

Safety, Tolerability, and Pharmacokinetic Evaluation of Single- and Multiple-Ascending Doses of a Novel Kappa Opioid Receptor Antagonist LY2456302 and Drug Interaction With Ethanol in Healthy Subjects

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Abstract

Accumulating evidence indicates that selective antagonism of kappa opioid receptors may provide therapeutic benefit in the treatment of major depressive disorder, anxiety disorders, and substance use disorders. LY2456302 is a high-affinity, selective kappa opioid antagonist that demonstrates >30-fold functional selectivity over mu and delta opioid receptors. The safety, tolerability, and pharmacokinetics (PK) of LY2456302 were investigated following single oral doses (2–60 mg), multiple oral doses (2, 10, and 35 mg), and when co-administered with ethanol. Plasma concentrations of LY2456302 were measured by liquid chromatography-tandem mass spectrometry method. Safety analyses were conducted on all enrolled subjects. LY2456302 doses were well-tolerated with no clinically significant findings. No safety concerns were seen on co-administration with ethanol. No evidence for an interaction between LY2456302 and ethanol on cognitive-motor performance was detected. LY2456302 displayed rapid oral absorption and a terminal half-life of approximately 30–40 hours. Plasma exposure of LY2456302 increased proportionally with increasing doses and reached steady state after 6–8 days of once-daily dosing. Steady-state PK of LY2456302 were not affected by coadministration of a single dose of ethanol. No clinically important changes in maximum concentration (C_{max}) or AUC of ethanol (in the presence of LY2456302) were observed.

Keywords

kappa opioid receptor, dynorphin, LY2456302

The efficacy of kappa opioid receptor (KOR) antagonists in producing antidepressant-like effects in pre-clinical models and early human studies has led to interest in developing a selective kappa antagonist for the treatment of depression. LY2456302 ((S)-3-fluoro-4-(4-((2-(3,5-dimethylphenyl)pyrrolidin-1-yl)methyl)phenoxy)benzamide¹) is a high-affinity antagonist at KOR, which demonstrates >30-fold pharmacological selectivity over mu and delta opioid receptors, as shown in in vivo receptor occupancy and pharmacology assays.²

Currently, antidepressants that modulate monoamine levels, while useful for some patients, are not effective in a substantial proportion (about 25–50%) of patients with major depressive disorder, and are not free from adverse events.^{3,4} Chronic stress is associated with increased risk for depression, and may be an underlying factor in depression.^{5–7} The KOR system is a key mediator of stress signaling in the brain, with the dysphoric component of stress being encoded by activation of the kappa opioid system.^{7–10} Based on evidence that KOR function influences emotional states^{8–10} selective antagonism of

KORs may provide therapeutic benefit in treating major depressive disorder, anxiety disorders, and substance use disorders.^{8–12} In pre-clinical models, kappa antagonists blocked the behavioral and neurochemical effects of stress, produced antidepressant-like effects, and reversed the prodepressive effects of kappa agonists on intracranial self-stimulation thresholds and other behavioral measures of anhedonia.^{11–16} Furthermore, kappa antagonists attenuated ethanol self-administration and reinstatement to

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ethanol-seeking in animal models.^{17,18} Although selective KOR antagonists have not been tested clinically for depression, Alkermes (Dublin, Ireland) has reported that in a Phase 2 clinical trial, ALKS-5461 improved symptoms in patients with major depressive disorder who had not responded to prior treatments.³ ALKS-5461 is a combination of the mixed opioid pharmacology compound, buprenorphine, with the mu opioid receptor antagonist, ALKS-33; a combination that is believed to produce functional kappa antagonist activity.³ The significant unmet need for effective treatments for depression has led to interest in the development of a selective KOR antagonist.

The aims of the present clinical studies were to assess the safety, tolerability, and pharmacokinetics (PK) of LY2456302. Study A was the first-in-human study of single escalating oral doses of LY2456302 administered to healthy subjects. The multiple-ascending dose study (Study B) of repeated daily doses of LY2456302 in healthy subjects used a dose range based on results of Study A.

