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RESEARCH PAPER

Role of mixed ion channel effects in the cardiovascular safety assessment of the novel anti-MRSA fluoroquinolone JNJ-Q2

G Eichenbaum[1]*, MK Pugsley[1]*, DJ Gallacher[2], R Towart[2], G McIntyre[3], U Shukla[1], JM Davenport[4], HR Lu[2], J Rohrbacher[2] and V Hillsamer[1]

[1]*Janssen Research & Development, LLC., a division of Janssen Pharmaceutical Companies of Johnson & Johnson, Drug Safety Sciences, Raritan, NJ, USA,* [2]*Janssen Research & Development, a division of Janssen Pharmaceutica N.V., Beerse, Belgium,* [3]*Furiex Pharmaceuticals, Blue Bell, PA, USA, and* [4]*Furiex Pharmaceuticals, Richmond, VA, USA*

Correspondence
G Eichenbaum, Janssen Research & Development, LLC., a division of Janssen Pharmaceutical Companies of Johnson & Johnson, 1000 Route 202 South, Raritan, NJ 08869, USA. E-mail: geichenb@its.jnj.com

*Co-first authors.

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BACKGROUND AND PURPOSE

JNJ-Q2, a novel broad-spectrum fluoroquinolone with anti-methicillin-resistant *Staphylococcus aureus* activity, was evaluated in a comprehensive set of non-clinical and clinical cardiovascular safety studies. The effect of JNJ-Q2 on different cardiovascular parameters was compared with that of moxifloxacin, sparfloxacin and ofloxacin. Through comparisons with these well-known fluoroquinolones, the importance of effects on compensatory ion channels to the cardiovascular safety of JNJ-Q2 was investigated.

EXPERIMENTAL APPROACH

JNJ-Q2 and comparator fluoroquinolones were evaluated in the following models/test systems: hERG-transfected HEK293 cells sodium channel-transfected CHO cells, guinea pig right atria, arterially perfused rabbit left ventricular wedge preparations and *in vivo* studies in anaesthetized guinea pigs, anaesthetized and conscious telemetered dogs, and a thorough QT study in humans.

KEY RESULTS

The trend for effects of JNJ-Q2 on Tp–Te, QT, QRS and PR intervals in the non-clinical models and the plateau in QTc with increasing plasma concentration in humans are consistent with offsetting sodium and calcium channel activities that were observed in the non-clinical studies. These mixed ion channel activities result in the less pronounced or comparable increase in QTc interval for JNJ-Q2 compared with moxifloxacin and sparfloxacin despite its greater *in vitro* inhibition of I_{Kr}.

CONCLUSIONS AND IMPLICATIONS

Based on the non-clinical and clinical cardiovascular safety assessment, JNJ-Q2 has a safe cardiovascular profile for administration in humans with comparable or reduced potential to prolong QT intervals, compared with moxifloxacin. The results demonstrate the importance of compensatory sodium and calcium channel activity in offsetting potassium channel activity for compounds with a fluoroquinolone core.

Abbreviations

APD, action potential duration; BID, twice daily drug administration; dP/dtmax, maximum rate of development of pressure in the left ventricle; EAD, early after depolarization; hERG, human *ether a-go-go*-related gene; I_{Kr}, delayed rectifier outward potassium current; I_{Na},voltage-gated sodium current; I_{Ca}, calcium current; MRSA, methicillin-resistant *Staphylococcus aureus*; QD, once daily drug administration; QTcI, corrected QT interval; QTcF, Frederica corrected QT interval; QTcB, Bazett corrected QT interval; QTcVdW, Van de Water corrected QT interval; Tp–Te, the interval between the peak and end of the T wave; VT, ventricular tachycardia; VF, ventricular fibrillation; TdP, Torsades de Pointes

Introduction

A significant number of approved drugs from different therapeutic classes may prolong the QT interval by as much as 10–20% at therapeutic doses in humans, without evidence suggestive of an increased risk for the development of cardiac arrhythmias (Redfern *et al.*, 2003; Champeroux *et al.*, 2011). These drugs, which are members of the quinolone, macrolide and ketolide classes of antibacterial agents (Chiba *et al.*, 2000; Owens, 2004; Wisialowski *et al.*, 2006), provide important therapeutic options for the treatment of mild to severe infections (Liu *et al.*, 2006; Zuckerman *et al.*, 2009).

When evaluating the cardiac safety of discovery- and development-stage compounds that prolong the QT interval, it is important to evaluate critically their proarrhythmic potential, such as their potential to cause early after depolarization (EAD) or torsades de pointes (TdP) (Redfern *et al.*, 2003). Most of the drugs that increase the duration of the QT interval do so by inhibiting the delayed rectifier outward potassium current (I_{Kr}) from cardiac cells (Yang *et al.*, 2001; Lawrence *et al.*, 2005). As a result, *in vitro* screening of the human *ether-a-go-go*-related gene voltage-dependent potassium channel assay (hERG assay) has been widely used to assess early-stage compounds and is required by the regulatory authorities for a drug to advance into clinical development under ICHS7A and ICHS7B (Pugsley *et al.*, 2008a; Pollard *et al.*, 2010). Screening studies of diverse chemical libraries in the hERG assay have shown that a wide range of chemotypes can bind with high affinity to this ion channel, explaining the chemical diversity of drugs that can prolong the QTc interval (Sanguinetti and Tristani-Firouzi, 2006; Polak *et al.*, 2009). Changes in a functional group of a given chemotype can alter the hERG binding profile significantly; for instance, binding of different fluoroquinolones can vary up to approximately 50-fold (Polak *et al.*, 2009).

For drugs with QT prolongation effects, it is now well recognized that the potency in the hERG assay coupled with QT prolongation alone are not predictive of a cardiac arrhythmogenic potential (Lawrence *et al.*, 2005). Offsetting activity of a drug at the sodium and calcium channels must also be considered (Redfern *et al.*, 2003; Kang *et al.*, 2004; Schneider *et al.*, 2010; Champeroux *et al.*, 2011). The cardiac action potential is determined by a temporal and spatial sum of the fluxes of sodium, calcium and potassium ions across the cell membranes of cardiac myocytes. The relative contributions of these ion fluxes are expected to show differences in the cells of the SA node, atria, AV node and ventricles and determine the action potential shape in these regions (Nerbonne and Kass, 2005). Inhibition of flux of sodium or calcium into the cardiac cells may therefore offset hERG-mediated delayed repolarization, limit dispersion and thereby mitigate the risk for cardiac arrhythmias (Kang *et al.*, 2004; Antzelevitch and Belardinelli, 2006).

Mixed ion channel activity on the sodium and calcium current can be detected *in vivo* by examining changes to the PR and QRS intervals of the ECG. The ECG effects of a sodium channel blocker, such as tetrodotoxin, are limited to prolongation of the QRS interval with little effect on the PR interval (Abraham *et al.*, 1989). In contrast, compounds that inhibit both sodium and calcium channels increase the QRS and PR intervals *in vivo* [e.g. propanolol and thioridazine (Guo *et al.*, 2009)]. Other examples of a diverse range of compounds from different therapeutic classes include verapamil (Zhang *et al.*, 1999), tolterodine (Kang *et al.*, 2004), tapentadol (Schneider *et al.*, 2010), nicardipine (Champeroux *et al.*, 2011) and moxifloxacin (Champeroux *et al.*, 2011).

Over the past 10–15 years, significant progress has been made in developing non-clinical models for evaluating EAD and biomarkers for TdP potential that go beyond only assessing *in vitro* hERG and *in vivo* QT effects (Kang *et al.*, 2004; Lawrence *et al.*, 2005; Liu *et al.*, 2006; Lu *et al.*, 2007; Carlsson, 2008; Pugsley *et al.*, 2008b; Schneider *et al.*, 2010; Champeroux *et al.*, 2011). One of the challenges in developing sensitive models is that TdP in the presence of a prolonged QTc interval is a very low-frequency event in both animals and humans (Rubinstein and Camm, 2002; Yap and Camm, 2003), and therefore, unless one alters the model or increases the concentrations, these effects may not be detected (Pugsley *et al.*, 2008b; Vos, 2008). Several proarrythmia models have been developed (Lawrence *et al.*, 2005; Liu *et al.*, 2006; Carlsson, 2008); however, these models have only been used selectively due to concerns over substantial inter-laboratory variability and other experimental factors that may confound predictive potential (Lawrence *et al.*, 2005).

