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Confirmation of the Cardiac Safety of $PGF_{2\alpha}$ Receptor Antagonist OBE022 in a First-in-Human Study in Healthy Subjects, Using Intensive ECG Assessments

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Abstract

OBE022, a new orally active prostaglandin $F_{2\alpha}$ receptor antagonist (OBE022) with myometrial selectivity is being developed to reduce uterine contractions during preterm labor. This first-in-human study evaluated the effect of OBE022 following multiple doses on the QT interval in 23 healthy postmenopausal women, using the effect of a meal on QTc to demonstrate assay sensitivity. We report the cardiac safety outcome performed during the multiple ascending part of this trial. OBE022 was administered after a standardized breakfast on day 1 and in the fasted state from day 3 to day 9 wth a standardized lunch 4 hours after administration. Concentration–effect modeling was used to assess the effect of prodrug OBE022 and parent OBE002 on QTc after a single dose (days 1 and 3) and multiple doses (day 9). The concentration–response analysis showed the absence of QTc prolongation at all doses tested. Two-sided 90% confidence intervals of the geometric mean C_{max} for estimated QTc effects of OBE022 and OBE002 of all dose groups were consistently below the threshold of regulatory concern. The sensitivity of this study to detect small changes in the QTc was confirmed by a significant shortening of the QTc on days 1, 3, and 9 after standardized meals. This study establishes that neither prodrug OBE022 nor parent OBE002 prolong the QTc interval. The observed food effect on the QT interval validated the assay on all assessment days. Both the change from predose, premeal and the change from premeal, postdose demonstrated the specificity of the method.

Keywords

OBE022, FP-receptor pharmacology, tocolytic, obstetrics, safety pharmacology, QTc, food effect, QTc shortening, QTc prolongation

The valine ester prodrug OBE022 (Figure 1A) and its structural parent OBE002 (Figure 1B) are both potent, novel, orally active and selective prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) receptor (FP) antagonists. OBE022 is converted in vivo to OBE002 by cleavage of the ester bond by enzymatic hydrolysis. OBE022 has been developed by ObsEva SA, Switzerland, and selected for development as a treatment for preterm labor.

Preterm labor is a major health issue. Premature delivery is the most important direct cause of mortality in the first month of life and is a major cause of death in children younger than 5 years old.[1,2] There is an urgent need to develop drugs that would prolong pregnancy up to a stage at which increased fetal maturation raises the chances of survival and decreases morbidity associated with preterm birth.

Thus, tocolytic drugs have an important role in the management of preterm delivery. These include calcium channel blockers, oxytocin-receptor antagonists,

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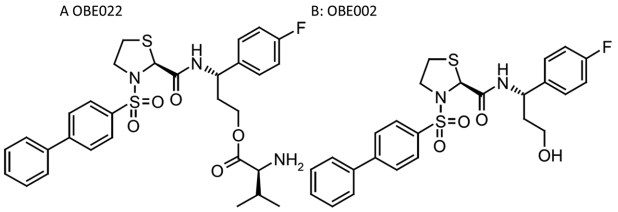

Figure 1. (A) Structural formula of OBE022. (B) Structural formula of OBE002. OBE022 is the valine ester of OBE002, to which it is hydrolyzed in vivo.

β-mimetics, $MgSO_4$, and prostaglandin synthesis inhibitors. β_2-agonists show potentially serious maternal adverse effects while being relatively innocuous for the fetus. Conversely, pan-prostaglandin synthesis inhibitors are associated with potential harm to the fetus, with milder gastrointestinal effects for the mother. The current first-line tocolytic therapy consists of the L-type calcium channel blocker nifedipine and the oxytocin-receptor antagonist atosiban. A recent meta-analysis showed that nifedipine maintenance tocolysis is not associated with improved perinatal outcome or pregnancy prolongation.[3] Another meta-analysis showed that atosiban is less effective than pan–prostaglandin synthesis inhibitors, calcium channel blockers, $MgSO_4$, or beta-mimetics in delaying labor.[4] Thus, a requirement for a new class of tocolytic persists.

OBE022 is designed to control preterm labor through specific antagonism of the FP, by reducing inflammation, decreasing uterine contractions, and preventing cervical changes and membrane rupture. OBE022 has the potential, based on its pharmacokinetic (PK) profile and efficacy observed in animal models, to become a first-in-class therapy to suppress premature labor and delay or avoid preterm birth while also being safe for the fetus.

Cardiac effects of administration of an FP antagonist to humans are a possibility, as the receptor is abundantly expressed in the heart,[5,6] and activity of the endogenous ligand $PGF_{2\alpha}$ on cardiac tissue in vitro has been demonstrated. In cultured neonatal rat cardiac myocytes, $PGF_{2\alpha}$ promotes arrhythmias.[7,8] This ligand has been shown to modulate cardiac ion channels because $PGF_{2\alpha}$ can depress contractile recovery and increase calcium accumulation of the globally ischemic heart,[9] and administration of $PGF_{2\alpha}$ induced phasic contractions in murine myometrium through ATP-sensitive potassium channels.[10] In vivo studies have also demonstrated the relevance of FP modulation for cardiac function. Genetic deletion of FP protects against inflammatory tachycardia in mice,[9] and $PGF_{2\alpha}$ antagonism partly blocks centrally administered arachidonic acid–evoked pressor responses.[11] A clinical study of an FP allosteric inhibitory modulator recorded isolated QT-interval prolongations that were probably not related to the investigational medical product.[12]

The electrophysiological effects of OBE022 and OBE002 on the human hERG potassium channel have been evaluated in vitro in patch clamp studies using HEK293 and CHO cell lines transfected with hERG (ObsEva, data on file). Both OBE022 and OBE002 inhibited the hERG-mediated potassium current, with IC_{50} values of 0.87 and 2.6 μM, respectively. A follow-up in vitro Purkinje fiber assay at up to 5 μM OBE022 showed that OBE022 does not cause any increase in the duration of the action potential at 50% repolarization (ObsEva, data on file). The results of an in vitro reaction phenotyping study using human recombinant enzymes suggest that OBE002 is primarily metabolized by cytochrome P450 (CYP) enzyme CYP3A4, with lesser metabolism by CYP2C19 and CYP2D6. Uridine 5′ diphosphate glucuronosyltransferase (UGT) reaction phenotyping only identified UGT1A9 as a metabolizing enzyme (ObsEva, data on file).