Potential PK and cognitive interactions between LY2456302 and concomitant ethanol were investigated in Study B because the metabolic pathway for LY2456302 has not been fully elucidated and potential effects of ethanol on the absorption of LY2456302 cannot be ruled out. In vitro solubility data for LY2456302 showed an increased solubility in ethanol compared to gastric pH levels, suggesting that ethanol consumption while taking LY2456302 may result in unwanted interactions (data on file, Eli Lilly and Company, Indianapolis, IN). Because of the possibility that subjects treated with LY2456302 may consume alcohol, it was important to investigate potential PK interactions between LY2456302 and ethanol, and to evaluate the safety and tolerability of combined administration of LY2456302 and ethanol, including the impact of LY2456302 on ethanol's effects on cognitive function.

Methods

Study Design

Both studies were conducted at Covance Clinical Research Unit (CRU), Inc., Evansville, IN; Study A from December 2008 to May 2009, and Study B from September 2010 to February 2011. The studies were conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The Covance CRU Institutional Review Board approved the protocol, and all subjects gave their written informed consent.

Subjects in each study included healthy males and females with no childbearing potential, aged 21–65 years, who had a body mass index (BMI) of 18–35 kg/m². Patients were excluded if they had current or previous

(within the past year) Axis I diagnosis of major depressive disorder, mania, bipolar disorder, psychosis, dysthymia, generalized anxiety disorder, alcohol, or eating disorders as determined by the investigator and confirmed by the Mini-International Neuropsychiatric Interview.¹⁹ Patients with current suicidal ideation as assessed by the Columbia Suicide Severity Rating Scale were also excluded.

In both studies, dose escalation was limited to maintain a 30-fold margin of safety to the no-observed-adverse effect level (NOAEL) for convulsions observed in a pre-clinical toxicology study in rabbits, such that at the highest administered dose the median maximum concentration (C_{max}) of LY2456302 did not exceed 180 ng/mL. Interim PK assessments were conducted to ensure maintenance of the margin of safety. Dose escalations were initiated only after completion of the interim assessment and review of safety data from all subjects receiving LY2456302 or placebo from the preceding cohort.

Study A. Study A was a Phase 1, single-ascending dose, randomized, placebo-controlled, subject- and investigator-blind, crossover study conducted in two alternating cohorts (Cohorts A and B) of healthy subjects to assess safety, tolerability, and PK of LY2456302. For each cohort, subjects were randomized to either LY2456302 or placebo in a block of 4:3 LY2456302 to one placebo. Each subject received up to three doses of LY2456302 and up to one placebo dose to achieve the greatest degree of within-subject control for evaluating outcomes and for controlling for potential bias.

Subjects underwent a maximum of four single-dose treatment periods with a minimum washout of 7 days between doses. Subjects were randomly assigned to receive LY2456302 or placebo such that at least six subjects received LY2456302 and at least two subjects received placebo during each treatment period. Subjects in Cohort A received 4, 25, and 60 mg LY2456302, and placebo. Subjects in Cohort B received 2 and 10 mg LY2456302, and placebo. Subjects entered the CRU on Day –1 (day before dosing) and were observed for at least 24 hours after dosing on Day 1 of each period.

Study B. Study B was a Phase 1, multiple-ascending dose, randomized, placebo-controlled, subject- and investigator-blind, parallel study in three cohorts (Cohorts A, B, and C) of healthy subjects to assess the safety, tolerability, and PK of multiple doses of LY2456302. The PK interactions between LY2456302 and ethanol were investigated in Cohort B. An interim assessment of PK data of LY2456302 was conducted after all Cohort B subjects completed to confirm the dose selected for Cohort C.

Subjects in Cohorts A and C were randomly assigned to receive LY2456302 or placebo as daily oral doses on 14 consecutive mornings. Cohort A received 2 mg LY2456302 or placebo and Cohort C received 35 mg

LY2456302 or placebo. Subjects entered the CRU on Day -1 (day before dosing) and were discharged on Day 15.