JNJ-Q2 is a novel broad-spectrum, fluoroquinolone with potent activity against gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and gram-negative pathogens (Morrow *et al.*, 2010). It is a bactericidal agent that inhibits the activity of bacterial type II topoisomerases, DNA gyrase and topoisomerase IV more than other compounds in the class (Morrow *et al.*, 2011). The two enzymes work together in the replication, transcription, recombination and repair of bacterial DNA (Wang, 1996).

The cardiovascular safety profile of fluoroquinolones, as a class, has been extensively evaluated, driven in part by the withdrawal from the market of both sparfloxacin and grepafloxacin, which caused QT prolongation and an increased risk for TdP (Ball, 2000). Therefore, any alterations in the ECG waveform such as QT prolongation, particularly for this class of compounds, needs to be investigated carefully and thoroughly to determine whether a potential for cardiac arrhythmias exists.

Here, we present the results from *in vitro* and *in vivo* non-clinical cardiovascular safety pharmacology studies with JNJ-Q2 (Figure 1), and compare them with results obtained in a human thorough QT study. We also compare the results with those obtained for moxifloxacin, ofloxacin and sparfloxacin (Figure 1). The results provide a basis for understanding the compensatory ion channel effects observed with JNJ-Q2 and the implications for its clinical cardiovascular safety.

Methods

All studies were conducted in accordance with good laboratory practices or best scientific principles. *In vivo* studies were conducted in an Association for Assessment and Accreditation of Laboratory Animal Care approved laboratory in accordance with Institutional Animal Care and Use Committee guidelines and were housed and treated in accordance with

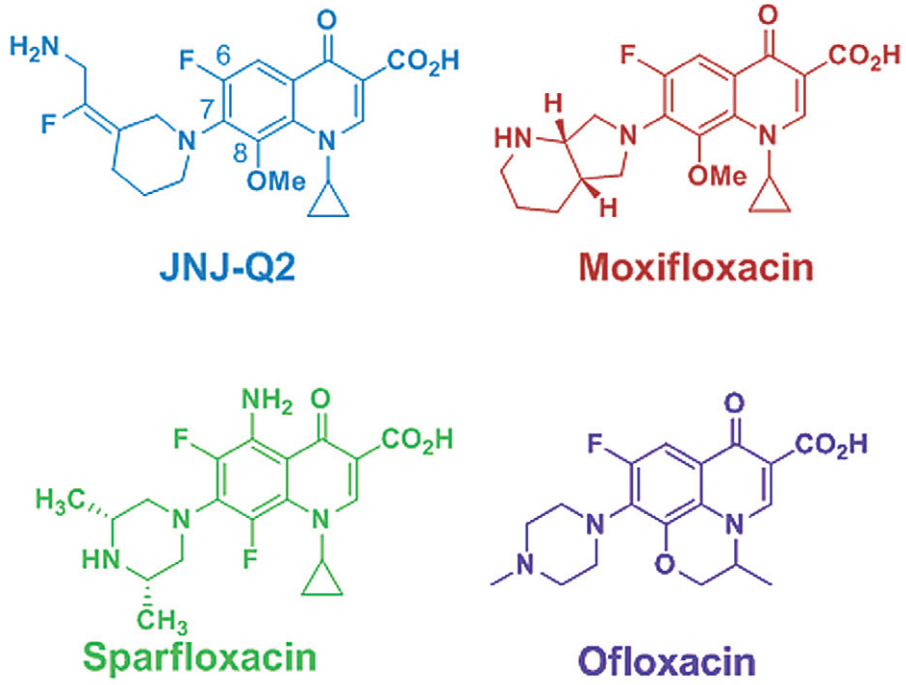

Figure 1
Chemical structure of JNJ-Q2 moxifloxacin, sparfloxacin and ofloxacin.

the Scientific Procedures Act of 1986. Animals were group housed where appropriate and provided with food and water in accordance with ethical guidelines. Rooms were in a cycle of 12 h of light and 12 h of darkness. The concentrations for all the *in vitro* and *in vivo* studies were selected to cover the target therapeutic and supratherapeutic exposures for clinical efficacy against their target pathogens, including MRSA for JNJ-Q2 (Morrow *et al.*, 2010). The nomenclature for the ion channels follows Alexander *et al.*, 2011.

In vitro *studies*

Plasma protein binding. Plasma samples from New Zealand White rabbits, beagle dogs, guinea pigs and healthy human volunteers were pooled separately for each species, with JNJ-Q2 added to yield concentrations of 2 and 20 $\mu g \cdot mL^{-1}$, and assessed for plasma protein binding using rapid equilibrium dialysis techniques. Each plasma sample was dialysed against phosphate buffered saline (pH 7.4) for an equilibration time of 4 h. Plasma and buffer samples were analysed for unchanged JNJ-Q2 concentration using a qualified liquid chromatographic-triple quadrupole mass spectrometric procedure (LC/MS/MS). Values for percentage of bound drug were calculated by taking the ratio of the peak area in the dialysate buffer to the peak area in the dialysate plasma.

Ion channel binding. Patch Express (I_{Na} and I_{Ca} carried by L-type calcium channels) and CEREP screens (using L-type calcium channels) were conducted. In the Patch Express assay, CHO cells (ATCC, Manassas, VA, USA) were stably transfected with the $Na_v1.5$ and $Ca_v1.2$ ion channels cDNA(s) and the current through these ion channels was measured at concentrations of 3, 10, 30, 100 and 300 μM (JNJ-Q2 only, with nifedipine as a positive control) as described previously (Lu *et al.*, 2010). For the CEREP binding assay (non-functional), ligand binding to the calcium channel (L-type, verapamil site) from rat cerebral cortex was defined as the difference between the total binding and the non-specific binding determined in the presence of an excess of unlabelled ligand (Krafte *et al.*, 1995). The DMSO concentration in these assays was ≤0.3%.

Membrane potassium current (I_{Kr}) in hERG-transfected HEK293 cells. HEK293 cells transfected with hERG were incubated with JNJ-Q2 at concentrations of 0.3, 3 and 10 μM, and JNJ-Q2, moxifloxacin, sparfloxacin and ofloxacin at concentrations of 30, 100 and 300 μM (dissolved in bath solution; four or six cells per concentration) for 5 min. hERG encodes a potassium channel ($K_v11.1$) mediating a current with biophysical properties similar to the rapidly activating delayed rectifier potassium current (I_{Kr}) in cardiac myocytes (Trudeau *et al.*, 1995) that contributes to the voltage-operated potassium currents modulating the duration of the repolarization phase of the cardiac action potential (Smith *et al.*, 1996; Snyders and Chaudhary, 1996). Astemizole, a compound known to inhibit the hERG-mediated current at nanomolar concentrations (Zhou *et al.*, 1999), was tested at concentrations of 3, 10 and 30 nM (dissolved in DMSO, 0.1%) as the positive control under identical conditions. Time-matched solvent controls were also included. The hERG-mediated membrane potassium current was measured at distinct membrane potentials using the single electrode, whole-cell voltage patch clamp technique (Hamill *et al.*, 1981). Membrane

current data were recorded on a computer and analysed using the programs Pulse and Pulsefit (version 8.30; HEKA Elektronik Dr. Schulze GmbH, Lambrecht), DataAccess (Bruxton Co., Seattle, WA, USA) and Igor (version 3.13; Wavemetrics Inc., Portland, OR, USA).

Membrane sodium current in CHO cells transfected with hH1a cDNA. CHO cells transfected with the α subunit of the sodium channel from human heart (hH1a cDNA) were incubated with JNJ-Q2 at concentrations of 10, 30, 100, 300 and 1000 μM (dissolved in bath solution) for 5 min. hH1a cDNA encodes a sodium channel (Na_v 1.5) with biophysical properties similar to the human cardiac sodium current in cardiac myocytes (Gellens *et al.*, 1992). Lidocaine, which is known as a preferentially inactivated-state blocker of sodium channels (Bean *et al.*, 1983), was tested at concentrations of 10, 100 and 1000 μM as a positive control. Time-matched solvent controls were included as a negative control. The sodium current (I_{Na}) was measured at distinct membrane potentials using the single electrode, whole-cell voltage patch clamp technique (Hamill *et al.*, 1981). To investigate the state- and concentration-dependent effects of JNJ-Q2, inward peak sodium currents were elicited with test pulses to –20 mV, from two different conditioning holding potentials of –140 and –40 mV. Data were recorded on a computer and analysed using the programs Patchmaster (version 2.05; HEKA), DataAccess (Bruxton Co.) and Igor (version 5.01; Wavemetrics Inc.).

