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A Double-Blind, Placebo-Controlled Trial to Evaluate the Safety, Tolerability, and Pharmacokinetics of Single, Escalating Oral Doses of JDTic

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Animal studies suggest that kappa opioid receptor antagonists (KORAn) potentially could treat a wide variety of addictive and depressive disorders. We assessed the KORAn JDTic for safety, tolerability, and pharmacokinetics in a double-blind, placebo-controlled, randomized trial evaluating single oral doses in healthy adult males. Predose and postdose safety assessments included orthostatic vital signs; 6-lead continuous telemetry monitoring (approximately 16 h predose to 24 h postdose); 12-lead electrocardiograms (ECGs); clinical chemistry, hematology, coagulation, and urinalysis; psychomotor functioning (using the Wayne Saccadic Fixator (WSF)); and adverse events. As a potential indicator of JDTic effects on affect, the POMS Standard instrument was administered predose and daily postdose Days 1–6. At 1 mg, 2 of the 6 JDTic (and 0/6 placebo) subjects experienced a single, asymptomatic event of multiple beats of nonsustained ventricular tachycardia (NSVT). Their events were temporally similar with respect to time postdose (and the postdose timing of an NSVT event in a monkey). These events triggered a study stopping rule. No differences were observed between the placebo and JDTic subjects with respect to clinical chemistry, hematology, coagulation, urinalysis, orthostatic vital signs, WSF, or 12-lead ECG parameters. Plasma JDTic levels were below the lower limit of quantitation (0.1 nM) in all subjects. There were no significant differences in POMS scores between the placebo and JDTic groups. Although the evidence is circumstantial, it suggests that NSVT is a potential JDTic toxicity in humans. Given the therapeutic potential of KORAn, further investigation is needed to determine whether a significant JDTic human cardiac effect indeed exists, and if so, whether it is specific to JDTic or represents a KORAn class effect.
Neuropsychopharmacology (2015) 40, 2059–2065; doi:10.1038/npp.2015.27; published online 4 March 2015

INTRODUCTION

The kappa opioid receptor (KOR) and its endogenous ligand dynorphin are enriched in brain regions involved in stress response, and potential clinical utility for KOR antagonists (KORAn) has been shown in depression and substance abuse (Carroll and Carlezon, 2013). JDTic is a potent and selective KORAn (Thomas et al, 2003) that in preclinical models shows antidepressant and anxiolytic activity and reduces the signs of nicotine withdrawal, the stress-induced reinstatement of cocaine-reinforced behavior, and manifestations of alcohol abuse (Carroll and Dolle, 2014). KORAn are a drug class of considerable pharmaceutical interest, and RTI International intended to develop JDTic for several indications. This article reports the results of the first-in-human safety study.

Kappa-active compounds also have cardiovascular effects; however, results are mixed. In most rat studies, kappa

opioid receptor agonists (KORAg) are cardioprotective (eg, Jaiswal et al, 2010; Zhou et al, 2001), reduce arrhythmias (eg, Jin-Cheng et al, 2008; Lishmanov et al, 2007; Peart et al, 2004; Xiu et al, 2013), have positive inotropic effects (eg, Pyle et al., 2001), and are a component of ischemic preconditioning (Yu et al, 1999; Zatta et al, 2008). Consequently, KORAn could have negative effects. One study reported that highly selective KORAn activate c-Jun N-terminal kinase (JNK; Bruchas et al, 2007). In another study, lipopolysaccharide activation of JNK caused disruption of fatty acid oxidation by a human ventricular-derived cardiomyocyte cell line and caused cardiac dysfunction in C57BL/6 mice (Drosatos et al, 2011). However, in dogs (Hartlage et al, 2006) and swine (Coles et al, 2003), KORAn are cardioprotective. Also, negative inotropic (Ventura et al, 1992) and pro-arrhythmic (Bian et al, 1998) effects of U-50,488H and cardioprotective effects of nor-BNI (Liu et al, 2005) are described in rats.