In vivo safety pharmacology studies conducted in telemetered beagle dogs aimed to verify any possible changes to cardiovascular parameters arising from single oral administration of OBE022 or single intravenous exposure to OBE002. No effects were observed up to the highest administered oral dose of 720 mg/kg of OBE022 or an intravenous dose of 15 mg/kg OBE002 (ObsEva, data on file).

Here we describe the results of a phase 1 study investigating the safety, tolerability, pharmacokinetics, and pharmacodynamics of rising doses of OBE022 in healthy postmenopausal women, including the application of a concentration–effect analysis on cardiac repolarization validated by meal effects. A preliminary report of OBE022's cardiac safety was presented as a poster at the ACCP meeting, September 17, 2017.[13]

Methods

Study Design

The present study was a single-center, randomized, placebo-controlled study conducted at Richmond Pharmacology, St George's, University of London, London, UK. The study aimed to assess the safety, tolerability, food effect, and pharmacokinetics of single-ascending (SAD) and multiple-ascending (MAD) oral doses of OBE022 in healthy postmenopausal women and pharmacodynamic effects in women of childbearing potential during menstruation. In this report the cardiac assessments conducted in the MAD part of the study will be described.

The MAD part of the study comprised a screening period within 20 days (days −21 to −2) before entering the study, an in-house period of 12 days and 11 nights (days −1 to 11), followed by 4 outpatient visits (days 12, 13, 14, and 15) and a follow-up visit (14 ± 3 days post–last dose). Eighteen subjects were planned to be randomized to receive daily oral doses of 100, 300, and

1000 mg OBE022 (6 per dose level) and 6 subjects to receive matching placebo (2 per dose level). A single dose was planned to be given on day 1 under fed conditions and on days 3–9 under fasted conditions. No treatment was administered on day 2, which served as washout.

Study Subjects

In total 46 women were enrolled in this study. In the MAD part, we included 23 postmenopausal women who participated in the cardiac safety study and are reported here. We targeted healthy postmenopausal women aged 50 to 65 years inclusive with a body mass index between 18.0 and 32 kg/m^2; the actual demographics are shown in Table 1. Natural (spontaneous) postmenopausal was defined as being amenorrheic for at least 12 months without an alternative medical cause with a screening follicle-stimulating hormone level > 25.8 IU/L and/or 17β-estradiol serum level < 49.8 ng/L.

Postmenopausal subjects were excluded if they had: (1) known structural cardiac abnormalities; (2) family history of long QT syndrome; (3) cardiac syncope or recurrent, idiopathic syncope; (4) exercise-related clinically significant cardiac events; or (5) any clinically important abnormalities in rhythm, conduction, or morphology of resting electrocardiogram (ECG) that might have interfered with the interpretation of QTc interval changes. These included but were not limited to sinus node dysfunction, clinically significant PR (PQ) interval prolongation, intermittent second- or third-degree atrioventricular block, complete bundle branch block, abnormal T-wave morphology, and QT interval corrected using the Fridericia's formula (QTcF)[14] > 450 milliseconds. Written and signed informed consent was obtained from each subject before taking part in the study.

The study protocol (EudraCT 2016-001957-42) was reviewed and approved by a National Health Service Research Ethics Committee (South Central-Berkshire B, UK) and the Medicines and Healthcare Products Regulatory Authority. The study was conducted according to the ethical principles enshrined in UK law, the Declaration of Helsinki, and Good Clinical Practice guidelines.

Breakfast served on day 1, composed according to the US Food and Drug Administration (FDA) standard,[15] contained 784.8 kcal with a ratio of 31.6% carbohydrate to 51.8% fat to 16.6% protein. The reference meal for the assessment of assay sensitivity on days 3 and 9 was lunch, which contained 606.6 kcal in a ratio of 75.8% carbohydrate to 3.3% fat to 20.9% protein.

Pharmacokinetic Assessments

Blood samples for plasma pharmacokinetics were collected on days 1, 3, and 9 at -0.5, 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 7, 8, 12, 16, and 24 hours, at predose on days 5, 6, 7, and 8, and 48, 72, 96, 120, and 144 hours following the last dose on day 9. Plasma samples for determination of OBE022 and OBE002 concentrations were analyzed by SGS CEPHAC (Saint Benoît, France), which employed a validated liquid chromatography–tandem mass spectrometry method with liquid–liquid extraction. This was developed and satisfactorily validated for the measurement of both analytes in human plasma over the calibration range 0.1–100 ng/mL. Plasma concentrations of OBE022 and OBE002 were determined with OBE022-D_5 and OBE002-D_3 as internal standards.

Chromatographic separation was performed through an Ascentis Express C8 column (2.7 μm, 2.1 × 50 mm; Millipore Sigma, Lyon, France) analytical column, with a linear gradient mobile phase consisting of acetonitrile/water, with acetonitrile varying from 30% to 80%. Both mobile phases contained 0.1% formic acid. The flow rate was 600 μL/min, and the total run time was 6 minutes. Detection and quantification were performed by mass spectrometry using an API 4000 mass spectrometer fitted with Turbo Ion Spray ion optics (AB Sciex, Villebon sur Yvette, France). Ions were monitored at transitions m/z 600.3 → 483.1 and 501.3 → 349.1 for OBE022 and OBE002, respectively. For the internal standards, transitions were monitored at 605.4 → 488.1 for OBE022-D5 and at 506.3 → 354.1 for OBE002. The retention time for OBE022 was 1.53 minutes, whereas for OBE002 it was 2.05 minutes.

