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CLINICAL TRIALS

Thorough QT study of the effect of intravenous amisulpride on QTc interval in Caucasian and Japanese healthy subjects

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Keywords amisulpride, Thorough QT study, QTc interval

AIM

The D_2/D_3 antagonist amisulpride has shown promising efficacy against postoperative nausea and vomiting (PONV) at low doses. We investigated whether intravenous amisulpride has an effect on the QTc interval in a formal Thorough QT study (TQT).

METHODS

This was a randomized, double-blind, placebo and positive-controlled, four-way crossover study. Forty healthy Caucasian and Japanese subjects were included to receive a single administration of 5 mg and 40 mg of i.v. amisulpride or a single oral dose of moxifloxacin or placebo per period.

RESULTS

The therapeutic dose of 5 mg amisulpride was associated with a slight, transient increase in mean ΔΔQTcF, from 2.0 ms prior to dosing to a peak of 5 ms (90% CI: 2.8, 7.1 ms) at 8 min, decreasing to 2.1 ms at 30 min after dosing. The supra-therapeutic dose of 40 mg given at twice the infusion rate was associated with prolongation in ΔΔQTcF peaking at 23.4 ms (90% CI: 21.3, 25.5 ms) at the end of infusion (8 min), returning below 10 ms within 1.5 h. Assay sensitivity was confirmed; ΔΔQTcF had increased by 12.3 ms (90% CI 10.1, 14.6 ms) at 4 h post-dose. The PK-PD relationship revealed no differences between Caucasian and Japanese subjects (p-value > 0.5).

CONCLUSIONS

Amisulpride has a plasma concentration-dependent effect on the QTc interval. The proposed therapeutic dose for management of PONV does not lead to a prolongation of QTcF above the threshold of regulatory concern, while such effect could not be excluded for the supratherapeutic dose.

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Amisulpride is an atypical antipsychotic approved for the treatment of acute and chronic psychoses.
- Overdoses of amisulpride are known to have effects on cardiac repolarization and the assessment of the potential for the drug to prolong the QT interval when administered intravenously remains to be elucidated.
- Much lower doses than those used to treat psychoses have been shown to be effective at preventing postoperative nausea and vomiting when given intravenously.

WHAT THIS STUDY ADDS

- At the therapeutic dose of 5 mg amisulpride, the attained C_{max} was comparable to the optimum plasma concentrations to treat psychoses using higher oral doses.
- The proposed therapeutic dose of 5 mg amisulpride given over 2 min does not prolong QTc interval.
- A supratherapeutic dose of 8 times higher than the therapeutic dose infused at double the rate led to ΔΔQTcF prolongation of 23.4 ms at the end of infusion and to a C_{max} concentration of 1305.44 ng ml^{-1}, similar to the largest available oral presentation of amisulpride.

Tables of Links

TARGETS	
GPCRs [2]	**Voltage gated ion channels [3]**
D_2 receptor	hERG
D_3 receptor	$Na_v1.5$

LIGANDS
Amisulpride

These Tables lists key protein targets and ligands in this article that are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2, 3].

Introduction

Amisulpride is a substituted benzamide that acts as a selective antagonist of dopamine D_2 and D_3 receptors [4]. It was introduced in the 1980s as an 'atypical' antipsychotic and has been approved in many countries for the treatment of acute and chronic psychoses at a dose range between 50 and 1200 mg per day, given orally or intramuscularly [5, 6]. The overall safety profile of amisulpride at its approved doses has been assessed in numerous clinical studies with no evidence of cardiac toxicity [6–8]. However, very large overdoses of oral amisulpride have been associated with the development of torsade de pointes (TdP) [9–13] and there is a solitary case report in the literature suggesting that TdP may have been caused by a low oral dose of 100 mg day^{-1} in a patient with a low body mass index [14].

In *in vitro* testing, amisulpride has been shown to have a low potential for blockade of the cardiac hERG channel, with a high IC_{50} value of approximately 100 μM for inhibition of the repolarizing delayed rectifier potassium current, I_{Kr}, and less than 20% displacement of [^{3}H]-astemizole binding [15]. Amisulpride has also been shown to bind to $Na_V1.5$ and the effects of late I_{Na} blockade ability have been suggested to reduce the impact on QT interval due to hERG blockade [16].

QT response may differ between ethnic groups and this may be attributable to inter-ethnic differences in the frequency of variant alleles of the genes SCN5A, KCNH2, KCNE2, KCNQ1 and KCNE1 encoding for the sodium channel $Na_V1.5$ and four potassium channels respectively [17–19].

Recently, an intravenous (i.v.) dose of 5 mg amisulpride was shown to be superior to placebo at preventing postoperative nausea and vomiting [20]. As part of the clinical development programme for amisulpride, we conducted a specifically designed Thorough QT/QTc (TQT) study, in accordance with the ICH E14 guideline, in order to quantify the effect of i.v. amisulpride on the QTc interval at the therapeutic dose proposed for PONV management and at an eightfold supratherapeutic dose. Additionally, the study compared the PK-QTc relationship in two ethnic groups: Caucasian and Japanese.