Biopsied human ventricular myocytes contain dynorphin (Ationu et al, 1993), and surgical specimens of human atrial tissue (Lendeckel et al, 2005) and pooled human heart extracts (Peng et al, 2012) contain KOR mRNA. In human hearts, there are KOR immunoreactive fine nerve fibers, cardiomyocytes, and cells resembling intrinsic

cardiac adrenergic cells (Sobanski et al, 2014), consistent with rabbit cardiomyocyte immunoprecipitation studies (Surendra et al, 2013). The KORAg U-50,488H has negative inotropic effects on isolated human right atrial strips not antagonized by the selective KORAn nor-BNI or the broader-acting naloxone (Llobell and Laorden, 1995). Similarly, U-50,488H has effects on transient outward K^+ and ultrarapid delayed rectifier K^+ currents in human pediatric atrial myocytes that are not antagonized by nor-BNI or naloxone (Xiao et al, 2003). However, in human subjects undergoing angioplasty, the acute ischemic preconditioning effect of the first of a pair of balloon occlusions was abolished by naloxone (Tomai et al, 1999). Ventricular tachycardia is listed under 'Precautions' and 'Adverse Reactions' in the product insert for naloxone (Hospira, 2007) and is described in case reports (eg, Hunter, 2005). In the first-in-human study of the KORAn LY2456302 (Lowe et al, 2014, p. 6), 'a 5-beat ventricular tachycardia occurring approximately 8 h after receiving a single 25-mg dose' was observed in 1 of the 21 subjects (5%) receiving the drug, which does not activate JNK (Melief et al, 2011).

Nonclinical studies (data on file, RTI International, Research Triangle Park, NC) identified potential JDTic electrocardiogram (ECG) toxicities but at a human equivalent dose (HED) of >6000 mg and plasma concentrations >920 nM. Review of the first 24 h of ECG following a 200 mg/kg intragastric dose of JDTic (HED of 4500 mg) revealed one of the six cynomolgus monkeys (17%) had three ventricular premature complexes in a row (by definition, nonsustained ventricular tachycardia (NSVT)),

at 11 h 51 min postdose (Figure 1a). NSVT in cynomolgus monkeys can be an incidental finding; 16- to 24-h Holter monitoring of a colony of 62 monkeys revealed ventricular tachycardia (VT) in 8 (13%) (Macallum and Houston, 1993). Asymptomatic NSVT can be observed in humans (cf. Min et al, 2010). NSVT prevalence in normal, healthy males is described in four publications using qualitatively different populations (eg, inclusion of females, significantly higher median age), with estimates in the range of 2–3.2% (Bjerregaard, 1982; Brodsky et al, 1977; Clarke et al, 1976; Hinkle et al, 1969).

Following the human trial, a preliminary analysis of JDTic effects in human iPSC cardiomyocyte cultures (approach described by Guo et al, 2011) estimated that the concentration at which >20% of the spontaneous beats were arrhythmic was between 2 and 20 nM (Supplementary Figure S1). These concentrations are well below the IC_{50} values observed for hERG channel, human cardiac sodium channel, and guinea pig L-type calcium channel (5000 nM) (data on file, RTI).

SUBJECTS AND METHODS

Study Design

The JDTic-001 study was a single-center, double-blind, placebo-controlled, randomized clinical trial to evaluate the safety, tolerability, and pharmacokinetics (PK) of single, escalating oral doses of JDTic (1, 3, and 10 mg) in healthy male subjects, in three separate cohorts using a semiadap-

Figure 1 Nonsustained ventricular tachycardia (NSVT) in preclinical and clinical subjects. (a) 3-Beat NSVT in male cynomolgus monkey 11.8 h after 200 mg/kg intragastric JDTic (timescale not available). (b) 9-Beat NSVT in male human subject 13.5 h after 1 mg oral JDTic (the smallest grid boxes represent 0.04 s). (c) 7-Beat NSVT in male human subject 11.5 h after 1 mg oral JDTic (same timescale as in panel (b)).

tive dosing plan (cohorts 1 and 2 comprised four subjects and cohort 3 comprised eight subjects, in each case randomized 3 : 1 JDTic:placebo). Prior to dosing a subsequent cohort, all safety and PK data were reviewed by a Safety Review Team (SRT) as guided by a Data and Safety Monitoring Plan (DSMP). If adverse events (AEs) considered to be at least possibly related to study medication occurred during the first two cohorts, an additional four subjects were dosed at that same dose level, unless otherwise determined by the SRT. A final amendment put in place after the initial eight subjects were dosed stipulated that if AEs requiring study termination occurred during an eight-subject cohort, an additional four blinded subjects would be dosed with placebo; consequently, this portion is single blind.