The precision (the coefficient of variation, OBE022 < 7%, OBE002 < 10%) and accuracy (relative error, OBE022, -4.5% to 2.0%; OBE002, -2.7% to 0.0%) of the method were found to be within the target limits. The selectivity of the method was found to be satisfactory, with no endogenous interference that may have adversely affected the analysis.

Pharmacokinetic analyses were performed using noncompartmental methods with SAS software, version 9.3 (SAS Institute Inc., Marlow, Buckinghamshire, UK).

Cardiac Assessments

Intensive cardiac assessments were performed on days 1, 3, and 9 of the multiple-ascending-oral-dose study. The purpose of the cardiac assessments was to evaluate the proarrhythmic risk posed by exposure to OBE022 and OBE002. All ECG recordings were obtained in triplicate from each time.

Analysis of drug-related QT/QTc-interval changes relative to plasma PK concentrations were conducted on all dose regimens in this part of the study. The ECGs used for this analysis required adjudication by qualified cardiologists in accordance with principles set out in the International Conference on Harmonisation (ICH) E14 guideline and subsequent

Table 1. Summary of Subjects' Characteristics

n		100 mg OBE022 8	300 mg OBE022 8	1000 mg OBE022 7	Overall 23
Age (years)	Mean ± SD	59.0 ± 3.4	56.8 ± 4.0	53.4 ± 2.6	56.5 ± 4.0
	Range	56.0–64.0	53.0–64.0	50.0–57.0	50.0–64.0
Height (cm)	Mean ± SD	162.8 ± 3.6	161.1 ± 5.3	165.0 ± 5.4	162.9 ± 4.8
	Range	156–168	154–168	157–174	154.00–174.00
Weight (kg)	Mean ± SD	70.20 ± 9.86	64.06 ± 8.78	66.7 ± 9.3	67.0 ± 9.3
	Range	57.0–84.6	55.2–80.4	54.1–83.1	54.1–84.6
BMI ($kg.cm^{-2}$)	Mean ± SD	26.5 ± 2.9	24.7 ± 3.6	24.5 ± 3.8	25.3 ± 3.4
	Range	22.2–31.1	20.1–29.3	20.6–30.9	20.1–31.1
Race, n (%)	Asian	1 (12.5)	0 (0.0)	1 (14.3)	2 (8.7)
	Black African	1 (12.5)	1 (12.5)	2 (28.6)	4 (17.4)
	Caucasian	6 (75.0)	6 (75.0)	4 (57.1)	16 (69.6)
	Other	0 (0.0)	1 (12.5)	0 (0.0)	1 (4.3)

SD, standard deviation; BMI, body mass index.

question-and-answer documents.[16–23] The principles of this analysis follow the statistical methods described by Garnett et al.[24] The effects of a meal on the ECG were used to establish assay sensitivity, that is, the ability of the study to detect small changes in the QTc.[22,25]

ECG Assessments and QTc Evaluation

Twelve-lead ECGs were recorded using a GE Marquette MAC1200 and stored electronically on the Medical MUSE information system (GE Healthcare). Only ECGs recorded electronically at a stable heart rate (HR) were valid for QT-interval measurements. ECG recordings were collected on days 1, 3, and 9 at 2, 1, and 0.5 hours predose and 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 7, 8, 12, and 24 hours postdose. All ECGs were recorded after the subjects had been resting in a supine position for at least 10 minutes. To obtain consistent ECG recordings, the clinical staff ensured that the subjects were awake and avoided any postural changes. At each point, the ECGs were recorded in triplicate to confirm the accuracy and precision of the measurements. Each ECG lasted 10 seconds. The triplicates were performed at 1-minute intervals over 3 minutes.

Each electronic ECG data file contained the ECG data as well as the result of the automated ECG analysis performed by the Marquette 12 SLTM ECG Analysis Program. All ECGs and their associated automated interval measurements were subsequently blinded and reviewed by one qualified cardiologist in accordance with the ICH guidance[16] before any of the ECGs were used for the subsequent statistical analysis. The uncorrected QT interval, the RR interval from which the HR was derived according to the formula HR = 60 000/RR, the PR interval and QRS duration, the presence or absence of U waves, and quantitative and qualitative ECG variations were assessed by the cardiologist, who has extensive experience with manual on-screen overreading using electronic calipers in MUSE, for correction of any implausible readings presented by the automated process. All ECGs were overread by the same cardiologist, who was blinded to the treatment and the timing of the recording being evaluated. If manual adjustments of the automated measurement became necessary and the first overreader requested adjudication, then a second cardiologist performed overreading and assessment. Similarly, if the second cardiologist requested further adjudication, then a third most senior cardiologist performed the assessment. Compensation for heart rate of the QT interval (QTcF)[14] was used. A total of 212 ECGs (19.2%) were corrected after adjudication from a total of 1104. Predose baseline values were obtained from 3 predose points (2, 1, and 0.5 hours before drug administration). The mean of the values obtained at these points was used as baseline.

Statistical Analysis

The analysis was based on QTcF and on all data from days 1, 3, and 9. On day 1 the subjects received a dose of OBE022 after a meal. On day 3 subjects received a dose in fasting condition after a day of washout, whereas on day 9 subjects received doses in the fasting condition for 7 days without interruption. All subjects who received study medication and who had valid ECG data for predose baseline and the times during days 1, 3, and 9 were included in the QTcF analysis set.