Data from the ion channel experiments were analysed with the Mann–Whitney *U*-test. A two-tailed probability of less than 0.05 ($P < 0.05$) was considered a statistically significant difference. IC_{50} values were calculated by fitting the concentration-response data to the Hill equation (% inhibition = $100/\{1 + [IC_{50}/(C)]^{nH}\}$) where [C], IC_{50} and nH represent the concentration of the test compound, concentration at which 50% inhibition is observed and the Hill coefficient, respectively. The upper and lower bounds for the IC_{50} values were calculated by taking the means plus or the means minus the SD at each concentration, respectively and determining the IC_{50} value.

Guinea pig right atrium. Isolated, spontaneously beating right atrium dissected from female Dunkin Hartley guinea pigs suspended in an organ bath filled with 50 mL of Krebs–Henseleit solution (NaCl 118.8 mM; KCl 4.7 mM; $MgSO_4$ 2.5 mM; KH_2PO_4 1.2 mM; $CaCl_2$*$2H_2O$ 2.5 mM; $NaHCO_3$ 25 mM; glucose 11.1 mM; CaEDTA 0.026 mM). The solution in the organ bath was kept at 37°C and gassed with a mixture of 95% O_2/5% CO_2. JNJ-Q2 was added at increasing concentrations of 10, 30 and 100 μM (dissolved at a concentration of 100 mM in 100% DMSO and diluted such that the maximum concentration of DMSO was less than 0.1%) for 30 min per concentration. The rate and force of contraction was continuously monitored. The electrical signal from force generation was amplified and sampled on a computer at a frequency of 500 Hz. The rate of contraction and contractile force was calculated online (beat-to-beat) using Notocord Hem-Evolution software (version 4.2.0.230). For each variable, the median value was calculated over the last minute of the stabilization period (baseline value) and the three subsequent periods with compound respectively. Values were expressed as percentages of baseline values. Thirty minutes after application of 100 μM JNJ-Q2, the atria were electrically stimulated every minute, starting at 1 Hz up to a maximum of 16 Hz. The frequency of electrical stimulation that could not be followed continuously by a contraction was taken as a measure of the effective refractory frequency. The compound group was compared with Fligner–Wolfe 95% prediction intervals for the median of three experiments (based on n = 505 time- and volume-matched solvent control experiments). DMSO controls were routinely included in the screening experiments.

Arterially perfused rabbit left ventricular wedge preparations. The rabbit wedge has been shown to be a predictive and practical model for evaluating the TdP potential of multiple compound classes including fluoroquinolone antibiotics (Liu *et al.*, 2006; Lu *et al.*, 2007). In a comprehensive examination of drug-mediated changes in repolarization parameters, including hERG inhibition, QT interval prolongation, and transmural dispersion of repolarization (Tp–Te) and QRS, the rabbit wedge was able to distinguish sparfloxacin, a compound with increased clinical TdP potential from moxifloxacin, a compound with reduced clinical TdP potential (Liu *et al.*, 2006; Lu *et al.*, 2007).

Isolated, arterially perfused left ventricular wedges dissected from the heart of female albino rabbits (Lu *et al.*, 2001) and placed in Tyrode's solution containing 4 mM K^+ and buffered with 95% O_2 and 5% CO_2 (T: 35.7 ± 0.1°C, perfusion pressure: 40–50 mmHg), were exposed to JNJ-Q2 or to ofloxacin at increasing concentrations of 0.1, 1, 10, 100 and 300 μM (dissolved in DMSO at ≤0.1% final concentration; n = 7 and 6 per group, respectively) for 40 min per concentration. Sparfloxacin (0.1, 1, 10 and 100 μM; n = 7) and moxifloxacin (3, 30, 100 and 300 μM; n = 7) were also tested under the same conditions. Three DMSO groups were tested under identical conditions as the vehicle control. The preparations were stimulated at basic cycle lengths of 1000 and 2000 ms. A transmural ECG signal was recorded, and the following parameters were measured: duration of the QT interval, the Tp–Te interval [a measure of transmural dispersion of repolarization (TDR)], the Tp–Te/QT ratio (reflects the potential for Phase 2 early after depolarization [EAD] development in the endocardium), the QRS duration and the occurrence of phenomena dependent on Phase 2 EAD (i.e. R on T extrasystole and TdP), ventricular tachycardia (VT) and ventricular fibrillation (VF). Statistical significance was tested using the Student *t*-test for paired and unpaired data; $P < 0.05$ was considered a statistically significant difference.

In vivo *studies*

Anaesthetized guinea pigs. Six female Hartley guinea pigs anaesthetized with pentobarbital (45 $mg \cdot kg^{-1}$ i.p.) and instrumented for recording ECG and arterial blood pressure were administered single, escalating i.v. bolus doses of 0.47, 0.94, 1.88, 3.75, 7.5 and 15 $mg \cdot kg^{-1}$ JNJ-Q2 (dissolved in 5% dextrose in water) at 15 min intervals for a total cumulative dose of 30 $mg \cdot kg^{-1}$. A separate control group of six female guinea pigs were given equivalent volumes of the vehicle. Mean, systolic and diastolic arterial pressures, heart rate and ECG intervals (PR, QRS and QT) were monitored. Bazett's formula

was applied to correct the QT interval for changes in heart rate and expressed as Bazett's corrected QT (QTcB). Data for each parameter were reported as percent change from baseline.

Data were analysed using the Wilcoxon rank sum test to compare percent change from baseline between the vehicle and test compound groups at 5 min after each administration; $P \leq 0.05$ was considered a statistically significant difference. Conclusions about biologically significant changes were based on evidence of dose-dependence, comparison with the corresponding changes in the vehicle group, and the magnitude of the change. In addition, heparinized blood samples obtained 5 min after each dose were analysed by LC/MS/MS methods for determination of JNJ-Q2 plasma concentrations.

Anaesthetized dog – intravenous. Mechanically ventilated, neuromuscular-blocked (1 mg succinylcholine i.v.), closed-chest, anaesthetized (0.015 mg·kg^{-1} scopolamine and 0.075 mg·kg^{-1} lofentanil i.v.) beagle dogs (2/sex) were administered escalating 60 min i.v. infusions of 3, 10 and 30 mg·kg^{-1} for JNJ-Q2; 5, 10, 20 and 40 mg·kg^{-1} for moxifloxacin and 0.3, 1, 10 and 30 mg·kg^{-1} for sparfloxacin (dissolved in 20% hydroxypropyl-beta-cyclodextrin in pyrogen-free water) at 60 min intervals. A control group (3 males and 1 female) received equivalent volumes of solvent. Cardiohaemodynamic parameters (heart rate, aortic blood pressure, systolic and pulmonary artery blood pressure, left ventricular end-diastolic pressure, the maximum positive and maximum negative rate of change of isovolumic left ventricular pressure, stroke volume, cardiac output, pressure rate product, systemic vascular resistance, pulmonary vascular resistance, time constant of relaxation, common carotid artery blood flow and common carotid artery vascular resistance) were measured as described previously (Van Deuren *et al*., 2009). Cardiac electrophysiological parameters measured included surface ECG time intervals (PQ, QRS, QT and QTc calculated according to the formulae of Van de Water, surface index of transmural dispersion of QT [duration of Tp–Te, rTp–Te (Tp–Te/QT*100)], monophasic action potential including the duration of the action potential at 90% repolarization and early and delayed afterdepolarizations, and ECG morphology (presence of a P wave, association between a P wave and a normal QRS complex, regular RR interval, normal morphology of the T wave, absence of supraventricular or ventricular ectopic beats, tachycardia or fibrillation). In addition, blood samples collected at 30 and 60 min after each dose were analysed by a qualified LC/MS/MS method for determination of plasma concentrations for JNJ-Q2 only.

Conscious telemetered dog – oral. In a single-dose cardiovascular safety study, four conscious male beagle dogs surgically implanted with ITS telemetry transducers were administered a single oral dose of 0 (vehicle control), 25, 75 or 150 mg·kg^{-1} per day JNJ-Q2 (dissolved in 0.5% hydroxypropyl methylcellulose) following a 4 × 4 Latin square design. Telemetry data was collected from at least 30 min before dosing and through approximately 22.5 h after dosing. Arterial blood pressure (systolic, diastolic and mean), heart rate, lead II ECG variables, and left ventricular pressure variables (left ventricular systolic blood pressure, left ventricular end-diastolic blood pressure, peak positive and negative dP/dt (rate of development of pressure in the left ventricle), and peak positive contractility index) were measured continuously. ECG time intervals (RR, PR and QT, and QTcF intervals and QRS duration) were determined. ECG waveforms were inspected for disturbances in rhythm and morphology from 30 min prior to dosing to 6 h after dosing. In addition, venous blood samples were collected at 3.25, 8.25 and 22.5 h after each dose and analysed by a validated LC/MS/MS assay to determine JNJ-Q2 plasma concentrations.