DSMP stopping rules included typical criteria for serious adverse events (SAEs) and adverse events of specific interest (AESI) (eg, terminating the study if $\geqslant 25\%$ of a dosing cohort experienced a possibly or definitely drug-related AESI, such as NSVT), as well as limitations on JDTic plasma maximum concentration (C_{max}) and area under the curve from zero to infinity ($AUC_{0-\infty}$). The starting dose in the trial, 1 mg, was predicted to result in a maximum plasma concentration of 0.04 nM (below the lower level of quantitation, 0.1 nM); this was reduced from a proposed 3 mg starting dose, in response to a Food and Drug Administration clinical hold. Also in response, protocol ECG exclusion criteria were made even more restrictive than normal (eg, PR interval >180 ms, QTcB interval >420 ms), and telemetry was expanded to begin the night before dosing and to continue for 24 h postdose.

Potential subjects presented to the clinic within 14 days of their intended dose date to undergo a battery of screening assessments. Eligible subjects came to the clinic the day before dosing for eligibility reconfirmation. Subjects remained in the clinic that night and for 6 days postdose for safety assessments and timed blood and urine collections. If there were no ongoing safety issues on Day 6, subjects were sent home from the clinic and they returned 14 days later for a follow-up safety assessment. Subjects were then discharged from the study.

This study was approved by the clinical site's Institutional Review Board and conducted in accordance with the International Conference on Harmonisation E6 Consolidated Guidance for Good Clinical Practice (1996), the US Code of Federal Regulations 21 parts 50 and 56, and the ethical principles of the Declaration of Helsinki (as amended in 1996). It was performed at Celerion in Tempe, AZ, and is reported at ClinicalTrials.gov (NCT01431586).

Participants

Inclusion criteria. Subjects were healthy males, 18–50 years, $\geqslant 50$ kg in weight, body mass index (BMI) 18–30 kg/m^2, with normal vital signs (heart rate, systolic and diastolic blood pressure, respiratory rate, oral temperature), a negative prestudy drug screen (alcohol, cannabinoids, amphetamines, benzodiazepines, cocaine, opioids, phencyclidine, barbiturates, cotinine), no history of use of illicit drugs or other substances of abuse within 12 months of the screening visit, no tobacco use for $\geqslant 90$ days prior to screening, and no history of cardiovascular disease. Routine 12-lead ECG at screening

(a 10-s single-lead rhythm strip and approximately 2.5-s samples of each lead) had to be without clinically significant abnormalities, as did laboratory analyses of hematology (hemoglobin, hematocrit, reticulocyte count, red blood cells, red blood cell indices, white blood cells, white blood cell differential), chemistry (aspartate aminotransferase, alanine aminotransferase, gamma glutamyl transferase, serum troponin I, blood urea nitrogen, creatinine, total bilirubin, alkaline phosphatase, creatine kinase, lactate dehydrogenase, uric acid, total protein, globulin, cholesterol, triglycerides, sodium, potassium, calcium, chloride, phosphorus), coagulation (prothrombin time, activated partial thromboplastin time), urinalysis (specific gravity, pH, bilirubin, nitrite, urobilinogen, glucose, protein, blood, leukocytes, ketones, microscopic examination), and serology (HIV, hepatitis B surface antigen, hepatitis C antibodies).

Exclusion criteria. Subjects were excluded for a history or evidence of hepatic, gastrointestinal, renal, respiratory, ophthalmic, cardiovascular, hematological, endocrine/metabolic, neurological, immunological, oncological, or psychiatric illness or significant abnormalities, or any other conditions or surgical interventions known to interfere with the absorption, distribution, metabolism, or excretion of drugs. Subjects were excluded for any active infection. Consumption of alcohol within 48 h of screening or clinic admission; of caffeine-containing beverages within 72 h of clinic admission; or of grapefruit or grapefruit-, poppy seed-, or quinine-containing substances within 14 days of clinic admission were exclusionary. Subjects were excluded for any clinically significant ECG finding prior to dosing (including bundle branch blocks, second- or third-degree heart block, arrhythmia other than respiratory sinus arrhythmia, or multiple premature atrial contractions or premature ventricular contractions but with the exception of nonclinically significant unifocal premature ventricular contractions), presence or history of early cardiac repolarization, or a history or family history of QT prolongation or arrhythmia. ECG parameters outside the following ranges were also exclusionary: heart rate 55–100, PR interval 120–180 ms, QRS duration 70–100 ms, QTcB interval $\leqslant 420$ ms.