A concentration–effect analysis was chosen as the primary analysis.[24,26] Four linear statistical models were investigated to correlate heart-rate-corrected QTinterval duration with exposure to OBE022 and/or the parent OBE002. All the investigated models had a centered baseline (ie, baseline minus mean of baseline across

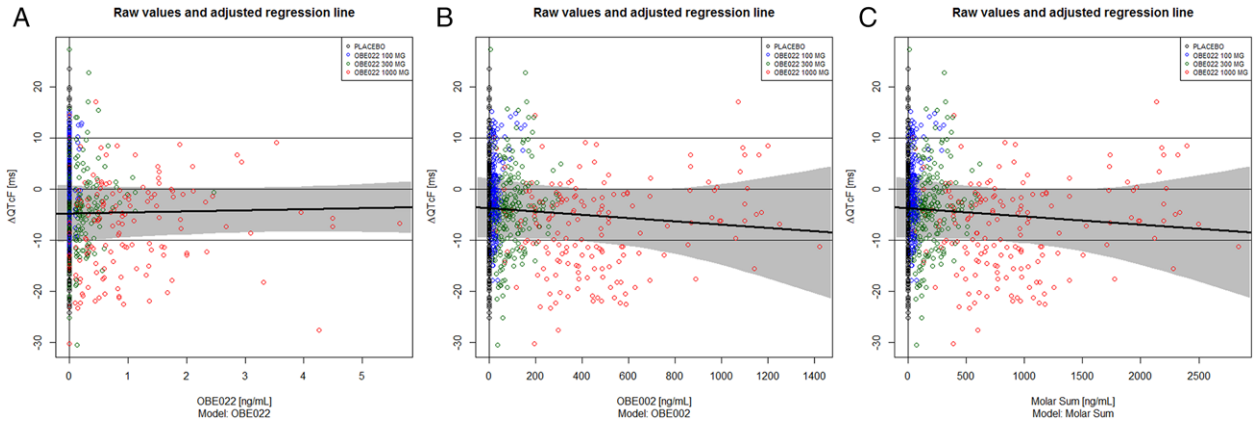

Figure 2. Scatterplots for models OBE022, model OBE002, and model molar sum. (A) Relationship between ΔQTcF and exposure to OBE022. As this parent substance was rapidly converted to OBE002 the concentration range for OBE022 is lower than that for OBE002 (B). Correspondingly, the range for the molar sum of OBE022 and OBE002 (C) is predominantly determined by the concentration of OBE002.

Table 2. Slope and Treatment Effect Estimates of Models Considered With AIC and Residual Error

Model	AIC	Residual Error	Parameter	Estimate	SE	DF	*t*	90%CI	
Both	5579.9	5.8	OBE022	0.4484	0.6926	6.6	−0.86	−0.0128	0.0064
			OBE002	−0.0027	0.0046	3.1	0.01	−4.2647	4.3346
			trt	−0.3085	2.5394	19.7	0.34	−0.8600	1.2802
OBE002	5578.9	5.8	OBE002	−0.0032	0.0037	2.5	−0.22	−4.5965	3.5616
			trt	0.0350	2.4901	19.5	−0.86	−0.0064	0.0032
OBE022	5574.9	5.8	OBE022	0.2101	0.6098	14.8	0.01	−4.2649	4.3325
			trt	−0.5174	2.3678	20.5	−0.86	−0.0128	0.0064
Molar sum	5574.9	5.8	msum	−0.0016	0.0018	2.5	0.01	−4.2647	4.3346
			trt	0.0338	2.4895	19.5	0.34	−0.8600	1.2802

trt, Treatment effect (active - placebo); msum, molar sum of OBE022 and OBE002. Slope units are in milliseconds per ng/mL.

subjects) as covariate and time and treatment (active or placebo) as discrete factors. In addition to models with concentrations of just one of the analytes included (models OBE022 and OBE002), a model with both concentrations of OBE022 and OBE002 as covariates (model both), and one with the molar sum of OBE022 and OBE002 concentrations as covariate (molar sum) were investigated (Figure 2). Each model included random effects per subject for the intercept and the concentrations included in the model (Table 2).

Based on these models, predictions of the effect at the geometric mean of the individual C_{max} values for OBE022, OBE002, and the molar sum were made for each dose group. For the model using both analytes, predictions were made for the geometric mean of both and OBE022 and OBE002 separately. The geometric mean C_{max} of OBE022 was combined with the arithmetic mean of the concentrations of OBE002 seen at the T_{max} with respect to OBE022 and vice versa. These predictions were given together with 90% confidence intervals.

The Absence of Hysteresis

The plot of the placebo-corrected change from mean baseline (ΔΔQTcF) compared with the mean plasma OBE022 and OBE002 levels against time (Figure 3) was used to judge the presence of a delay between change in cardiac repolarization rate and rise in drug concentrations. The absence of hysteresis was confirmed by a visual assessment of QTc changes over time versus the pharmacokinetic time profile.

Assay Sensitivity

Tests for assay sensitivity were performed on the basis of the estimates of the time course of ΔQTcF obtained from the primary models described above. In these estimates, the influence of drug plasma concentrations has already been removed. On day 1 breakfast was served 30 minutes before dosing and was finished 10 minutes before dosing, and change in ΔQTcF from the value 0.5 hours prior to drug administration to values 1.5 and 3.5 hours after drug administration was used. On days 3 and 9 the study drug was administered in the fasted