In a separate 4 week repeat-dose dog toxicity study, ECG evaluation (9-lead ECG with analysis of lead II) was performed 1–4 h post-dose during week 3 and plasma concentrations were evaluated.

Clinical thorough QT study. The study was a randomized, double-blind, placebo- and positive-controlled, double-dummy, four-period crossover study in male and female human subjects (n = 58) to evaluate the effect of JNJ-Q2 on cardiac repolarization. In each treatment period, the study drug was administered for 4 consecutive days to achieve approximate steady state of the pharmacokinetic profile. The doses of JNJ-Q2 evaluated in this study were 500 mg once daily (QD) (the maximum tolerated dose) and 250 mg twice daily (BID) (the projected efficacious dose). A 400 mg dose of moxifloxacin, a positive control known to prolong QT/QTc intervals, was given as a single dose on Day 4 to validate assay sensitivity. A placebo control was used to evaluate the effect of JNJ-Q2 on QT/QTc intervals compared with placebo.

For each subject, the study consisted of three phases: a screening phase, a double-blind treatment phase and a post-treatment phase. Subjects were randomly assigned to one of four treatment sequences (Sequences 1, 2, 3 or 4) and received one of four treatments (Treatments A, B, C or D) in each treatment period (Periods 1, 2, 3 and 4) in the order specified by the randomization scheme. Subjects received study drug on Days 1 through 4 in each treatment period, followed by a minimum 7 day washout between the last dose of study drug in a treatment period and the first dose of study drug in the next treatment period.

In each treatment period, venous blood samples were collected for determination of JNJ-Q2 plasma concentrations. On Days 1 and 3, blood samples for pharmacokinetic analysis were collected before dosing and at 4 h after dosing, and on Day 2, samples were collected before dosing and at approximately 2 and 4 h after dosing. On Day 4, pharmacokinetic samples were collected before dosing and at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 15, 18, 22.5, 24, 36 and 48 h after dosing, relative to the morning dose. Pharmacokinetic samples were analysed by a validated LC/MS/MS assay to determine JNJ-Q2 plasma concentrations.

All QTc data represent the means of three individual QTc intervals, based on ECGs taken at approximately 2 min intervals. Corrections used for all analyses of the QT data are as follows:

- Individual subject (subject-specific) correction. All pairs of QT and RR interval data collected on Day –1 (Baseline) for each subject were analysed by linear regression: log (QT) = log (a) + b*log (RR). The resulting slope (b_i) for the i^{th}

Table 1
Plasma protein binding of JNJ-Q2

Concentration (μg·mL^{-1})	Mean % bound[a] Guinea pig	Rabbit	Dog	Human
2	66 ± 7[b]	83 ± 2.5	69.3 ± 1.6	70.5 ± 1.2
20	NA	80 ± 1.8	58.5 ± 1.4	62.7 ± 1.4

[a]Percentage bound to proteins in pooled male and female plasma samples, as assessed by rapid equilibrium dialysis. means ± SD from 4 observations per concentration for each species.
[b]Guinea pig at 2.3 μg·mL^{-1} run by rapid equilibrium dialysis.
NA, not applicable – not measured.

subject was then used to calculate the individual correction for that subject as follows: QTcI = QT/RR^b
- Fridericia correction: QTcF = QT/RR$^{(1/3)}$

Each individual QT and RR was used to calculate a QTc interval. These individual QTc intervals were then averaged for analysis.

The primary hypothesis is that the largest time-matched difference in change from the pre-dose baseline in QTcI between each JNJ-Q2 dose and placebo is 10 ms versus the alternative that it is less than 10 ms. The primary analysis is a repeated measures mixed effects linear model that includes the effects of treatment sequence, subjects within treatment sequence, study drug, study period, ECG time point, study drug-by-ECG time point interaction, and period-specific pre-dose baseline QTcI. The response for this model, ΔQTcI represents the change from the period-specific pre-dose baseline, time represents each ECG extraction time, treatment represents the four treatment groups, period represents study period, sequence represents treatment sequence and base QTcI is the period-specific pre-dose baseline QTcI. The period-specific pre-dose baseline QTcI is the mean of the three sets of triplicate QTcI intervals collected within 1 h pre-dose (mean of ECGs extracted at –60, –30, and –15 min prior to dosing).

Results

In vitro *studies*

Plasma protein binding. JNJ-Q2 at a concentration of 2 μg·mL^{-1} was moderately bound (approximately 66–83%) to the plasma proteins of guinea pig, rabbit, dog and human plasma (Table 1). Plasma protein binding was lower in all species at a higher concentration of 20 μg·mL^{-1}.

Ion channel binding. Patch Express ($Na_V1.5$) and CEREP screen (L-type calcium channel). In the Patch Express assay at a concentration of 300 μM the tonic inhibition of I_{Na} by JNJ-Q2, moxifloxacin, sparfloxacin and ofloxacin was (mean±SD) 24.8 ± 6.9%, 4.7 ± 5.2%, 7.5 ± 6.1% and 1.7 ± 1.0% respectively.

In the CEREP screen, there was a 25% binding to L-type calcium channels with JNJ-Q2 at a concentration of 10 μM. This value is indicative of weak to moderate effects.

In vitro *incubations I_{Kr} in hERG-transfected HEK293 cells.* The concentration-dependent inhibition of I_{Kr} in hERG-transfected HEK293 cells by JNJ-Q2, moxifloxacin, sparfloxacin and ofloxacin is shown in Table 2. For JNJ-Q2, no notable effects were found up to a concentration of 3 μM, while at 10 and 30 μM, hERG current was inhibited 29.8 ± 6% and 61.3 ± 4.3% (SEM ± SD), respectively, with an IC_{50} of 19 μM. In comparison, the IC_{50} values for moxifloxacin and sparfloxacin were 122 and 45.9 μM respectively. An IC_{50} for ofloxacin could not be determined because there was only 29% inhibition at a concentration of 300 μM.

Membrane sodium current in CHO cells transfected with hH1a cDNA. Testing JNJ-Q2 on human I_{Na} at 10, 30, 100, 300 and 1000 μM in CHO cells resulted in inhibition of 4.8 ± 2.2%, 9.4 ± 1.1%, 21.6 ± 2.5%, 45.8 ± 7.4% and 66.0 ± 5.0% (mean ± SD), respectively, at –140 mV (resting membrane potential associated with the closed state of the channel) and 11.2 ± 3.1%, 16.6 ± 2.9%, 39.6 ± 2.6%, 62.4 ± 6.0% and 79.0 ± 1.0% (means ± SD), respectively, at –40 mV (potential associated with the inactivated state of channel). The IC_{50} values were 420 and 170 μM, at –140 and –40 mV respectively (Hill coefficients of 0.8).

Guinea pig right atrium. In the guinea pig right atrium, JNJ-Q2 decreased the spontaneous rate of beating at 10, 30 and 100 μM to 93.8, 87.4 and 81.4% of baseline, respectively and the effects at 30 and 100 μM were outside the Fligner–Wolfe 95% prediction intervals. JNJ-Q2 also significantly reduced the maximum stimulation frequency that could be successfully detected, which is a measure of the effective refractory frequency not followed by a contraction (JNJ-Q2 = 8 Hz vs. control range = 11–14 Hz).

Arterially perfused rabbit left ventricular wedge preparations. No EADs, R on T extrasystole, TdP, VT, VF (non TdP) or inexcitability were detected following incubation with JNJ-Q2 (0.1–300 μM) and moxifloxacin (3–300 μM). However, sparfloxacin (tested at 0.1, 1, 10 and 100 μM) elicited EADs in two and seven of the seven preparations each at 10 μM and 100 μM (vs. none of the seven preparations with solvent), and ofloxacin elicited EADs in one of the six preparations only at 300 μM (vs. none of the six preparations with solvent).