Safety and Tolerability End Points

Observational. Orthostatic vital signs were obtained predose and at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h postdose, as well as at 14 days postdose follow-up. Body weight was measured on admission to the clinic, at 48 and 120 h postdose, and at 14 days postdose follow-up. Physical examinations of all major organ systems (including neurological examination) were performed at admission, at 120 h postdose, and at 14 days postdose follow-up. AEs were monitored from dosing until 14 days postdose or resolution, whichever was longer. Psychomotor function was evaluated with the Wayne Saccadic Fixator (WSF; Wayne Engineering, Skokie, IL) on admission and at 120 h postdose.

Electrocardiogram. 6-Lead ECG telemetry was monitored from admission (approximately 16 h predose) until 24 h postdose. Supine, standard 12-lead ECGs (a 10-s single-lead rhythm strip and approximately 2.5-s tracings of each lead)

were obtained predose; at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96, and 120 h postdose; and at 14 days postdose follow-up.

Clinical laboratory. Urine for urinalysis and fasted blood samples for chemistry, hematology, and coagulation were obtained on admission, at 48 and 120 h postdose, and at 14 days postdose follow-up.

Pharmacokinetics

Blood samples were obtained at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 12, 16, 24, 30, 36, 48, 60, 72, 96, and 120 h postdose. Cumulative urine specimens were collected over the intervals 0–6, 6–12, 12–24, 24–36, 36–48, 48–72, 72–96, and 96–120 h postdose.

Potential Surrogate Marker of Activity

As a potential indicator of JDTic effects on subject affect, the Profile of Mood States Standard instrument (POMS; Multi-Health Systems, North Tonawanda, NY) was administered (in the 'Right Now' recall period) on admission to the clinic and on Days 1–6 postdose.

RESULTS

Study Population

The first subject enrolled on 8 August 2011, and the final subject completed the trial on 12 December 2011. The demographic profile of the study population is summarized in Table 1. All subjects in the study were male. Six (50%) of the study subjects were of Hispanic ethnicity. The majority (9 of the 12 subjects) were white, 2 were black/African American, and 1 was Asian. Mean age was 31 years (range 19–44 years) and mean BMI was 25 kg/m^2 (range 22–28 kg/m^2).

Table 1 Summary of Subject Demographics

Subject demographics	Placebo (*n* = 6)	1 mg JDTic (*n* = 6)	Overall (*n* = 12)
Race, n (%)			
Asian	0	1 (17%)	1 (8%)
Black or African American	0	2 (33%)	2 (17%)
White	6 (100%)	3 (50%)	9 (75%)
Ethnicity, n (%)			
Hispanic or Latino	4 (67%)	2 (33%)	6 (50%)
Not Hispanic or Latino	2 (33%)	4 (67%)	6 (50%)
Age in years, mean (SD)	27 (6)	35 (7)	31 (8)
Range	19–34	22–44	19–44
Height in cm, mean (SD)	167 (3)	174 (5)	170 (6)
Range	161–171	169–184	161–184
Weight in kg, mean (SD)	71 (8)	76 (9)	74 (9)
Range	62–83	61–89	61–89
BMI in kg/m^2, mean (SD)	26 (2)	25 (2)	25 (2)
Range	23–28	22–27	22–28

Abbreviation: BMI, body mass index.

The demographic profiles of those subjects who received JDTic 1 mg and those subjects who received placebo were similar, with the exception of race. All 6 subjects who received placebo were white *vs* 3 (50%) of the 6 subjects who received JDTic 1 mg.

Safety and Tolerability

There were no deaths and no SAEs reported during the study, and no AE led to an individual subject discontinuing the study prematurely.

Overall, there were 13 reported AEs: 9 AEs in the subjects who received JDTic 1 mg and 4 AEs in the subjects who received placebo. Four unique subjects exposed to 1 mg JDTic (67%) and 4 unique subjects who received placebo (67%) experienced at least one AE (Table 2). Six AEs were considered to be possibly related to the study medication, and four AEs were in three subjects who received JDTic: VT (9 beats, 13.5 h postdose; Figure 1b), bradycardia and VT (7 beats, 11.5 h postdose; Figure 1c), and postural dizziness. The arrhythmias were asymptomatic and were detected the following mornings via review of the entire telemetry tracings by a cardiologist.