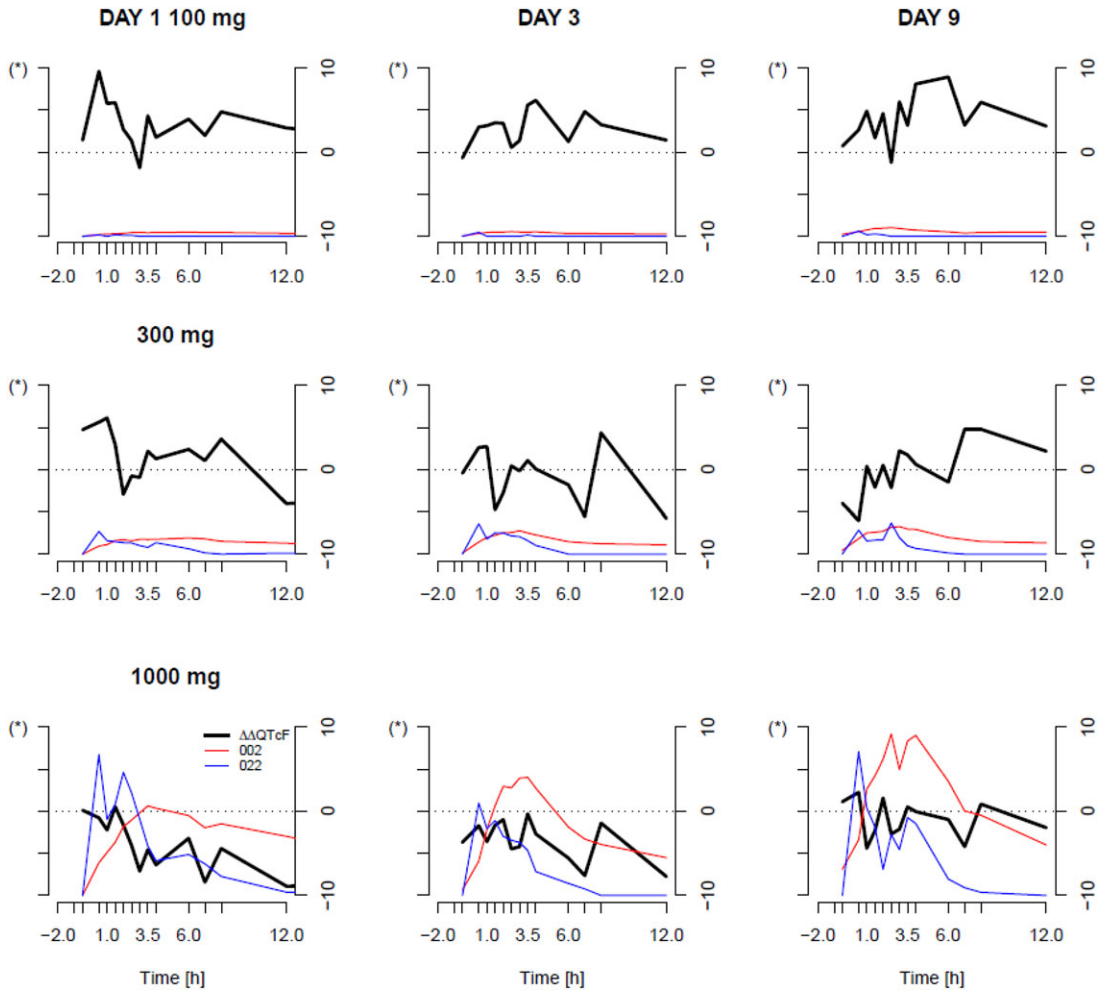

Figure 3. Drug concentration of the dose groups and ΔΔQTcF for each dose group, plotted against time. Each row represents 1 dose group, and each column represents 1 day. A peak in ΔΔQTcF would follow the peaks of the drug concentrations if the drug effect on QTcF were delayed. This effect should be uniform across dose groups and most pronounced in the highest dose group. This is not the case. *Top value of the concentration scale represents 3 ng/mL for OBE022 and 1000 ng/mL for OBE002.

state, and the first meal was lunch 4 hours postdose. On these days, the effect of the meal was tested by comparing the time effects 2, 3, and 4 hours after lunch (6, 7, and 8 hours after drug administration, respectively) with the average over the last 3 preprandial times (ie, 3, 3.5, and 4 hours after drug administration). The study was declared to be adequately sensitive to show a small change in mean QTc if a shortening significant on the 1-sided 5% level could be shown for all times separately for each of the 3 study days.

Safety Assessments

Adverse events (AEs) were continuously monitored throughout the study from the date of informed consent until the end of each subject's participation. The intensity and potential relationship with the study drug of each of the reported AEs were assessed. Subjects underwent physical examinations and clinical laboratory tests (hematology, coagulation, biochemistry, and urinalysis). Telemetry, 12-lead ECGs, vital signs, blood pressure (systolic and diastolic), and heart rate were regularly evaluated during the study. Any clinically significant abnormalities were reported as AEs. All AEs were graded using the National Cancer Institute-Common Terminology Criteria for Adverse Events version 4.0.

Results

Subject Disposition and Demographics

The multiple-ascending-dose (MAD) part of the study that was carried out with postmenopausal women (aged between 50 and 64 years) is reported here. Fifty-seven subjects were screened in total, of whom 30 did not meet the screening criteria and 4 withdrew consent prior to enrollment. The most common criteria not met by the 30 subjects who failed screening were urine