Methods

The study was given a positive opinion by an authorized Research Ethics Committee (NRES Committee South Central – Berkshire B, ref: 13/SC/0496), approved by the Medicines and Healthcare products Regulatory Authority and registered with EudraCT (ref: 2013-002 669-20) and ClinicalTrials.gov (ref: NCT02661594). The trial was conducted at a single centre (Richmond Pharmacology Ltd, UK) between 22 November 2013 and 31 March 2014. Its conduct was in accordance with the principles of the Declaration of Helsinki, current UK law and Good Clinical Practice (GCP) guidelines. Each subject received verbal and written information followed by signing of the Informed Consent Form (ICF) prior to any procedures taking place.

Subjects

General eligibility of 102 subjects volunteering for participation in this study was assessed at screening, which took place

within 21 days of the first study drug administration. Male and female subjects were included if they were healthy, non-smoking, Caucasian or Japanese, aged between 20 and 45 years and had a body mass index ranging between 18 and 25 kg m^{-2} inclusive. Subjects were excluded if they had: (i) a medical or family history of congenital long QT syndrome; (ii) a clinically significant abnormal ECG (PR consistently <120 or >230 ms, QRS consistently ≥120 ms, QTcB >430 ms for males and >450 ms for females, heart rate consistently <45 beats min^{-1} or >100 beats min^{-1}); (iii) if they used any concomitant medication that impaired drug metabolizing capacity or was known to have a QT-prolonging effect; or (iv) had any intolerance to moxifloxacin or amisulpride.

Study design

This was a randomized, double-blind, placebo and positive-controlled, four-way crossover study using features of an adaptive study design, including the potential to add subjects depending on the standard deviation obtained during a blinded ECG review allowing the sample size to be adapted optimally to the variability occurring in this particular trial [21]. The 40 eligible subjects were randomized to one of four sequences of the four study treatments (Figure 1): oral moxifloxacin 400 mg (M), i.v. amisulpride 5 mg (A5), i.v. amisulpride 40 mg (A40) and i.v. normal saline as a placebo (P). The four sequences (A5-A40-M-P, A40-P-A5-M, P-M-A40-A5 and M-A5-P-A40) were balanced for period and preceding treatment (Williams squares). Each treatment period consisted of a placebo baseline day (Day −1), followed by the treatment day (Day 1). A washout period of at least seven days separated treatment periods. ECGs and blood samples for PK analysis were taken at the same time points on Days −1 and 1 of every period. Subjects attended for a follow-up visit scheduled 7–14 days after the last dose.

Intravenous study treatments were fully blinded and administered from two syringe drivers starting at the same time, one infusing 2.5 ml over 2 min, the other infusing 20 ml over 8 min. The 5 mg amisulpride dose was given as 2.5 ml of 2 mg ml^{-1} amisulpride over 2 min in parallel with normal saline in the other syringe driver. The 40 mg amisulpride dose was given as 20 ml of 2 mg ml^{-1} amisulpride over 8 min in parallel with normal saline in the other syringe driver. For the placebo treatment, both syringe drivers contained normal saline. Moxifloxacin was given unblinded as a single, commercially-sourced, 400 mg oral tablet, preceded on Day −1 by a single, inactive moxifloxacin-placebo tablet.

ECG assessment and QTc evaluation

Twelve-lead ECGs were recorded using a MAC1200® ECG recorder (GE Healthcare, Buckinghamshire, UK) and stored electronically on the Medical MUSE® information system (GE Healthcare, Buckinghamshire, UK). ECG recordings were made at the following time points: pre-dose, 2, 8 and 30 min, 1, 1.5, 2, 3, 4, 5, 6, 8, 12 and 24 h post-dose of each treatment period. Before any ECG recording, the subjects maintained an undisturbed supine resting position for at least 10 min and avoided postural changes during the ECG recordings. At each time point, the ECGs were recorded in triplicate, to reduce