The results for QT effects of JNJ-Q2 (n = 6), moxifloxacin (n = 7), sparfloxacin (n = 7) and ofloxacin (n = 6) are shown in

Table 2

Blockade (as % inhibition with IC_{50} values) of I_{Kr} in hERG-transfected HEK293 cells

Concentration (μM)	JNJ-Q2	Moxifloxacin	Sparfloxacin	Ofloxacin
0.3	3.3 ± 3.2 (n = 6)	NA	NA	NA
3	12.5 ± 4.0 (n = 6)	NA	NA	NA
10	29.8 ± 6.0 (n = 5)	NA	NA	NA
30	61.3 ± 4.3 (n = 4)	18 ± 1.7 (n = 3)	38.0 ± 9.6 (n = 4)	12.7 ± 2.0 (n = 3)
100	91.5 ± 0.9 (n = 4)	43.3 ± 1.9 (n = 3)	71.3 ± 7.5 (n = 4)	20.7 ± 2.3 (n = 3)
300	98.3 ± 0.3 (n = 4)	74.0 ± 1.5 (n = 3)	88.5 ± 5.1 (n = 4)	29.3 ± 2.3 (n = 3)
IC_{50} (μM)	19.3	122	45.9	NC
Lower Bound (μM)[a]	7.5	15	21.4	NC
Upper Bound (μM)[a]	8.4	17	44.3	NC

Inhibition data are shown as means±SEM, from the number (n) of assays shown.
[a]SDs for the IC_{50} values could not be determined because of the different number of replicates at each concentrations. Therefore an upper bound for the IC_{50} values was calculated by taking the mean plus the SD at each concentration and determining the IC_{50} and the lower bound was calculated by taking the mean minus the SD at each concentration and determining the IC_{50}.
NA, no data available; NC, not calculated due to insufficient data points.

Figure 2A. Sparfloxacin markedly and concentration-dependently prolonged QT interval (1.26-, 1.75- and 2.31-fold from baseline at 1, 10 and 100 μM, respectively, $P < 0.05$) with high incidence of EADs at 10 and 100 μM, while moxifloxacin prolonged QT interval only starting at 30 μM (1.27-, 1.77- and 2.41-fold from baseline at 30, 100 and 300 μM, respectively, $P < 0.05$), but without any incidence of EADs or TdP. JNJ-Q2 had mild effects on QT interval (1.12- and 1.29-fold from baseline only at 10 and 100 μM, respectively, $P < 0.05$) without any incidence of EADs or TdP. At the highest concentration tested (300 μM), the increase in QT interval by the compound was diminished (1.27-fold; $P > 0.05$). Ofloxacin also significantly prolonged QT interval (1.16- and 1.41-fold at 100 and 300 μM only, respectively; $P < 0.05$).

The results for Tp–Te effects of JNJ-Q2, moxifloxacin sparfloxacin and ofloxacin are shown in Figure 2B. Sparfloxacin largely increased Tp–Te (1.82-, 2.71- and 3.5-fold from baseline at 1, 10 and 100 μM, respectively, $P < 0.05$), moxifloxacin mildly increased Tp–Te (1.32-, 1.93- and 2.93-fold from baseline at 30, 100 and 300 μM, respectively, $P < 0.05$), and ofloxacin increased this parameter (1.09-, 1.16-, 1.36- and 1.9-fold at 10, 30, 100 and 300 μM, respectively; $P < 0.05$). However, JNJ-Q2 had much less effect on Tp–Te (1.17- and 1.46-fold from baseline at 10 and 100 μM only, $P < 0.05$). At 300 μM, the increase in Tp–Te was also diminished by the compound (1.05× of baseline).

The results for QRS effects of JNJ-Q2, moxifloxacin, sparfloxacin and ofloxacin are shown in Figure 2C. Relative to vehicle, the QRS interval was prolonged by JNJ-Q2 (1.15- and 1.96-fold from baseline at 100 and 300 μM, respectively, $P < 0.05$), moxifloxacin (1.12- and 1.27-fold from baseline at 100 and 300 μM, $P < 0.05$), and sparfloxacin (1.10-fold from baseline only at 100 μM, $P < 0.05$) respectively. Ofloxacin had no effect on the QRS interval at any concentration tested.

In vivo *studies*

Anaesthetized guinea pigs. JNJ-Q2, administered i.v. to anaesthetized guinea pigs, had no effect on arterial pressure except for transient increases immediately following bolus dosing at cumulative doses of 7.5, 15, and 30 mg·kg^{-1}. JNJ-Q2 caused dose-related decreases in heart rate that were accompanied by prolongation of QT interval. However, the lack of effect on QTcB indicates that the effects on the QT interval were predominantly heart rate dependent. JNJ-Q2 had no effect on the PR interval or QRS duration of the ECG up to a cumulative dose of 30 mg·kg^{-1}.

In anaesthetized guinea pigs, moxifloxacin produced dose-related decreases in mean arterial blood pressure (MABP) at cumulative doses of 7.5, 15 and 30 mg·kg^{-1}, which were statistically significant at 30 mg·kg^{-1} when compared with vehicle controls 5 min after compound administration. Heart rate was decreased in a dose-dependent fashion at the same doses. The effects on heart rate were also significant at 30 mg·kg^{-1}. The compound also produced dose-related increases in QT and QTcB intervals, which were statistically significant at 15 and 30 mg·kg^{-1} for QT interval and at 30 mg·kg^{-1} for QTcB interval.

A comparison of the effects of JNJ-Q2 and moxifloxacin on QTc, QRS and PR-I is shown in Figure 3. As shown by the magnitude of the error bars, there was significant variability in the data as a result of the effects being more prominent in some animals than others. Nevertheless, the data are included to qualitatively illustrate potential trends that are observed with respect to ATc, ARS and PR-I. Ofloxacin is not shown because it did not produce any dose-related, statistically significant effects on MABP, heart rate or on the ECG at cumulative doses up to 30 mg·kg^{-1}, when compared with vehicle controls 5 min after administration of each dose of compound.

Anaesthetized dog – intravenous. Representative ECGs for JNJ-Q2, moxifloxacin and sparfloxacin compared with vehicle control at the end of infusion for maximum dose administered are shown in Figure 4. JNJ-Q2 at 3 mg·kg^{-1} infused over 60 min (C_{max} = 1.64 μg·mL^{-1}) did not produce any significant effects on cardio-haemodynamic, cardio-electrophysiological

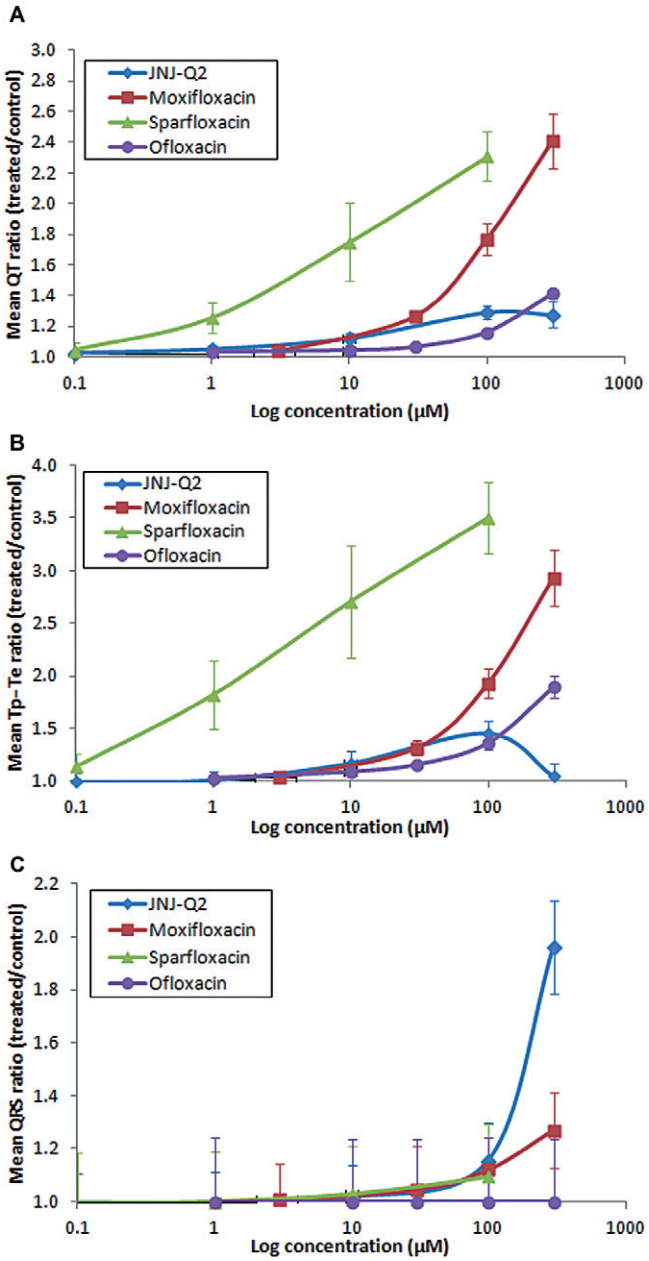

Figure 2

Effect of JNJ-Q2, moxifloxacin, sparfloxacin and ofloxacin on the (A) QT interval [QT statistical significance vs. pretreatment ($P < 0.05$): JNJ-Q2 at ≥ 10 μM, moxifloxacin at ≥ 3 μM, sparfloxacin at ≥ 1 μM and ofloxacin at ≥ 30 μM]; (B) Tp–Te [Tp–Te statistical significance vs. pretreatment ($P < 0.05$): JNJ-Q2 at 100 μM not at 0.1, 1, 10 or 300 μM; moxifloxacin at ≥ 30 μM; sparfloxacin at ≥ 1 μM; and ofloxacin at ≥ 30 μM]; and (C) QRS in rabbit wedge preparations [QRS statistical significance vs. pretreatment ($P < 0.05$): JNJ-Q2 ≥ 100 μM, moxifloxacin at ≥ 300 μM, sparfloxacin not significant and ofloxacin not significant]. Individual data points are mean values ± SD for $n = 6$ or 7.