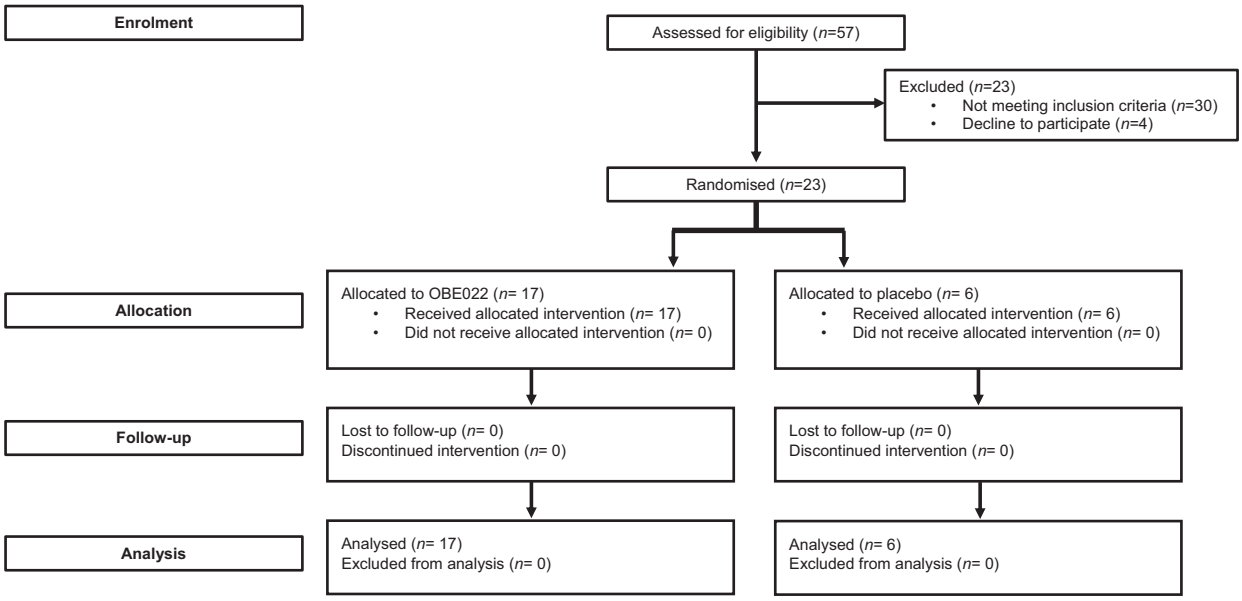

Figure 4. Consort diagram.

and blood parameters, ECG, and Holter parameters. Twenty-three subjects fulfilled the eligibility criteria and were randomized to treatment. All 23 subjects enrolled completed the study and were included in all the analysis sets. Demographic data and subject disposition are summarized in Table 1 and Figure 4.

Pharmacokinetics

Concentration–time curves of OBE022 and OBE002 after single and multiple dosing are presented in Figure 5.

After single doses, OBE022 was well absorbed, and OBE022 and OBE002 were both observed in plasma but with markedly higher levels of OBE002 compared with OBE022. In view of a rapid hydrolysis of OBE022 to OBE002, OBE022 reached T_{max} within 0.3 to 1.0 hours, and $t_{1/2}$ was below 1.6 hours. Maximum plasma concentrations of OBE002 exceeded those of OBE022 by 100- to 200-fold, with up to 1000-fold higher AUC values. The T_{max} of OBE002 was reached within 2.6 and 3.4 hours, and $t_{1/2}$ was between 8.3 and 10.7 hours.

Following multiple doses of OBE022 there was no accumulation of the prodrug OBE022, which is in line with its short half-life. Dose accumulation ratios of OBE002 had a 2-fold increase in AUC_{0-24h} (2.1) at the 100-mg dose and a 1.3 increase at 300 and 1000 mg. The terminal half-life of OBE002 showed a 2- to 3-fold increase over time to 22.2–29.2 hours on day 9, which may be because of half-life estimates after single dosing being based on 24 hours, whereas after multiple dosing this could be extended to 6 days of sampling. Peak concentrations of OBE002 increased by 1.5 (100 mg) and 1.2 (300 and 1000 mg) over time.

Coadministration of food resulted in 20% and 84% higher exposure (C_{max} and $AUC_{0-\infty}$, respectively) to OBE022. For OBE002, $AUC_{0-\infty}$ was slightly increased (+15%) following administration with food, but within accepted bioequivalence limits, and C_{max} was reduced by 20%.

Cardiac Assessments

The primary analysis was conducted following the statistical methods described by Ferber et al[27] and employed the change from average baseline of the QT interval corrected by Fridericia's formula[14] for changes because of heart rate (QTcF). In addition, the time course of all ECG parameters was described by summary statistics.

All the subjects in the safety data set who had valid ECG data for at least 1 postdosing time were included in the primary analysis set. A total of 24 values had to be excluded because no valid concentration data were available, and 2 values were exclude because the time difference between the ECG and the blood sampling exceeded the predefined limit. To demonstrate the sensitivity of the assay, a Hochberg procedure[28] was applied to the results of the 2-, 3-, and 4-hour times of the difference between food effect and fasting. The baseline was calculated from the day -1 values of the respective period. Two types of baseline were used: the mean baseline for the primary analysis and a time-matched reference baseline for a secondary analysis.

The QTc values for placebo and the 3 treatment groups tended to be similar on days 1, 3, and 9. The