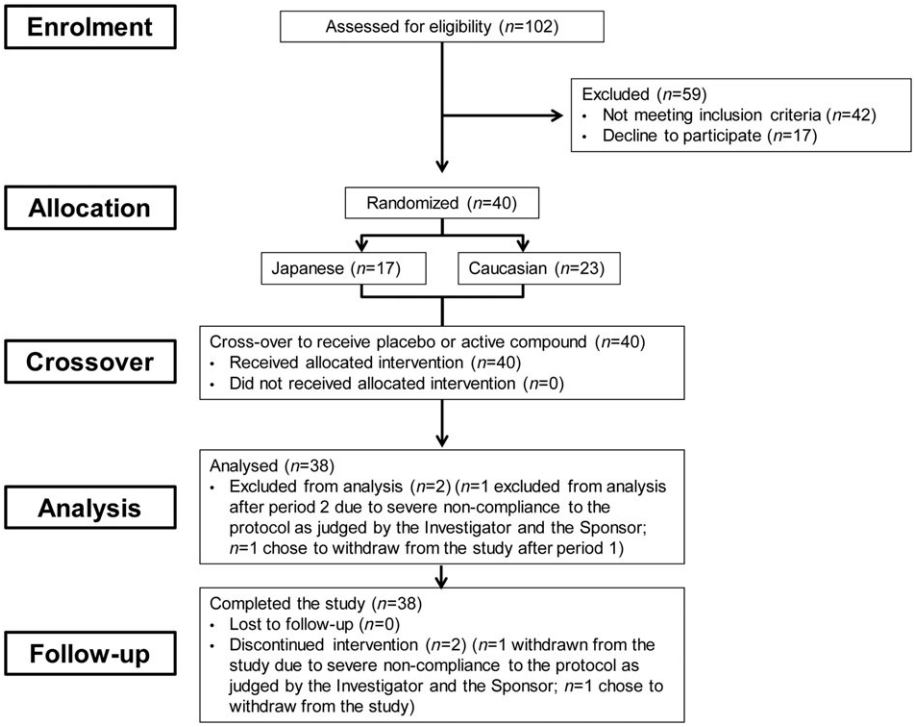

Figure 1
Study flow chart

variance and improve the precision of measurement. Each ECG lasted 10 s. The triplicates were performed at one-minute intervals over 3 min. The QT interval, RR interval and heart rate, PR interval and QRS duration, the presence or absence of U-waves, quantitative and qualitative ECG variations were assessed by cardiologists with extensive experience of manual on-screen over-reading with electronic callipers using the commercially available MUSE® in its latest version to correct any implausible readings presented by the automated process. During hospitalization, subjects were served breakfast before dosing, lunch and dinner with similar nutritional composition at approximately 6 h and 12 h post-dose, respectively. On baseline and treatment days, breakfast was identical across all periods and was served 1 h and completed 30 min prior to allocated dose administration.

Statistical analysis

The primary analysis was based on the crossover design of the study and used the change from average baseline of QTcF of the baseline day. For each time point, a linear mixed effects model with sequence, period, gender and race and treatment as fixed effects and baseline as covariate was adapted, with subject (nested in sequence) as a random effect. Two-sided 90% confidence intervals for the difference between each dose of amisulpride and placebo (safety) and moxifloxacin and placebo (assay sensitivity) were derived. All subjects in the safety dataset who had valid ECG data for at least one post-dosing time point during periods 1–4 were included in the primary analysis set. To show assay sensitivity, a Hochberg procedure was applied to the results of the 2, 3 and 4 h time points of the difference between placebo and positive control (moxifloxacin). Baseline was calculated from the Day −1 values of the respective period. Two types of baseline were used: the averaged baseline for the primary analysis and a time-matched one for a secondary analysis. Baseline ECGs were scheduled to match the on-treatment clock time points for each treatment period. Categorical analyses were performed to determine the number of subjects per treatment regimen and time who had values >450 ms as well as an increase from baseline QTc interval ≥30 ms and ≥60 ms by gender. Furthermore, a linear concentration response model was fitted to all QTcF data in the two amisulpride groups. The placebo-corrected change from average baseline (ΔΔQTcF) was used as a dependent variable. The model had a fixed intercept and slope but random intercepts only. The absence of hysteresis was confirmed graphically. For each dose group the placebo-corrected change from baseline in QTcF was plotted over time as well as the drug concentration of the respective dose group. If maximum ΔΔQTcF was delayed compared with the respective plasma concentration by at least 1 h, a model with an additional effect compartment was to be used to replace the mixed effects model described above. However, this replacement was only to be done if the maximal effect of amisulpride (point estimate) as estimated in the primary analysis was 5 ms or above. To assess differences between the Caucasian and Japanese subjects, a factor ethnicity and its interaction with concentration were added to the model and tested against a null hypothesis of no effect. The models were graphically presented by a regression line together with a two-sided 90% confidence region. For the model with ethnicity, separate regression lines for Caucasians and Japanese were given.

The appropriateness of a linear model was assured by inspecting the goodness of fit, for example by looking at normal QQ plots for the residuals and plots of the residuals over predicted values. If there was an indication that a linear model was inappropriate, the nonlinearity detected was to be taken into account by an appropriate transformation of the concentration values (e.g., log(conc/lloq)) or by the use of an appropriate nonlinear model.

Pharmacokinetic assessments

Pharmacokinetic samples were taken over a 24 h period on Day 1 of each period. Timings for pharmacokinetic blood sampling were coincident with ECG assessment time points. Plasma samples for determination of amisulpride concentration were analysed by Quotient BioAnalytical Sciences. A validated liquid chromatography/tandem mass spectrometry (LC/MS/MS) method with liquid–liquid extraction has been used, developed and satisfactorily validated for the measurement of amisulpride in human plasma over the calibration range 2–2000 ng ml^{-1} with a lower limit of quantification of 2 ng ml^{-1}. Validation procedures were based on those outlined in the 'Guideline on Bioanalytical Method Validation' [22].

Chromatographic separation was performed through a Waters CSH C18 (1.7 µm, 2.1 × 50 mm, Waters Corporation, Milford, Massachusetts, USA) analytical column at 40°C, with a mobile phase consisting of 10 mM ammonium formate in 0.2% formic acid. The flow rate was 0.6 ml min^{-1}, and the total run time was 3 min. Detection and quantification were performed by mass spectrometry using a Sciex API5000 mass spectrometer (AB Sciex LLP, Framingham, Massachusetts, USA).

The precision and accuracy (expressed as the coefficient of variation (% CV) and as the relative error (% RE) respectively) of the method was found to be within the target limits of ±20% at the lower limit of quantification and within ±15% at all other concentrations. The recovery of amisulpride from human plasma was reproducible and acceptable. The selectivity of the method was found to be satisfactory with no endogenous interference which may adversely affect the analysis. Amisulpride was found to be stable in human plasma for up to 28 h at room temperature, after four freeze–thaw cycles from −20°C and for up to 119 days stored at nominally −20°C. Amisulpride was found to be stable in whole human blood for up to 2 h at room temperature, at 30°C and at 37°C. No significant matrix effect was observed, and the method was found satisfactory for the analysis.

