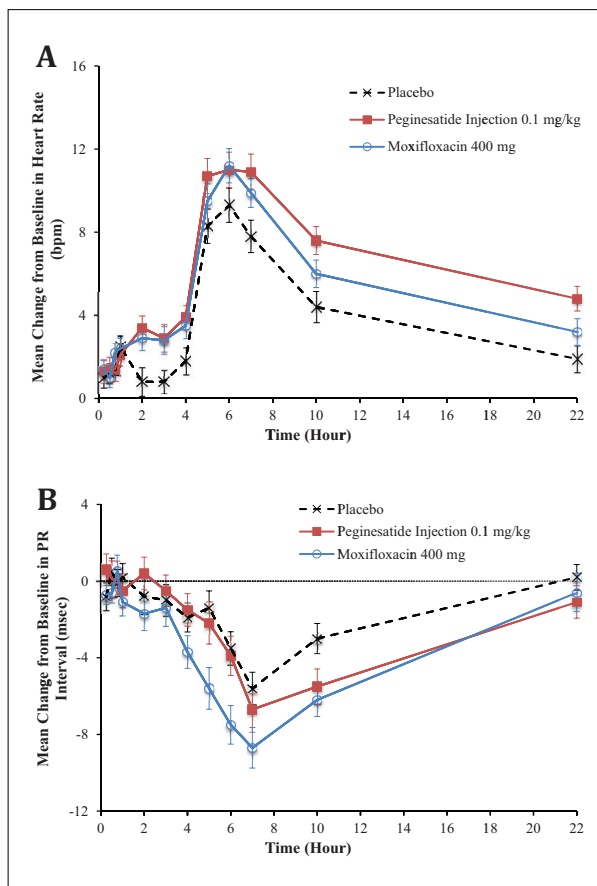
Heart Rate, PR Interval, and QRS Interval

There were apparent increases in HR between Hours 5 and 10, which were similar for all three regimens (Figure 2A). These increases were not drug related and occurred after the observed mean T_{max} for peginesatide (0.8 hours) or moxifloxacin (2.4 hours). There were small decreases in PR interval starting at Hour 5 for all three regimens. The mean changes from baseline in PR interval were <10 milliseconds and were similar for all three regimens (Figure 2B). There were no apparent changes from baseline in QRS interval with any of the three regimens (data not shown).

QT Assessments

Of the three correction methods evaluated, the QTcF method gave the best HR correction for QT intervals for each of the three regimens (peginesatide injection, moxifloxacin, placebo) as well as all three regimens combined. The mean of the squared individual QTcF/RR slopes for three-regimen combined data was only 0.00059.

Evaluation of the effect of peginesatide on QTcF, QTcI, and QTcB yielded consistent results. The results from QTcF are presented here.

Figure 2. (A) Mean (SE) change from baseline in heart rate for each regimen. (B) Mean (SE) change from baseline in PR interval for each regimen. Times are relative peginesatide/placebo injection (administered at Hour 0); moxifloxacin was administered at Hour 1.

Decreases from baseline in QTcF were observed at all timepoints following peginesatide injection and these changes were similar to those observed following placebo. In contrast, following moxifloxacin administration, increases from baseline were observed at all timepoints except at Hour 7 (Figure 3A).