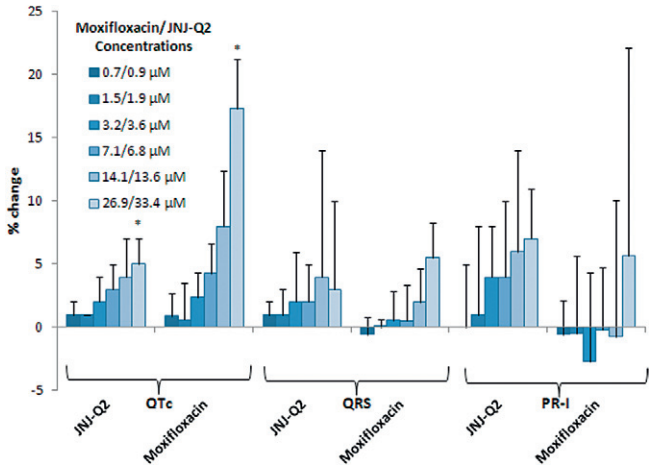

Figure 3

Effect of JNJ-Q2 and moxifloxacin on cardiovascular parameters in pentobarbital-anaesthetized guinea pigs following single escalating i.v. bolus doses at 15 min intervals. Each bar is the mean value and the error bars are the SD. *$P \leq 0.05$ versus solvent control, $n = 6$.

and respiratory parameters. The compound, at doses of 10 and 30 mg·kg^{-1} (C_{max} ranging from 7.15 to 23 μg·mL^{-1}), tended to increase common carotid, systemic and pulmonary vascular resistance, and decrease cardiac contractility. These effects were accompanied by a tendency for an increase in diastolic aortic blood pressure. Furthermore, the amplitude of the T wave increased 70% at 10 mg·kg^{-1} and 168% at 30 mg·kg^{-1} at the end of the infusion. Regarding the cardio-electrophysiological parameters, at 10 mg·kg^{-1}, the compound did not induce prolongation of the PQ, QRS and QT intervals of the ECG. However, at 30 mg·kg^{-1}, JNJ-Q2 induced a slight, but non-statistically significant prolongation of the duration of the heart rate-corrected QT intervals and of the monophasic action potential duration at 90% repolarization (MAP_{90}).

Moxifloxacin at 5 mg·kg^{-1} increased heart rate, and at 10 mg·kg^{-1} increased aortic blood pressure and the duration of the QT interval of the ECG. Administration of 20 mg·kg^{-1} of moxifloxacin increased the global dispersion of repolarization (Tp–Te and relative Tp–Te) and the action potential duration at 90% repolarization (APD_{90}). Infusion of 40 mg·kg^{-1} produced an increase in heart rate (+75%), an increase in QTc (+31%) and the QRS interval on the ECG. Moxifloxacin, at the dose range tested and relative to vehicle, did not induce supraventricular or ventricular arrhythmias, or other changes in the ECG (lead II), except for a decrease in T wave amplitude of 31% at 40 mg·kg^{-1}. The compound induced EADs in the right ventricular endocardial monophasic action potential in one of the six animals after the highest dose of 40 mg·kg^{-1}.

Sparfloxacin at doses of 0.3 to 30 mg·kg^{-1} (total dose = 44.3 mg·kg^{-1}; C_{max} = 27.1 μg·mL^{-1}) did not affect left ventricular contraction (LV dP/dtmax), the PQ and QRS intervals of the ECG, and arterial blood parameters. Relative to vehicle and starting at 1–3 mg·kg^{-1} (C_{max} = 1.2 to 4.2 μg·mL^{-1}), sparfloxacin dose-dependently prolonged the repolarization parameters of the heart such as the APD_{90} and the QT, QTcB, QTcF and QTcVDW intervals. Concomitantly, the compound

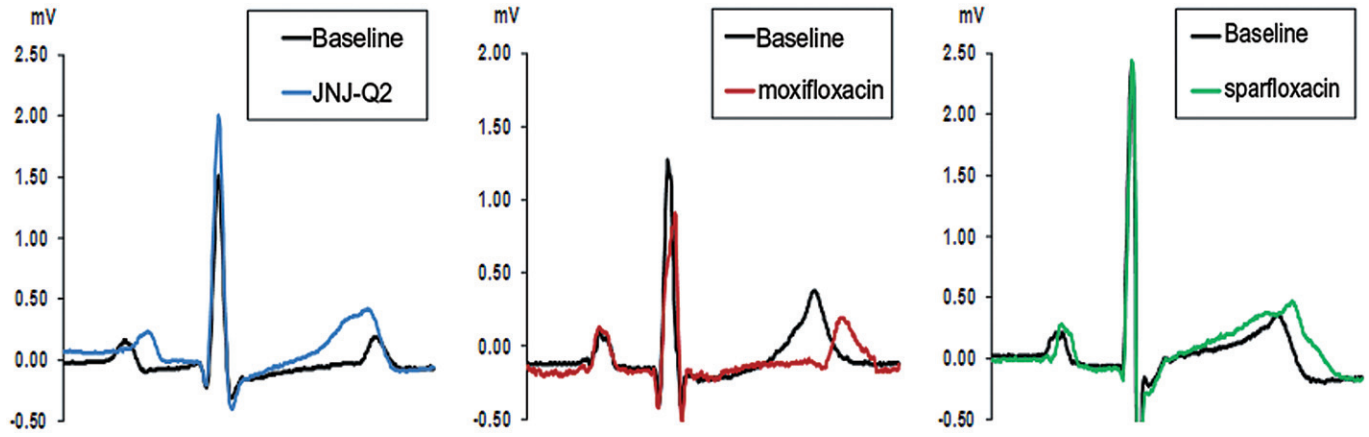

Figure 4

Representative ECGs for JNJ-Q2 (30 mg·kg^{-1}), moxifloxacin (40 mg·kg^{-1}) and sparfloxacin (30 mg·kg^{-1}) compared to vehicle control at the end of infusion for maximum dose administered in anaesthetized dogs.

increased spatial dispersion (Tp–Te and relative Tp–Te), and tended to increase the T wave amplitude of the ECG. At 10 and 30 mg·kg^{-1} (C_{max} = 10.1 and 27.1 μg·mL^{-1}), sparfloxacin dose-dependently increased heart rate, and systolic and diastolic aortic blood pressure. At these doses of the compound, there was a tendency to decrease LV dp/dtmax and the RR interval of the ECG. In the dose range tested and relative to vehicle, the compound did not produce an increase of ventricular and supraventricular arrhythmias. The compound induced an increase in the T wave amplitude of the ECG (lead II). Induction of EADs on the endocardial monophasic action potential signal was seen in one of four dogs after 0.3 mg·kg^{-1}, one of four dogs after 1 mg·kg^{-1} and one out of four dogs after 30 mg·kg^{-1}.

Conscious telemeterized dogs – oral. Following oral gavage administration of JNJ-Q2 to four conscious dogs at doses of 0 (vehicle), 25, 75 or 150 mg·kg^{-1} observed for 22.5 h after dosing there were no significant or biologically relevant changes in arterial blood pressure (systolic, diastolic and mean), heart rate, lead II ECG variables (RR, PR, QRS, QT, QTcF intervals), and or left ventricular variables. ECG wave form analysis revealed no evidence of significant changes in gross morphology or rhythm as a result of administration of either the vehicle or JNJ-Q2 at 150 mg·kg^{-1}. The plasma values at 3.25 h post-dose were 2.2, 5.4 and 6.8 μg·mL^{-1} at doses of 25, 75 and 150 mg·kg^{-1}, respectively. In the 4 week repeat-dose dog toxicity study, which included ECG evaluation, there were no significant effects on PR, QRS or QTc at any doses tested (high dose = 150 mg·kg^{-1} per day) in males and females (C_{max} 11.2 and 10.1 μg·mL^{-1} and AUC_{24h} 147.5 and 106.4 μg×h mL^{-1} respectively).