Pharmacokinetic analyses were performed using non-compartmental methods using SAS™ version 9.2 (SAS Institute, Buckinghamshire, UK).

Pharmacokinetic parameters were calculated using non-compartmental analysis of the plasma concentration–time data: observed maximum plasma concentration (C_{max}), time to C_{max} (t_{max}), apparent terminal half-life ($t_{½}$) calculated by $0.693/\lambda z$, area under the plasma concentration–time curve from zero to time t of the last measured concentration (C_{last})

above the limit of quantification (AUC_{0-t}) calculated by the linear up-log down trapezoidal method and area under the plasma concentration–time curve from zero to infinity ($AUC_{0-\infty}$) calculated by $AUC_{0-t} + AUC_{t-\infty}$, where $AUC_{t-\infty}$ is the residual area under the plasma concentration vs. time curve, extrapolated by $C_{last}/\lambda z$.

Safety assessments

All adverse events (AEs) were continuously monitored throughout the study from the date informed consent was signed until the end of each subject's participation. The intensity and potential relationship with the study drugs of each of the reported AEs were assessed. Vital signs, blood pressure (systolic and diastolic) and heart rate were regularly measured during the study. Laboratory investigations including haematology, biochemistry and urinalysis were performed during the screening period, Day −2 and follow-up. Any clinically relevant abnormalities were reported as AEs.

Results

Subject characteristics and disposition

Subject disposition and characteristics are summarized in Table 1 and in Figure 1. Of the 40 subjects enrolled, 23 subjects were Caucasian (12 females and 11 males) and 17 were Japanese (5 females and 12 males). Thirty-eight subjects completed the study, two subjects being withdrawn. One subject received 5 and 40 mg amisulpride before withdrawal due to non-compliance with the protocol and one received moxifloxacin before choosing to withdraw. All subjects were included in all the analysis sets, with the exception of the subject withdrawn due to protocol non-compliance, who was included in the safety set but not in the PK set.

Effect of amisulpride 5 mg and 40 mg on QTcF

The ΔQTcF profiles (Figure 2A) of 5 mg and 40 mg amisulpride and moxifloxacin demonstrated a dose-dependent effect of amisulpride on QTcF at early time points. At the end of the infusion of amisulpride 5 mg, ΔQTcF was slightly changed, with a difference relative to baseline of 0.9 ms (90% CI: 1.1, 3.0 ms) at 2 min. At 8 min ΔQTcF increased by 2.4 ms (90% CI: 0.6, 4.2 ms). Following administration of a 40 mg dose, ΔQTcF increased 5.2 ms (90% CI: 3.2, 7.3 ms) at 2 min and had a peak value of 20.8 ms (90% CI: 19, 22.6 ms) at 8 min, the end of infusion for the higher dose. The upper bound of the confidence interval remained above 10 ms at 30 min and 1 h (16.9 and 10.5 ms, respectively). The ΔQTcF profile of amisulpride 40 mg returned to levels similar to baseline after 2 h. Following administration of placebo, ΔQTcF showed a maximum shortening at 4 h, with a difference relative to

Table 1

Summary of subjects' characteristics

			A5-A40-M-P (n = 10)	A40-P-A5-M (n = 11)	M-A5-P-A40 (n = 10)	A5-A40-M-P (n = 9)
Age (years)	Mean (SD)		27.50 (4.22)	26.82 (4.24)	30.20 (8.07)	27.44 (4.93)
	Range		21–33	21–34	21–45	23–38
Gender, *n* (%)	Female		4 (40.0)	5 (45.5)	4 (40.0)	4 (44.4)
	Male		6 (60.0)	6 (54.5)	6 (60.0)	5 (55.6)
Ethnicity, *n* (%)	Caucasian		6 (60.0)	6 (54.5)	6 (60.0)	5 (55.6)
	Japanese		4 (40.0)	5 (45.5)	4 (40.0)	4 (44.4)
Height (cm)	Mean (SD)		170.10 (8.27)	172.55 (6.79)	171.90 (6.37)	171.44 (9.30)
	Range		158–182	161–180	162–181	157–182
	Mean (SD)	Caucasian	170.67 (9.11)	176.5 (4.09)	172.17 (7.49)	173.6 (9.84)
	Range		162–182	169–180	162–181	157–182
	Mean (SD)	Japanese	169.25 (8.06)	167.8 (6.53)	171.5 (5.26)	168.75 (9.18)
	Range		158–176	161–178	164–176	161–182
Weight (kg)	Mean (SD)		65.24 (8.43)	63.42 (10.73)	64.22 (9.14)	66.27 (9.40)
	Range		49.1–79.5	46.9–76.8	50.0–79.1	52.5–83.3
	Mean (SD)	Caucasian	65.93 (10.64)	71 (7.34)	66.13 (8.79)	70.58 (9.62)
	Range		49.1–79.5	59.7–76.8	52–79.1	56.3–83.3
	Mean (SD)	Japanese	64.2 (4.71)	54.32 (5.52)	61.35 (10.16)	60.88 (6.53)
	Range		57.2–67.4	46.9–61.5	50–72	52.5–67.2
BMI (kg m^{-2})	Mean (SD)		22.49 (1.83)	21.19 (2.51)	21.66 (2.26)	22.46 (1.74)
	Range		18.5–24.3	18.1–24.8	18.5–24.6	19.3–25.1
	Mean (SD)	Caucasian	22.53 (2.32)	22.8 (2.31)	22.25 (2.04)	23.3 (1.17)
	Range		18.5–24.3	18.4–24.8	19.1–24.6	22.2–25.1
	Mean (SD)	Japanese	22.43 (1.01)	19.26 (0.7)	20.78 (2.57)	21.4 (1.9)
	Range		21.4–23.6	18.1–19.9	18.5–23.2	19.3–23.2