As specified in the JDTic-001 protocol and DSMP, the two instances of NSVT in two subjects after administration of JDTic (a 33% incidence in subjects receiving JDTic) triggered a study termination rule. Both of these subjects subsequently underwent cardiac evaluations (21–41 days postdose), including echocardiograms, exercise myocardial perfusion studies, and treadmill stress tests. They remained asymptomatic, and no abnormalities were revealed. Protocol Amendment 3 (which allowed for the dosing of four additional subjects with placebo) was drafted to provide additional control subjects to better ascertain a relationship between the occurrence of NSVT and JDTic administration. None of the four additional subjects dosed under Amendment 3 experienced NSVT during the study.

Other safety results were unremarkable: clinical chemistry, hematology, coagulation, urinalysis, orthostatic vital signs, 12-lead ECGs (including heart rate (Figure 2a), PR interval, QRS duration, and QTcB interval (Figure 2b)), and psychomotor functioning. As these figures show, the mean HR and QTcB duration were consistently slower and shorter for JDTic than placebo subjects at all predose and postdose time points, although the differences were not statistically significant ($p > 0.05$).

Pharmacokinetics

JDTic was not detected in the plasma of any subject (lower limit of quantitation 0.1 nM).

Potential Surrogate Marker of Activity

There were no significant differences in POMS scores between the placebo and JDTic groups (Supplementary Table S1).

DISCUSSION

In this first-in-human clinical trial of the KORAn JDTic, six subjects received the starting oral dose of 1 mg and six

Table 2 Adverse Events

Subject	Adverse event	Clinical principal investigator's assessment of causality	Treatment
11102	Ventricular tachycardia (9 beats)	Possibly related	1 mg JDTic
	Presyncope (unrelated to ventricular tachycardia)	Definitely not related	
11103	Dizziness postural	Possibly related	Placebo
12101	Bradycardia	Possibly related	1 mg JDTic
	Ventricular tachycardia (7 beats)	Possibly related	
12103	Somnolence	Possibly related	Placebo
12104	Vessel puncture site pain	Definitely not related	1 mg JDTic
	Vessel puncture site pain	Definitely not related	
	Dizziness postural	Possibly related	
	Dermatitis contact	Definitely not related	
12105	Excoriation	Definitely not related	1 mg JDTic
13101	Dermatitis contact	Definitely not related	Placebo
13103	Headache	Definitely not related	Placebo
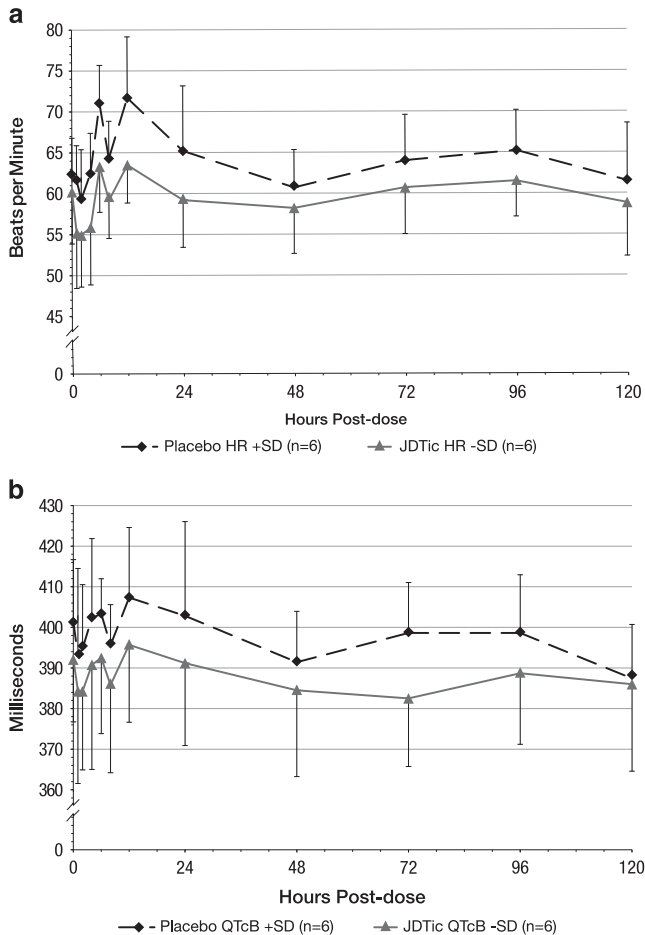
Figure 2 Subject electrocardiogram (ECG) heart rate and QTcB interval over time. (a) ECG heart rate. (b) QTcB interval.