Subjects in Cohort B underwent two treatment periods (1 day, and 14 days in duration). In Period 1, on Day -2, subjects were admitted to the CRU and, on Day -1, attended training sessions for cognitive/motor assessments. On Day 1, after administration of a single ethanol drink, subjects underwent serial blood sampling for determining blood ethanol concentrations. Cognitive/motor tests were administered prior to ethanol dosing and at regular intervals for 6 hours after dosing. Subjects were discharged after completion of Period 1 and underwent a washout of 3–9 days prior to the start of Period 2.

In Period 2, on Day -1, Cohort B subjects were admitted to the CRU and on Day 1 were randomly assigned to once-daily dosing of 10-mg LY2456302 or placebo for 14 days. Placebo-dosing was to retain the blind. The placebo results are not reported. On Day 13, when LY2456302 was at steady state, subjects received LY2456302 or placebo followed immediately by an ethanol placebo. On Day 14, subjects were administered LY2456302 or placebo followed immediately by an ethanol drink. On both days, serial blood samples were collected for determination of LY2456302 and ethanol concentrations, and cognitive/motor tests were administered for 6 hours after dosing. Subjects were discharged from the CRU after completion of study procedures on Day 15.

Study drug formulation and administration. Study drug was provided as capsules containing 2 and 25 mg of LY2456302 or matching placebo (Eli Lilly and Company). After an overnight fast of at least 8 hours, LY2456302 or placebo capsules were administered orally, with water, in the morning of each dosing day.

Ethanol dose and administration. In Study B, the ethanol dose was 0.6 g/kg for women and 0.7 g/kg for men. The respective doses were selected as a social dose of alcohol (equivalent to approximately 4 units for a 70-kg subject), which is known to cause a measurable and consistent decrease in cognitive function.²⁰ The ethanol drink consisted of vodka (40% ethanol) mixed with orange drink for a final volume of 400 mL. The ethanol placebo was orange drink with 1 mL supernatant of ethanol for blinding. In each case, the drink was divided in two equal portions. Subjects drank the entire first cup of ethanol/placebo within 5 minutes (± 1 minute) of LY2456302- or placebo-dosing. After 5 minutes (± 1 minute), they drank the second cup of ethanol/placebo within 5 minutes (± 1 minute).

Blood sampling. In Study A, venous blood sampling for measuring LY2456302 occurred at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 48, 72, 96 hours following each dose of study drug.

In Study B for Cohorts A and C, serial blood samples for determining concentrations of LY2456302 were

collected after single-dose administration (Day 1) and at steady state (Day 14) at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 hours post-dose. Further samples were collected at 48, 72, 96, 120, 144, 168, and 192 hours post-Day 14 dose and pre-dose on Days 6, 8, 10, 12, and 13. Cohort B underwent blood collection on Day 1 of Period 1 and Day 14 of Period 2, for determining ethanol concentrations at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, and 6 hours post-dose. In Period 2, on Day 1, Day 13, and Day 14, blood samples were drawn to evaluate LY2456302 at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 hours post-dose. Sampling was repeated pre-dose on Days 6, 8, 10, and 12 for steady-state assessment.

For analysis of neurohormones, in Study A, venous blood samples were taken 0, 2, 4, and 24 hours post-dose. In Study B, samples were taken on Days 1 and 14, at 0, 2, 4, and 24 hours post-dose for luteinizing hormone (LH) and prolactin, and 0, 1, 2, 3, 4, 6, 8, and 24 hours post-dose for cortisol and adrenocorticotropic hormone (ACTH).

Safety assessments. After each dose of LY2456302, subjects underwent safety assessments for approximately 4 days in Study A and throughout Study B with appropriate follow-up upon presentation of clinical signs or symptoms. Safety measures included: adverse events (AEs), vital signs, electrocardiograms (ECGs), clinical laboratory tests, and blood sampling for neurohormones. Neurohormones (cortisol, prolactin, LH, and ACTH) were measured in both studies (ACTH in Study B but not Study A) to assess any potential effects of LY2456302 on the hypothalamic–pituitary–adrenal (HPA) axis.