A5: amisulpride, 5 mg; A40: amisulpride, 40 mg; BMI: body mass index; M: moxifloxacin, 400 mg; P: placebo; SD: standard deviation

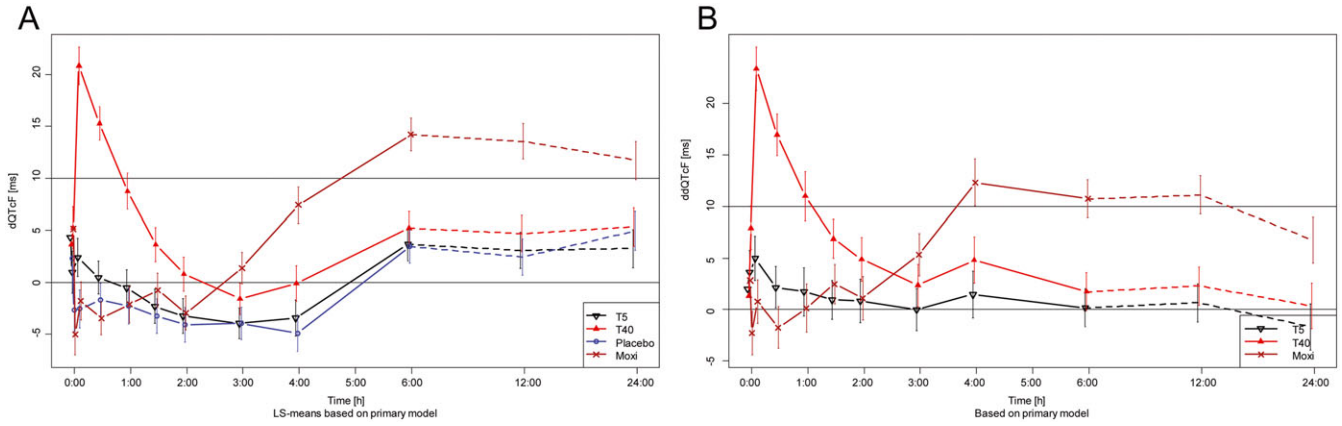

Figure 2

(A) Mean change from average baseline (ΔQTcF) and (B) Time matched placebo corrected change from baseline (ΔΔQTcF). Amisulpride 5 mg (T5) is represented by a black line, amisulpride 40 mg (T40) is represented by a red line, placebo is represented by a blue line and moxifloxacin 400 mg by a brown line. The 90% CIs are shown as vertical lines and the threshold of 10 ms is shown as a horizontal line

baseline of −4.9 ms (90% CI: −6.7, −3.2), returning to baseline level at 6 h.

The highest mean difference to time-matched placebo of change of QTcF from average baseline (ΔΔQTcF) for all treatments is summarized in Table 2.

Following i.v. administration of amisulpride 5 mg, the ΔΔQTcF profile demonstrated no changes of concern. At the end of infusion (2 min), mean ΔΔQTcF was 3.6 ms (90% CI: 1.5, 5.8 ms), and the maximal mean ΔΔQTcF was 5.0 ms (90% CI: 2.8, 7.1 ms), occurring at 8 min. The increase was transient and at 30 min the mean difference was 2.1 ms (90% CI: 0.1, 4.2 ms).

A 40 mg infusion over 8 min resulted in a mean ΔΔQTcF of 7.9 ms (90% CI: 5.8, 10.0 ms) at 2 min and 23.4 ms (90% CI: 21.3, 25.5) at the end of the infusion (Table 2). The ΔΔQTcF returned to below 5 ms by 2 h and was at baseline levels by 6 h (Figure 2B).

After an oral dose of 400 mg moxifloxacin, ΔΔQTcF was unchanged until 2 h post-dose, then increased above 5 ms between 3 h and 24 h post-dose, peaking at 4 h (12.3 ms; 90% CI: 10.1, 14.6 ms) (Figure 2B). The Hochberg procedure applied to the results of the 2, 3 and 4 h time points showed a statistically significant difference between placebo and moxifloxacin ($p < 0.001$), confirming study sensitivity. The moxifloxacin profile was consistent with previous studies where moxifloxacin was administered in the fed and fasted states, whereby the peak of the QTc effect is delayed if oral moxifloxacin is administered after a meal [23, 24].

In terms of heart rate on the ECG recordings, the largest change from baseline for all treatments occurred at 24 h: 4.4 bpm for APD421 5 mg, 3.8 bpm for APD421 40 mg, 4.3 bpm for placebo and 4.6 bpm for moxifloxacin. At the 8 min time point (end of infusion), heart rate was 64.6 bpm after APD421 40 mg and 60.4 bpm after placebo. Change from baseline for these two treatments at this time point was 3.1 bpm and −2.1 bpm, respectively, which is a difference of about 5 bpm.