Clinical thorough QT study. The doses of 250 mg BID and 500 mg QD represent a maximum tolerated dose as higher doses resulted in emesis. Mean differences in heart rate from pre-dose baseline were minor and of no clinical consequence. There were no significant effects on the PR or QRS intervals. The mean concentrations at 2–5 h post-dose are shown in Table 3. Both JNJ-Q2 treatment regimens increased the QTcI interval and the 95% upper confidence bounds exceeded 10 ms over a period of 2–12 h after the dose. The upper confidence bound reached 12 ms in both treatment regimens. Therefore, the primary hypothesis is rejected and the study is considered positive according to the ICH guideline. The observed changes in QTcI following each JNJ-Q2 dose were less than those observed following a single 400 mg dose of moxifloxacin at all time points after 0.5 h except one. The observed changes in QTcI and QTcF following each JNJ-Q2 dose were less than those observed following a single 400 mg dose of moxifloxacin 2–5 h post-dose (Figure 5A and B).

Discussion and conclusion

Role of mixed ion channel effects in mitigating QT prolongation

There is substantial evidence to suggest that sodium and calcium channel block can offset the QT prolongation and TdP risk of compounds binding to hERG channels (Carmeliet, 1977; Kang *et al.*, 2004; Antzelevitch and Belardinelli, 2006; Schneider *et al.*, 2010) and this has been explicitly suggested to mitigate the potential arrhythmogenic effects of ranolazine, vernakalant and verapamil (Yang *et al.*, 2001; Redfern *et al.*, 2003; Savelieva and Camm, 2008). These compensatory ion channel activities at cardiac sodium and calcium channels are evident in the *in vitro* and *in vivo* studies with JNJ-Q2 and comparators described later (Table 4).

Evidence of mixed ion channel effects in cell based assays. In the cell-based assays, JNJ-Q2 showed no notable effects on hERG-transfected HEK293 cells up to a concentration of 3 μM but slight to moderate effects at 10, 30 and 100 μM. Similarly, JNJ-Q2 had a small inhibitory effect on the human cardiac sodium current at 10 μM that increased in a concentration-dependent manner from 30 to 1000 μM and with a greater affinity for the inactivated compared with the resting state

Table 3

Mean ±SD plasma concentrations of JNJ-Q2 in the clinical thorough QT study

Time (h)	250 mg BID plasma concentration ($\mu g \cdot mL^{-1}$)	250 mg BID plasma concentration (μM)	500 mg QD plasma concentration ($\mu g \cdot mL^{-1}$)	500 mg QD plasma concentration (μM)
2	2.81 ± 0.58	6.16 ± 1.27	3.39 ± 0.64	7.44 ± 1.40
3	2.72 ± 0.58	5.97 ± 1.27	3.51 ± 0.7	7.70 ± 1.54
4	2.62 ± 0.54	5.75 ± 1.18	3.35 ± 0.66	7.35 ± 1.45
5	2.49 ± 0.51	5.46 ± 1.12	3.19 ± 0.65	7.00 ± 1.43
C_{max}	2.87 ± 0.60	6.30 ± 1.32	3.63 ± 0.69	7.96 ± 1.51
T_{max} (h) median (min, max)	2.0 (1.0, 4.0)		3.0 (1.0, 6.0)	

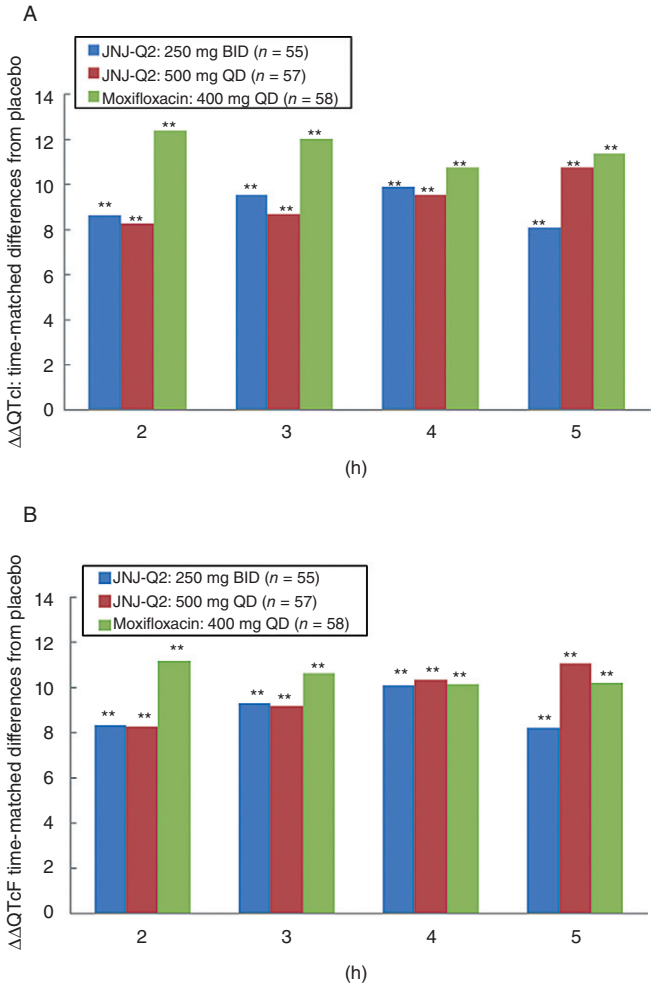

Figure 5

Mean time-matched differences from placebo in change from pre-dose baseline for JNJ-Q2 and moxifloxacin for (A) QTcI (msec) and (B) QTcF (msec) at 2, 3, 4 and 5 h post dose in the clinical thorough QT study; $^{**}P < 0.001$ versus placebo.

(IC_{50} = 420 and 170 μM, at –140 and –40 mV respectively). Additionally, JNJ-Q2 showed 25% binding to L-type calcium channels at 10 μM in the CEREP versus 29.2% hERG inhibition and 29.8% L-type calcium channel inhibition at 100 μM in the Patch Express assay.

It is well documented that the IC_{50} values derived from *in vitro* methods used to assay ion channel inhibition (i.e. static high throughput assays vs. manual patch clamp) can vary significantly due to differences in experimental conditions which may explain the differences in the absolute magnitude of the concentration responses that are observed across models (Polak *et al.*, 2009). Nevertheless the relative activities at the different ion channels within each assay are consistent. Taken together, these data suggest that mixed sodium and calcium ion channel effects may act to offset QT prolongation by I_{Kr} and may mitigate the potential for TdP. The offsetting ion channel effects on I_{Kr} by I_{Na} and I_{Ca} have also been observed for moxifloxacin; at 100 μM, inhibition of hERG channels, I_{Na} and L-type calcium channels were 37, 20 and 10% respectively (Champeroux *et al.*, 2011).

Evidence of mixed ion channel effects in rabbit wedge. The QT, Tp–Te and QRS effects in the rabbit wedge for JNJ-Q2 were also indicative of mixed ion channel activity. As shown in Figure 2A, JNJ-Q2 mildly increased the QT interval to a similar extent as moxifloxacin up to concentrations of 30 μM, but above this plateaued with a maximum increase of 1.29-fold at 100 μM. In contrast, moxifloxacin increased the QT interval at 100 and 300 μM to 1.77-fold and 2.41-fold, respectively without evidence of a plateau. Sparfloxacin increased the QT interval in a dose-dependent manner but at much lower concentrations (1.26-fold at 1 μM vs. no effects for the other compounds and 2.3-fold at 100 μM).