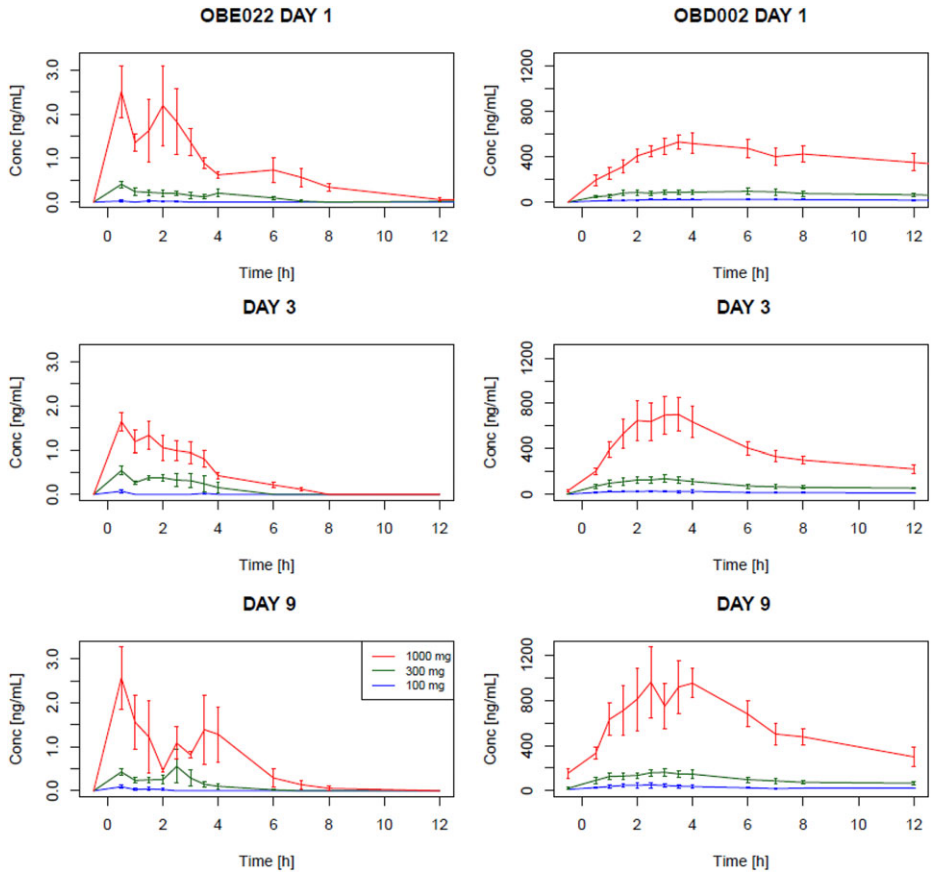

Figure 5. Arithmetic means and SEs of plasma concentrations of OBE022 and OBE002 at 3 different doses, on days 1 (single dose, fed), 3 (single dose, fasted), and 9 (steady state, fasted). The red line indicates a 1000-mg dose, the green line a 300-mg dose, and the blue line a 100-mg dose.

maximal mean values for 100-, 300-, and 1000-mg doses and placebo were observed between 1 and 1.5 hours postdose and the minimal values between 5 and 8 hours postdose.

On day 1 RR intervals exhibited escalating fluctuations at 4 and 8 hours. These increases in mean HR correlate with the effect on HR that can be attributed to food[23] and are not related to OBE022 administration. The time courses of HR and QTcF exhibited no systematic difference between the groups that had received OBE022 and those that had received placebo; thus, no drug-related changes in these parameters were observed; explicitly, all HR changes were related to food administration, and these changes were in line with previously published data.[23,25,29]

Model-Based Concentration–Effect Analyses

To identify any PK-pharmacodynamic (PD) hysteresis, that is, a delay between the effect of a drug on the QTcF and the plasma concentrations of the 2 analytes, the drug concentration of the respective dose group as well as the ΔΔQTcF for each dose group was plotted against time (Figure 3). The plots do not show any systematic relationship between PK and PD, and in particular, there was no indication for a delayed effect on QTcF; thus, hysteresis can be excluded.

In all models, a slight positive but nonsignificant relationship between the concentration of OBE022 and the change in QTcF was seen, whereas those of OBE002 and of the molar sum were negative. All *t* values for the estimates were in the range between -1 and 1, and therefore none of the 2-sided 90% confidence intervals excluded zero. The predictions derived from the primary model are given in Table 2. Most predictions are slightly negative, and all are in the range between -2.5 and 1 milliseconds. All 90% confidence intervals are well below the threshold of 10 milliseconds.

A graphical comparison of the results is displayed in Figure 2, where the observed ΔQTcF values are displayed as scatterplots over the concentration of OBE022, OBE002, and the molar sum. In addition, the regression line from the respective mixed-effects model is displayed. This regression line has been adjusted to the average of the time and treatment effects to match the data displayed. The key results of the joint model are shown in Table 2.

The Sensitivity of the Assay

The sensitivity of the assessment to consistently detect changes of QTcF was established by measuring the effect of ingesting a standardized meal in the MAD part of the study. A high-fat breakfast, composed according to FDA recommendations,[15] was served prior to dosing on day 1, whereas on days 3 and 9 lunch was served 4 hours after administering OBE022; thus, the study subjects fasted prior to dosing on those latter 2 days, which provides the opportunity to evaluate assay sensitivity on each of the 3 ECG assessment days.

The time course of the food effect on QTcF was estimated from the estimates of the time effect in the models. Compared with the value measured before the start of breakfast, the QTc consistently dropped 2 to 4 hours after this point on day 1, whereas it remained virtually unchanged on both days 3 and 9. The estimated changes based on the primary model using change from predose baseline are shown in Table 2. All 95% confidence intervals for the day 1 estimators were well below zero, and therefore assay sensitivity could be considered attained. Because of different meal times, the food effect was confirmed 6, 7, and 8 hours after dosing, corresponding to 2 to 4 hours after the start of the meal, and it showed a similar reduction in QTcF (Table 3) and within the prospectively determined range.

Discussion

The study established the cardiac safety of the prodrug OBE022 and its active metabolite and parent compound, OBE002, in an older female postmenopausal population during the MAD part of the study using a 10-fold increase in dose and a plasma exposure from 29 to 926 ng/mL. OBE022 is rapidly metabolized to the equally pharmacologically active OBE002; therefore, the analysis assessed both substances separately and jointly. Neither exposure to OBE022 nor to OBE002 inhibited cardiac repolarization at these plasma levels. The observed food effect validates the cardiac repolarization assay on all days on which QTc effects were tested and is within the range of QTc values reported in the literature. Both the change from predose, premeal and from premeal, postdose in the present study demonstrated the sensitivity of the study to detect small changes in the QTc.