subjects received placebo. JDTic was undetectable in their plasma (LLOQ 0.1 nM). There were no deaths and no SAEs. Subjective AEs possibly related to study drug in placebo subjects were postural dizziness (one instance) and somnolence (one instance). In JDTic subjects, AEs comprised postural dizziness (one instance). There were no significant differences in clinical laboratory parameters (chemistry, hematology, coagulation, urinalysis), 12-lead ECG parameters, or WSF measures of psychomotor function. POMS scores did not differ between placebo and JDTic subjects. However, two JDTic subjects (33%) had asymptomatic single instances of NSVT on telemetry, triggering a study stopping rule.

We were surprised by the surfacing of a potential NSVT safety signal at the initial 1 mg dose. Applying the published 2–3.2% prevalence range of NSVT to the study population, the project statistician calculated the probability range of having exactly two otherwise healthy people with NSVT in a random sample of six people to be 0.006–0.013. In terms of study site experience with NSVT prevalence during the previous 9 years, it is estimated that approximately one in 3000 subjects showed an episode of NSVT. This is based on numerous studies which had cardiac rhythm monitoring either as a function of safety telemetry or as a function of thorough QT analysis. However, the full ECG/telemetry/Holter record was not reviewed by a cardiologist in all of these studies. Nevertheless, these site-specific data imply a very low prevalence.

Although the evidence is circumstantial, based on the temporal similarity of the 2 individual occurrences of NSVT out of 6 subjects (33%) who received JDTic, the statistical unlikelihood that the 2 observed occurrences of NSVT were preexisting conditions in the affected subjects, the temporal similarity to the episode of NSVT observed in 1 of the 6 (17%) monitored monkeys dosed with JDTic, and the existence of a possible causal mechanism, NSVT could be a JDTic toxicity in humans. Certainly, there is no longer any commercial interest in the clinical development of JDTic.

Given the preclinical and clinical data presented in the Introduction, it is plausible that JDTic could have a direct cardiac interaction in human beings, although perhaps not through the classic KOR, which itself has myriad potential

cellular interactions (Bruchas and Chavkin, 2010). Although plasma JDTic levels were unquantifiable ($< 0.1\,\text{nM}$) in this human study, there is preclinical data to suggest that 1 mg could be an efficacious human dose for KOR antagonism. The intragastric JDTic AD_{50} for inhibition of the effects of exogenous enadoline-induced behaviors (15 min postdose) in cynomolgus monkeys was 0.025 mg/kg, which would equate to an HED of 0.5 mg (with a predicted human C_{max} of 0.02 nM) (data on file, RTI). Once again, these concentrations are much less than the lowest observed to cause prolongation of QTc in cynomolgus monkeys ($> 920\,\text{nM}$).

JDTic activates JNK, which can in turn cause disruption of fatty acid oxidation in a human ventricular-derived cardiomyocyte cell line and cardiac dysfunction in mice, a potential mechanism through which JDTic could cause cardiac dysfunction in humans. However, LY2456302 does not activate JNK (Melief *et al*, 2011); nevertheless, a single episode of NSVT was observed among 21 subjects (5%) who received at least one dose of LY2456302 in the first-in-human study (Lowe *et al*, 2014). The '5-beat ventricular tachycardia (occurred) approximately 8 h after a single 25-mg dose' in one of the eight subjects receiving that dose, but NSVT was not observed in any of the seven subjects receiving a 60-mg dose. The numbers are few, but here NSVT is not dose-related, so again the single episode might be unrelated to the KORAn activity of LY2456302. Still, given its occurrence and the NSVT that have been associated with naloxone, there remains the possibility that a human cardiac response to these agents could be associated more directly with the KOR itself and be a class effect of KORAn rather than specific toxicities associated with the molecules. It simply is impossible to know from the currently available data.

Given the therapeutic potential of the KORAn agents, it is imperative that further preclinical studies using human materials (eg, *in vitro* experiments with human cardiomyocytes) be conducted to clarify whether there is in fact a human cardiac effect of JDTic or its metabolites; and, if so, the mechanism by which it occurs (ie, class effect or specific JDTic-related toxicity). Depending on these *in vitro* data, further human trials of JDTic might be considered.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)