The plot of dispersion of repolarization (Tp–Te) versus concentration (Figure 2B) in the rabbit wedge is similar for JNJ-Q2, moxifloxacin and ofloxacin up to concentrations of 30 μM. Sparfloxacin shows clear concentration-dependent increases in the Tp–Te ratio that are evident beginning at 1 μM with incidence of EADs in two and seven of the seven preparations each at 30 and 100 μM respectively. JNJ-Q2 is distinctly different from the other compounds tested at similar concentrations: the Tp–Te ratio was mildly increased and reached a plateau at 100 μM and then decreased at

Table 4

A comparison of the concentrations of JNJ-Q2 at which non-clinical and clinical cardiovascular effects are observed

Cardiovascular/ clinical endpoint	Test system	% change or inhibition (%)	Concentration (μM)
L-type calcium channel	CEREP	–25	10
hERG potassium channel	HEK293	–50	19.3
I_{Na} (–40 mV)	CHO	–50	170
L-type calcium channel	CHO	–50	300
I_{Na} (–140 mV)	CHO	–50	420
PR	Anaesthetized guinea pig	4	3.6
PR	Anaesthetized guinea pig	6	13.6
PR	Anaesthetized guinea pig	7	33.4
QRS	Anaesthetized guinea pig	2	3.6
QRS	Rabbit wedge	1	10
QRS	Anaesthetized guinea pig	4	13.6
QRS	Rabbit wedge	15	100
QRS	Rabbit wedge	96	300
QTcF	Humans (250 mg BID)	2.5	5.75
QTcI	Humans (250 mg BID)	2.4	5.75
QTcB	Anaesthetized guinea pig	3	6.8
QTcF	Humans (500 mg QD)	2.7	8
QTcI	Humans (500 mg BID)	2.5	8
QT	Rabbit wedge	12	10
QTcB	Anaesthetized guinea pig	5	33.4
QT	Rabbit wedge	29	100
QT	Rabbit wedge	28	300
Tp–Te	Rabbit wedge	17	10
Tp–Te	Rabbit wedge	46	100
Tp–Te	Rabbit wedge	5	300
ECG – no effect-QTcQ	Conscious dog	7	16.3
ECG – no effect-QTcV	Anaesthetized dog	3	50.0

The human exposures are from the thorough QT study.
QTcF pre-dose baseline = 402.5 ms; QTcI pre-dose baseline = 404.5.
Human QTcF Change at 5.75 μM = 10 ms and 8 μM = 11 ms.
Human QTcI Change at 5.75 μM = 9.91 ms and 8 μM = 10.22 ms.

300 μM. This difference may be due to the effects of JNJ-Q2 on I_{Na} at these concentrations, shown as significantly increased QRS duration at 100 and 300 μM.

It is interesting to note that the changes in the QT and Tp–Te ratios versus baseline shown in Figure 2A and B are comparable at all concentrations (Tp–Te/QT ratio was also comparable at all concentrations – data not shown) except at 300 μM for JNJ-Q2 where the reduction in Tp–Te is slightly larger. This similarity between the changes in QT and Tp–Te is expected because they are both reflective of I_{Kr}-mediated repolarization. The more pronounced reduction of Tp–Te for JNJ-Q2 at 300 μM could be a result of increased sodium channel activity affecting Tp–Te. The QRS interval in the rabbit wedge is comparable for all of the compounds tested up to 100 μM but at 300 μM there is a large increase in QRS for JNJ-Q2 (1.96-fold) and a modest increase for moxifloxacin (1.27-fold).

Evidence of mixed ion channel effects in guinea pig right atria. Further supporting a mixed ion channel effect are results from a screening assay using the guinea pig right atrium. The reduction in the maximum following frequency and reduced spontaneous beating rate with JNJ-Q2 are suggestive of inhibition of sodium and calcium channels at 30 and 100 μM.

Evidence of mixed ion channel effects in anaesthetized guinea pig. Mixed ion channel effects of JNJ-Q2 are also evident in the anaesthetized guinea pig. There was a trend for an increase in mean QRS and PR interval for JNJ-Q2 and moxifloxacin that did not reach statistical significance but that increased with dose (PR interval for moxifloxacin only increased at the highest concentration). Changes in QRS and PR interval were evident at lower concentrations for JNJ-Q2 but not for moxifloxacin. These mixed ion channel effects

may explain why there was a trend for an increase in QTc for both compounds with increased concentrations that did not reach statistical significance except at the highest concentrations of 26.9/33.4 μM (Figure 3). The magnitude of the change in QTc with concentration was much greater for moxifloxacin compared with JNJ-Q2 (approximately 17 vs. 5%) (Figure 3).

Evidence of mixed ion channel effects in dog. The absence of any statistically significant QTc or other notable cardiovascular effects with JNJ-Q2 in the anaesthetized and conscious dog safety pharmacology and toxicity study is consistent with potential sodium and calcium channel inhibition offsetting any effects on hERG in the dog. In contrast, in both anaesthetized and conscious dogs (Mittelstadt and Hart, 2005), moxifloxacin dose-dependently altered autonomic control (increased heart rate and vascular resistance), affected QT, Tp–Te, and QRS and produced negative inotropic effects and instability resulting in pro-arrhythmic tendencies (EADs).

The dog is a less sensitive model for detecting QT or other cardiovascular effects of JNJ-Q2 than the rabbit wedge or anaesthetized guinea pig. There are a number of published studies showing significant interspecies differences in ion channel properties and distribution between dogs compared with rabbits and guinea pigs that may explain the differences observed with JNJ-Q2 (Szigligeti *et al.*, 1998; Takagishi *et al.*, 2000; Nerbonne and Kass, 2005). It has also been suggested that increased sympathetic tone may reduce the sensitivity of the dog to hERG-induced QT prolongation (Champeroux *et al.*, 2011). Therefore, it cannot be ruled out that in the anaesthetized dog treated with JNJ-Q2, the slight increase in sympathetic tone contributes to offsetting any QT prolongation, in addition to the mixed ion channel effects.

Evidence of mixed ion channel effects in humans. There is also evidence of compensatory mixed ion channel effects for JNJ-Q2 in humans. The QTc prolongation appears to have reached a plateau at the clinical maximum tolerated dose (limited by emesis). The QTc prolongation at a dose of 500 mg QD (C_{max} = 8 μM) was similar or less than the QTc at 250 mg BID (C_{max} = 5.5 μM) (Figure 5A and B). This plateau was also observed the rabbit wedge, in which QTc decreased between 100 and 300 μM.

The mixed ion channel activity that is evident with JNJ-Q2 in the cardiovascular safety studies is a characteristic of other compounds that prolong the QT interval but do not cause overt arrhythmias, such as moxifloxacin, ranolazine, risperidone and nicardipine (Champeroux *et al.*, 2011)]. Generally, a distinguishing feature between arrhythmogenic and non-arrhythmogenic compounds with mixed ion channel activities is that there are proarrhythmic changes (Tp–Te and instability) other than QT observed *in vivo* (see data for sparfloxacin Figure 5B) and the free therapeutic plasma concentrations of the arrhythmogenic compounds is often greater than the I_{Kr} IC_{50} values (Champeroux *et al.*, 2011). Interestingly, for the non-arrhythmogenic compounds, the sodium and calcium channel IC_{50} values in isolated single ion channel expression systems are in many cases 5–100-fold lower than those for I_{Kr} but are sufficient to mitigate TdP risk (Champeroux *et al.*, 2011). This difference could be explained by inhibition of sodium channels in the non-inactivating state (not evaluated) (Ju *et al.*, 1992).

Comparison of non-clinical and clinical cardiovascular effects

The ECG effects and the corresponding concentrations at which they were observed in the non-clinical and clinical studies are summarized in Table 4. A correction for protein binding was not applied because the protein binding across species is similar (Table 1), the *in vitro* effects occur at much higher concentrations than are achieved *in vivo*, the free concentration in the cardiac tissue is unknown (Smith *et al.*, 2010) and, if it were applied, it would increase the *in vitro* to *in vivo* margins.

The data in Table 4 show that in the different test systems, the QRS and QTc changes are similar in magnitude and in the concentration at which they occur. Changes in the QRS interval were only observed in the non-clinical models but not in humans. The QRS changes were 1–4% in the 1–10 μM range and 15–96% between 100 and 300 μM. The QTcF interval changed 2.2–12% in the 1–10 μM range and 5–32% between 34 and 300 μM. When viewed in aggregate, these data suggest that there are offsetting effects of sodium and calcium channel blockade on I_{Kr} inhibition that are comparable in magnitude at JNJ-Q2 concentrations up to 8 μM (C_{max} at the maximum tolerated dose in humans = 500 mg QD).

Conclusion

The integrated cardiovascular data demonstrate that JNJ-Q2 has an acceptable safety profile for administration in humans. The trend for effects of JNJ-Q2 on Tp–Te, QT, QRS and PR intervals in the non-clinical models and the plateau in QTcF with increasing plasma concentration in humans are consistent with offsetting sodium and calcium channel activities that were observed in the non-clinical studies. These mixed ion channel activities result in the less pronounced or comparable increase in QTc interval for JNJ-Q2 compared with moxifloxacin and sparfloxacin despite its greater *in vitro* I_{Kr} inhibition. The integrated cardiovascular safety assessment that we have presented herein provides a valuable basis for understanding the role that compensatory cardiac ion channel effects can have on cardiovascular function.

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Conflict of interest

The co-authors are employees of either Janssen R&D, LLC or Furiex Pharmaceuticals. Furiex Pharmaceuticals is working on the development of JNJ-Q2 for the treatment of complicated skin and skin structure infections and community acquired pneumonia and both companies stand to benefit financially from the successful development of the compound.

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