The maximum upper bound of the one-sided 95% CI for the difference in the least-square means between peginesatide and placebo (baseline adjusted) from the linear mixed-effect model was 2.2 milliseconds at Hour 0.75 for ddQTcF and 2.2 milliseconds at Hour 0.25 for ddQTcI. The time-matched least squares mean difference in ddQTcF between moxifloxacin and placebo began to increase at Hour 2 (i.e., 1 hour after moxifloxacin dosing) and the lower bound of the one-sided 95% CI was >5 milliseconds from Hours 3 to 10 (i.e., 2–9 hours after moxifloxacin dosing). The peak effect occurred at Hour 3 (Figure 3B). Similar prolongations were observed for QTcI after moxifloxacin administration (data not shown).
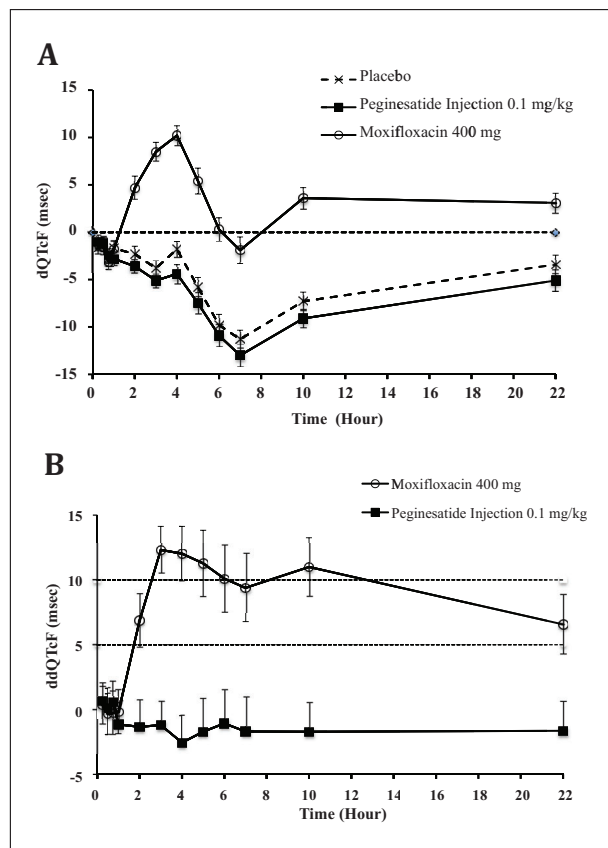
Figure 3. (A) Mean (SE) change from baseline in QTcF (dQTcF) for each regimen. (B) Mean (95% CI) placebo-adjusted change from baseline (ddQTcF) for peginesatide and moxifloxacin. The difference from placebo (ddQTcF) in least-square means of the change from baseline (dQTcF) are from the linear mixed-effects model with repeated measures. Times are relative to peginesatide/placebo injection (administered at Hour 0); moxifloxacin was administered at Hour 1.

QTcF intervals >450 but ≤480 milliseconds were observed for one subject (1.6%) following placebo, 0 subjects following peginesatide injection, and five subjects (8.3%) following moxifloxacin. No subjects had QTcF interval >480 milliseconds and no subject had a >30 milliseconds increase in QTcF following any regimen. No U-wave was observed in any subject.

Relationship Between Change in QTc Interval and Peginesatide Plasma Concentrations

The mean C_{max} values for peginesatide and moxifloxacin were 3334 and 2270 ng/mL, respectively. As shown by the concentration-time data for both drugs (Figure 4), QT was measured at the time of C_{max} for both peginesatide and moxifloxacin.

Using the linear mixed-effects modeling to evaluate the relationship between ddQTcF and peginesatide plasma concentrations showed that at the mean peginesa-
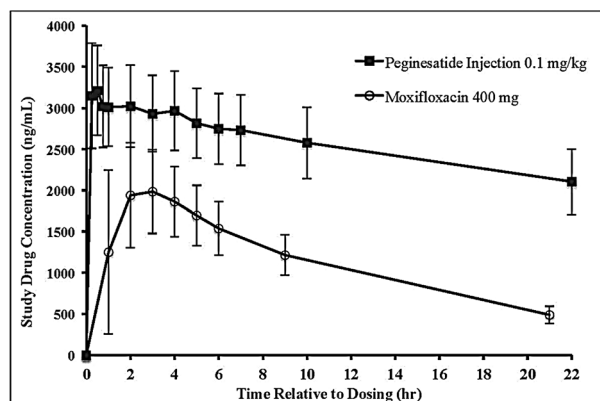
Figure 4. Mean (SD) peginesatide and moxifloxacin plasma concentrations over time. Times are relative to actual administration of each drug.

tide C_{max} for 0.1 mg/kg, the ddQTcF was −0.9 milliseconds, 90% CI: (−2.0, 0.3 milliseconds). The linear relationship between ddQTcF and peginesatide concentration was not statistically significant (slope = 0.001, $P = 0.126$, 90% CI: [<−0.0005, 0.002]).