The MAD arm of the study was chosen over the SAD arm for investigation of the effect of OBE002 and OBE022 exposure on cardiac repolarization because the cohort size of the MAD study arm is generally greater than that for the SAD arm, thus providing a greater sample size at doses considered relevant for future clinical development. Further important advantages are that the MAD arm showed less autonomic interference arising from subjects' anxieties, as eventful study days often include many other assessments, restricting the careful recording of ECG data. The longer exposure is capable of revealing the effects of known as well as unknown metabolites. Investigational Medicinal Product (IMP) exposure to steady state often results in higher exposures than those seen with marginally higher single doses.

Modeling showed that there was no statistically significant prolongation of QTcF (Table 3). Some of the concentration–QTcF models for parent OBE002 (models both and OBE022; Table 2) tended to indicate a nonsignificant negative slope. An effect on cardiac function of exposure to the FP antagonists OBE002 or its parent OBE022 could be excluded despite the reported action of FP agonists on cardiac function in vitro[8,9,11,30–32] and reports of blood pressure elevation in response to topical application of FP agonists.[33] This is consistent with previous clinical studies of allosteric FP antagonists that showed no QTc prolongation attributable to drug administration.[12] The predicted C_{max} values from the concentration–response analysis model were in close agreement with the empirical data gathered in this study.

Moxifloxacin is widely employed as a pharmacological positive control,[17] whereas the present study used the effect of a meal to demonstrate confidence that the QT interval assay was capable of detecting a small change, as mandated by the regulatory requirements.[34] The effect of a meal shortens the QT interval, whereas moxifloxacin administration lengthens it. This reduces the length of the waveform. The influence of this shortening on assessment accuracy depends on the measurement technique. We used a global beat analysis — whereby the automatic algorithm determined a global beat across all leads.[35] Although technical considerations indicate a difference of 1–3 milliseconds in QT-interval length, this small systematic variation is within QT-interval measurement precision limits.[35–39] The T-wave length is artificially extended when the T wave is flattened compaed with a more peaked waveform, when these are measured using the tangential method.[40] For this reason we used the former approach.

The robust change in QT interval of 6–10 milliseconds produced by eating a meal[25,41] ensures that the assay possesses sufficient sensitivity to reveal an effect level close to the threshold of regulatory concern (5 milliseconds).[34] The rules we use to confirm assay sensitivity are robust in that we use 2 or 3 times 2 to 4 hours after a meal and require all of those to be between 5 and 10 milliseconds and be significantly below zero, shown by 2-sided 90% confidence intervals or a single-sided 95% confidence interval.

The QT-interval reduction response to standardized meals further demonstrated that this response is reproducible, and employing lunch as the standard meal

Table 3. QTcF Prolongation — Effect of a Meal

Model	Day	Hours After Time of Start of Breakfast	Effect	SE	DF	*t*	90%CI	
Both	1	2	−10.9	1.78	69.1	−6.10	−13.9	−7.9
		4	−10.1	1.81	71.1	−5.56	−13.1	−7.0
	3	2	−1.0	1.74	808.0	−0.57	−3.8	1.9
		4	−2.6	1.75	801.4	−1.49	−5.5	0.3
	9	2	−2.5	1.83	808.7	−1.38	−5.5	0.5
		4	−3.0	1.82	802.6	−1.68	−6.0	−0.1
OBE002	1	2	−10.3	1.76	70.2	−5.86	−13.3	−7.4
		4	−9.8	1.79	72.3	−5.49	−12.8	−6.8
	3	2	−0.7	1.74	804.9	−0.40	−3.6	2.2
		4	−2.4	1.76	786.6	−1.35	−5.3	0.5
	9	2	−2.1	1.84	808.2	−1.15	−5.1	0.9
		4	−2.9	1.82	803.2	−1.60	−5.9	0.1
OBE022	1	2	−11.0	1.72	81.7	−6.39	−13.8	−8.1
		4	−10.4	1.71	79.9	−6.07	−13.2	−7.5
	3	2	−1.2	1.73	807.7	−0.67	−4.0	1.7
		4	−2.7	1.73	807.8	−1.55	−5.5	0.2
	9	2	−2.7	1.83	808.6	−1.48	−5.7	0.3
		4	−3.2	1.81	807.9	−1.74	−6.1	−0.2
Molar sum	1	2	−10.3	1.76	70.2	−5.86	−13.3	−7.4
		4	−9.8	1.79	72.3	−5.49	−12.8	−6.8
	3	2	−0.7	1.74	804.7	−0.40	−3.6	2.2
		4	−2.4	1.76	786.2	−1.35	−5.3	0.5
	9	2	−2.1	1.84	808.2	−1.15	−5.1	0.9
		4	−2.9	1.82	803.3	−1.60	−5.9	0.1

further demonstrated that this can be used independently of time of day. This offers a choice of fasted or fed dosing, meaning that the method does not interfere with the preferred mode of drug administration. This study further confirms that application of the effect of feeding is a viable nontoxic alternative to the use of a pharmacological positive control to validate QTc assay sensitivity. It allows multiple assessments on any relevant study day, employing the data of all subjects.

Conclusion

The study met the criteria for a negative QT study, with the upper boundary of a 2-sided 90%CI falling below 10 milliseconds with respect to the full range of doses tested.

OBE022 was generally well tolerated. The most frequent AEs were headache and constipation. A previous study with an allosteric FP antagonist[12] recorded 2 instances of QT-interval prolongation that were not considered related to the IMP, which is concordant with the findings of the present study.

In summary, the study shows that OBE022 and its parent, OBE002, are well tolerated by postmenopausal women in the dose range given and are not associated with any QTcF prolongation of regulatory concern.

Declaration of Conflicting Interests

Jörg Täubel, Ulrike Lorch, Simon Coates, Sara Fernandes, and Paul Foley are employees of Richmond Pharmacology Ltd. Georg Ferber is an employee of Statistik Georg Ferber GmbH who has received honoraria for consulting from Richmond Pharmacology. Jean-Pierre Gotteland and Oliver Pohl are employees of ObsEva SA. OBE022 is being developed by ObsEva SA.

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