Categorical analysis

There were no absolute QTcF values >500 ms or QTcF values >480 ms at any point during the study and no subjects had a QTcF value >450 ms after administration of 5 mg amisulpride. QTcF values >450 ms occurred with placebo ($n = 2$), before administration of amisulpride ($n = 1$) and after 40 mg amisulpride administration, at the 8 min ($n = 4$) and 30 min ($n = 2$) time points. Moxifloxacin QTcF values greater than 450 ms were recorded in six occasions at 4 h ($n = 1$), 6 h ($n = 2$), 12 h ($n = 2$) and 24 h ($n = 1$) after administration. QTcF increases from baseline greater than 30 ms occurred at 8 min ($n = 4$), 30 min ($n = 1$) and 90 min ($n = 1$) after the start of infusion of 40 mg amisulpride.

Table 2

Maximum difference to time-matched placebo of change of QTcF from average baseline

				90% CI	
Treatment	**Time of peak effect(hh:mm)**	**Mean**	**SE**	**Lower**	**Upper**
Amisulpride 5 mg	00:08	5.0	1.29	2.8	7.1
Amisulpride 40 mg	00:08	23.4	1.28	21.3	25.5
Moxifloxacin 400 mg	04:00	12.3	1.37	10.1	14.6

SE: standard error. Based on a linear mixed effects model with treatment, sex and race as factors and average as covariate

Pharmacokinetics

Table 3 summarizes the PK data for amisulpride. PK parameters for both Caucasian and Japanese are also listed in Table 3. Mean $AUC_{0-\infty}$, AUC_{0-t} and C_{max} values were consistent with dose proportionality: at the 8-fold higher dose, $AUC_{0-\infty}$ increased by approximately 9-fold, AUC_{0-t} increased by approximately 10-fold and C_{max} increased by approximately 6.5-fold.

Concentration response analysis

The relationships between QTcF and the amisulpride plasma concentration using all study subjects' data and separated by ethnicity are presented in Figure 3A and B, respectively. Slope estimates of the effect of amisulpride together with a 90% CI, *p*-values for the differences in slopes for Caucasian and Japanese and predictions at the two concentrations are presented in Table 4.

At the geometric mean C_{max} (169 ng ml^{-1} for 5 mg amisulpride and 1276 ng ml^{-1} for 40 mg), the predicted increase in QTcF was 5.1 ms for the 5 mg dose and 24.4 ms for 40 mg, using a linear concentration effect model. The PK-PD relationship was linear and dose proportional with no differences between the ethnic groups, with neither the difference between slopes (*p*-value = 0.540) nor that between intercepts for Caucasian and Japanese being statistically significant (Figure 3B). Predictions at 5 mg amisulpride also showed no ethnic differences (*p*-value = 0.434). The marginally significant difference in the prediction at the higher dose of amisulpide can be explained by the differences in pharmacokinetics as the differences in slopes to only contribute minimally. Regression analysis of PK-PD predicted that each additional increase of 10 ng ml^{-1} in plasma amisulpride concentration would lead to an increase in QTcF of 0.175 ms. Mean PK (concentration) and PD (placebo-corrected change from time-matched baseline) time courses for the two doses of amisulpride suggested no evidence of hysteresis.

Safety and tolerability

A total of 74 adverse events (AEs) were reported in 34 of 40 subjects, 8 with moxifloxacin, 11 in the placebo arm, 12 with 5 mg amisulpride and 43 with 40 mg amisulpride. There were no serious adverse events (SAEs), no AEs which led to withdrawal and no AEs with severe intensity.

The most frequently reported treatment-emergent adverse events (TEAEs) were infusion-related reactions reported four times in the placebo group by two Caucasian and two Japanese subjects, three times in the 5 mg amisulpride group by one Caucasian subject and two Japanese, and 31 times in the 40 mg amisulpride group by 16 Caucasians and 15 Japanese. The infusion-related reactions were a result of the higher infusion rate. In general, infusion-related reactions with amisulpride were essentially associated with site pain and began as soon as the infusions started and lasted the

Table 3

Pharmacokinetic parameters for amisulpride following single intravenous doses of 5 mg and 40 mg amisulpride

	Amisulpride 5 mg over 2 minMean (SD)	Amisulpride 40 mg over 8 minMean (SD)
n	39	39
$AUC_{0-\infty}$ (ng ml^{-1} h^{-1})	154.00 (30.17)	1374.14 (239.47)
AUC_{0-t} (ng ml^{-1} h^{-1})	134.59 (30.17)	1334.01 (228.36)
C_{max} (ng ml^{-1})	200.49 (139.16)	1305.44 (329.35)
t_{max} (h)[a]	0.033 (0.033–0.133)	0.133 (0.067–0.183)
$t_{½}$ (h)	4.05 (0.78)	5.04 (0.66)
	Caucasian	
$AUC_{0-\infty}$ (ng ml^{-1} h^{-1})	153.64 (35.04)	1376.79 (276.45)
AUC_{0-t} (ng ml^{-1} h^{-1})	133.12 (33.92)	1334.31 (261.55)
C_{max} (ng ml^{-1})	186.33 (92.19)	1216.41 (229.37)
t_{max} (h)[a]	0.033 (0.033–0.133)	0.133 (0.067–0.167)
$t_{½}$ (h)	4.0056 (0.69)	5.0795 (0.69)
	Japanese	
$AUC_{0-\infty}$ (ng ml^{-1} h^{-1})	154.44 (23.88)	1370.70 (189.37)
AUC_{0-t} (ng ml^{-1} h^{-1})	136.48 (25.39)	1333.63 (184.55)
C_{max} (ng ml^{-1})	218.81 (184.94)	1420.65 (404.43)
t_{max} (h)[a]	0.033 (0.033–0.133)	0.133 (0.133–0.183)
$t_{½}$ (h)	4.11 (0.8918)	4.99 (0.6317)