Adverse Events

The percentage of subjects experiencing at least one TEAE was comparable between the placebo and peginesatide regimens, but slightly higher with moxifloxacin (29.0%, 31.3%, and 41.9% for placebo, peginesatide, and moxifloxacin, respectively). The types of TEAEs were generally similar across regimens; TEAEs reported for >5% of subjects for any regimen were headache (9.7–14.1%), dizziness (1.6–6.5%), and nausea (1.6–8.1%). No subject had a cardiac disorder TEAE following peginesatide injection and all TEAEs following peginesatide injection were mild or moderate in severity. Two subjects discontinued the study due to TEAEs, one with sinus arrhythmia after receiving placebo and one with elevated creatine kinase after receiving peginesatide injection. Neither event was related to study drug. No serious adverse events or deaths were reported.

Discussion

This single-dose, randomized, double-blind, placebo- and positive-controlled, three-period crossover study was undertaken to confirm the cardiovascular safety of peginesatide injection with regard to the QT interval following a single dose of peginesatide injection 0.1 mg/kg IV in healthy subjects. The study demonstrated that peginesatide does not have a prolongation effect on QTc interval or on any other ECG parameters of clinical concern.

Three correction methods, QTcI, QTcF, and QTcB, were used in this study. They were evaluated using the method proposed by the FDA's interdisciplinary review

team,[7] where the appropriateness of each correction method is evaluated accounting for the evaluation of the correction for individual subjects. Examination of the means of the squared individual QTc/RR slopes for the different correction methods showed the lowest (i.e., the flattest slope) was for QTcF, which therefore gave the best correction for HR changes in this study.

Assay sensitivity was demonstrated through the QTcF effect of the positive control, moxifloxacin, for which the lower bound of ddQTcF was above 5 milliseconds at all four prespecified timepoints (Hours 2, 3, 4, and 5).[2,8] The mean difference in dQTcI and dQTcF between moxifloxacin and placebo peaked 2 hours after administration (at Hours 3 and 4) at around 12 milliseconds and was approximately 10 milliseconds at all timepoints from 2 to 9 hours post-moxifloxacin dose.

This study demonstrated that peginesatide injection does not prolong the QT interval in healthy adult subjects. According to ICH guidelines,[2] the threshold for regulatory concern for mean QT prolongation is around 5 milliseconds, as evidenced by an upper bound of the one-sided 95% CI of >10 milliseconds. In this study, across all the evaluated postdose time points, the highest upper bound for the one-sided 95% CI of the difference in the least-square means of the baseline adjusted QTcI between peginesatide injection and placebo was 2.2 milliseconds at 0.75 hours postdose for QTcF and 2.2 milliseconds at 0.25 hours for QTcI. In addition, no subject had a QTcF >450 milliseconds or an increase of >30 milliseconds in QTcF at any time following peginesatide injection.

The linear relationship between ddQTcF and peginesatide concentrations was not statistically significant, which clearly supports the lack of effect of peginesatide on the changes in QTc interval. Therefore, it is not appropriate to project the QTc effect at higher peginesatide concentrations due to the lack of association.

Increases in HR and decreases in PR interval were observed with all three regimens between Hours 5 and 10, and these changes were similar for all regimens at all timepoints. The HR changes were likely due to the increased freedom of movement allowed for the subjects after the intense monitoring during the first few hours after study drug administration.

The most common TEAEs in this study were headache and dizziness for each regimen, and all TEAEs with peginesatide injection were mild or moderate in intensity.

One limitation of this study was the choice of the therapeutic peginesatide injection dose (0.1 mg/kg) rather than a supratherapeutic dose. This was based on the results of an earlier study demonstrating that this dose was associated with clinically and statistically significant increases in Hb in patients with CKD.[11] Supratherapeutic doses were not used in order to minimize the risk to subjects of either an excessive absolute Hb level or an

excessive rate of rise in Hb levels, either of which could increase the potential for cardiovascular events such as hypertension and thrombotic events. The mean C_{max} for peginesatide (3334 ng/mL) in this study was similar to that observed for a 0.1 mg/kg dose in previous clinical studies.[12,13] The mean C_{max} for moxifloxacin (2270 ng/mL) was also similar to that published elsewhere.[3]

Preclinical studies indicated that peginesatide would be unlikely to exert electrophysiological effects in humans. The results of this study confirm this finding and demonstrate that peginesatide injection 0.1 mg/kg IV does not cause QTc prolongation and that there is no drug-related effect on the HR, PR interval, or QRS interval. Thus, there is no anticipated cardiovascular effect of peginesatide injection 0.1 mg/kg in patients with CKD.

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