[a]Median (range) presented for t_{max}

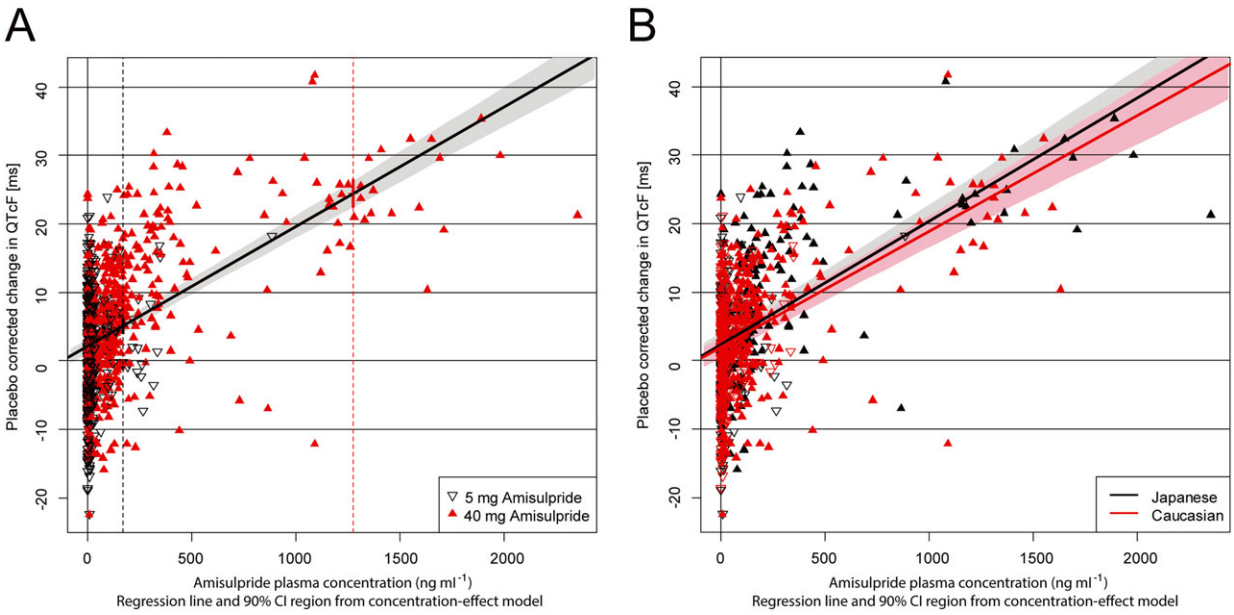

Figure 3

Concentration-effect relationship. (A) Scatter plot of the placebo-corrected change in QTcF against the amisulpride plasma concentration (ng ml^{-1}). The 90% CIs are represented by shades of grey; (B) PK-PD relationship by ethnicity. The regression lines for amisulpride derived from a linear mixed effect model. The 90% CIs are represented by grey shading for the Japanese slope and red shading for the Caucasian slope. The difference between the Caucasian and the Japanese is not statistically significant (*p*-value = 0.540)

Table 4

Slope estimates and predictions of amisulpride effect at 5 and 40 mg and differences between ethnic groups

Parameter	Ethnicity	Conc. (ng ml^{-1})	Estimate	SE	*p*-value	90% CI	
Slope (ms (ng ml))	Caucasian		0.0169	0.0012	<0.001	0.0149	0.0190
	Japanese		0.0180	0.0012	<0.001	0.0160	0.0200
	Difference		0.0011	0.0017	0.540	−0.0018	0.0039
Pred. at 5 mg (ms)	Caucasian	160	4.6	0.9	<0.001	3.2	6.1
	Japanese	181	5.6	0.9	<0.001	4.0	7.2
	Difference		1.0	1.3	0.434	−1.1	3.2
Pred. at 40 mg (ms)	Caucasian	1206	22.3	1.6	<0.001	19.7	25.0
	Japanese	1369	27.0	1.8	<0.001	24.0	30.0
	Difference		4.7	2.4	0.056	0.7	8.7

duration of the infusion, resolving within a few minutes after the end of infusion. In previous clinical studies with i.v. amisulpride, infusion site reactions were also shown to be short-lived and resolved when the infusion was slowed down or stopped. Infusion-related reactions were not specific to any ethnic group.

Most AEs were of Grade 1 on the CTCAE scale and of mild intensity, with the exception of two events of Grade 2 (diarrhoea in one female Japanese subject and anxiety in one male Caucasian subject) that were reported after 5 mg amisulpride. These were isolated events and both resolved without corrective therapy. There were no clinically relevant changes in the laboratory parameters or physical examinations in this study.

Discussion

This was the first study to evaluate the effects of therapeutic and supratherapeutic doses of an i.v. formulation of amisulpride on cardiac repolarization in line with a specifically designed TQT study performed according to ICH E14 [25]. The effect of moxifloxacin on QTcF confirmed the sensitivity of the study to detect a relevant increase in QTcF. In respect of a single i.v. dose of amisulpride at 5 mg, the study met the criteria for a negative TQT study, with the upper bound of 90% two-sided confidence falling well below 10 ms. The effect of the 5 mg dose on the QT duration was small and very short-lived, with values returning to pre-dose levels within 30 min of administration. A more rapidly

infused eight-fold supratherapeutic dose of amisulpride did cause a meaningful prolongation of the QT interval. None of the subjects had QTcF values >500 ms or >480 ms at either dose; increases from baseline in QTc of >30 ms occurred only with 40 mg amisulpride. Both doses were well tolerated by Caucasian and Japanese subjects. The statistical analysis of this study suggested that ethnicity has no influence on QT response to amisulpride. Although the slopes of amisulpride effect were not statistically significant, the difference in the predicted effect at 40 mg amisulpride was marginally significant. One limitation of this study is the fact that 12 of the 17 Japanese subjects were male. The most frequently reported AE was pain in the arm during infusion which is dependent on the rate of infusion and can be mostly avoided by reducing the infusion speed.

The capability for hERG blockade has been shown as the primary mechanism to predict QT prolongation associated with antipsychotic drugs [26]. Amisulpride, in common with some other substituted benzamides, was found to inhibit hERG current with an IC_{50} of around 100 μM [15], a markedly lower potency than some D_2 antagonists. For example, droperidol, a butyrophenone used both as an antipsychotic and an antiemetic, has an IC_{50} two to three orders of greater, at 32.2 nM [27]. Statistical models designed to predict the extent of QTc as a function of the blocking effects on hERG and $Na_V1.5$ may explain the QTc variability between antipsychotic drugs, suggesting that inhibition of late I_{Na} can act as a counterbalance of the effect of inhibition of I_{Kr} which can have an effect on the drug's pro-arrhythmic potential triggered by hERG blockade [16]. However, the effect of amisulpride on sodium channels remains to be fully elucidated.

Literature on oral amisulpride used to treat psychiatric disorders since its first marketing authorization in 1986 supports amisulpride's low propensity for clinical cardiac toxicity in practice. A review of 11 studies involving 1933 patients, of whom 905 had received amisulpride for acute exacerbations of schizophrenia at a mean daily dose of 670 mg (range 100–1200) and 342 had received amisulpride for predominant negative schizophrenia at a mean daily dose of 118 mg, confirmed the satisfactory cardiovascular safety profile of amisulpride. Overall, blood pressure variations were devoid of clinical significance and no arrhythmia was reported [6]. The occurrence of torsade de pointes associated with amisulpride has been reported [9–13]. However, all cases are related to extremely large acute overdoses of amisulpride, ranging from 4000–80 000 mg, leading to high plasma concentration of amisulpride. The plasma concentrations observed in these cases are two to three orders of magnitude above the mean C_{max} plasma levels reported at the highest dose in this study, indicating that the supratherapeutic dose to treat PONV is not associated with torsade de pointes.

The predicted C_{max} concentrations from the concentration–response analysis model fit were in close agreement with the observed data in this study. The observed C_{max} values were 200 ng ml^{-1} and 1305 ng ml^{-1} for the 5 and 40 mg amisulpride doses, respectively. These findings are in agreement with observed amisulpride plasma concentration levels in schizophrenic patients ranging from 20–1904 ng ml^{-1} after oral administration of 150–1600 mg day^{-1} of amisulpride [28]. Additionally, plasma levels of 424 ng ml^{-1} (SD 292.8 ng ml^{-1}) were reported as therapeutic for schizophrenia after an average daily dose of 772.3 mg (SD 346.7 mg) amisulpride [28]. The largest available oral presentation of amisulpride, a 400 mg tablet, was reported to deliver a C_{max} of approximately 1300 ng ml^{-1} [29]. This is more than five times higher than the C_{max} of the 5 mg i.v. infusion and analogous to the C_{max} of the 40 mg i.v. infusion. Regarding the use of amisulpride as an anti-emetic, a bell-shaped dose–response showed that a 5 mg dose is more effective than lower and higher doses [20]. The C_{max} of 200 ng ml^{-1} obtained after administration of 5 mg amisulpride at 2.5 mg min^{-1} in this study can be associated with clinical effectiveness in the treatment and prevention of PONV. Interestingly, therapeutic drug monitoring has demonstrated that the plasma concentration of approximately 200 ng ml^{-1} is associated with optimal clinical response when oral doses of 400–800 mg day^{-1} of amisulpride are administered in schizophrenia and schizoaffective disorder cases [30, 31].

The linear and proportional PK-PD relationship observed indicated that the attained C_{max} in this study is dependent on dose and rate of infusion. However, this study investigated only two dose regimens and two infusion rates, limiting conclusions on the effects on QTcF of higher doses of amisulpride administered at lower infusion rates; and, in particular, the infusion rate which may be needed to avoid a significant QT/QTc interval prolongation with higher doses of amisulpride.

In summary, this study shows that the proposed therapeutic dose of 5 mg amisulpride for the management of PONV, given intravenously over 2 min, is well tolerated by healthy Caucasian and Japanese subjects and is not associated with a QTcF prolongation of regulatory concern.

Competing Interests

All authors have completed the Unified Competing Interest form at www.icmje/coi_disclosure.pdf (available on request from the corresponding author) and declare: GF had support from Richmond Pharmacology Ltd for the submitted work, GF had support from Acacia Pharma for the submitted work; GF is an employee of Acacia Pharma Ltd with stock options in the previous 3 years; GF holds the issued patent no WO2011110854 A3.

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