Cognitive and motor assessments. In Cohort B, after ethanol/ethanol placebo administration subjects took a battery of tests from the Cognitive Drug Research (CDR) System.^{21–24} Briefly, the tests included; *simple reaction time* where patients pressed the “YES” button as quickly as possible every time the word “YES” appeared on the screen; *digit vigilance* in which the subject pressed the “YES” button as quickly as possible every time the digit on the screen matched a randomly selected target digit; *choice reaction time* where the subject responded to the words “YES” and “NO” as they appeared on screen by pressing the corresponding button as quickly as possible; *tracking* where the subject used a joystick to track a randomly moving target on the screen; and *postural stability* (body-sway) where the ability to stand upright without moving was assessed. The tests were supervised by trained site-staff pre-dose (0 hour), 0.5, 1.5, 2.5, 4, and 6 hours post-dose on Day 1 of Period 1, and Days 13 and 14 of Period 2.

Bioanalytical Methods

Study A. Plasma samples were analyzed for LY2456302 using a validated liquid chromatography with tandem mass spectrometry (LC/MS/MS) method (Advinus Therapeutics, Bangalore, India). The lower

limit of quantification was 0.20 ng/mL, and the upper limit of quantification was 202.70 ng/mL. The interassay accuracy (% relative error) during validation ranged from -4.55% to 3.19%. The interassay precision (% coefficient of variation [CV]) during validation ranged from 2.10% to 4.76%.

Study B. Blood samples were analyzed for LY2456302 using a validated LC/MS/MS detection method (Covance, Indianapolis, IN). The lower limit of quantification was 0.20 ng/mL, and the upper limit of quantification was 200.00 ng/mL. The inter-assay accuracy (% relative error) during validation ranged from -11.0% to -5.0%. The interassay precision (% CV) during validation ranged from 5.4% to 11.2%.

Whole blood samples were analyzed for ethanol using a validated College of American Pathologists/Clinical Laboratory Improvement Amendments (CAP/CLIA) chromatographic method. The lower limit of quantification was 25 mg/dL and the upper limit of quantification was 400 mg/dL. The inter-assay precision (% CV) during validation was 7% for quality control (QC) level 1 (64.9 mg/dL) and 7.6% for QC level 2 (153.2 mg/dL). Accuracy of ethanol was established by analysis of survey material of known concentrations with an acceptance range of 90–110%.

Pharmacokinetic Analyses

Pharmacokinetic parameter estimation. Pharmacokinetic parameter estimates for LY2456302 in both studies and for ethanol in Study B were calculated by standard non-compartmental methods of analysis and computed using WinNonlin Enterprise 5.3 (Pharsight, St. Louis, MO). All concentrations below quantification limit (BQL) between time zero and the first quantifiable concentration were replaced with 0, while all remaining BQL values were treated as missing. Actual sampling times were used in the estimation of individual PK parameters, with the exception of predose sampling times on the dosing day, which were set to zero. At each sample collection time point, mean concentration values were calculated only when at least two-thirds of the subjects had measurable plasma/blood concentrations that were collected within $\pm 15\%$ of the intended protocol sampling time.

In Study A, the primary parameters for analysis of LY2456302 were C_{\max} and area under the concentration–time curve (AUC). The log-linear trapezoidal rule was used to estimate AUC. Individual PK parameters estimated were C_{\max} , time of observed C_{\max} (t_{\max}), AUC from time zero to the last quantifiable time point [$AUC_{(0-t_{\text{last}})}$], and AUC from time zero to infinity [$AUC_{(0-\infty)}$].

In Study B, the primary parameters for LY2456302 analysis included: on Day 1, C_{\max} , t_{\max} , and the AUC over the dosing interval (from time zero to 24 hours) [$AUC_{(0-24)}$]; and at steady state, C_{\max} ($C_{\max,ss}$), and AUC

over the dosing interval [$AUC_{(0-\tau)ss}$]. Plasma accumulation was evaluated by the ratio of $AUC_{(0-\tau)ss}$ over $AUC_{(0-24)p}$, and the $C_{\max,ss}$ over Day 1 C_{\max} .

PK parameters determined for ethanol were $C_{\max,b}$, $t_{\max,b}$, AUC from time 0 until the time of the last measurable concentration [$AUC_{(0-t_{\text{last}})}$], for ethanol [$AUC_{(0-6)}$].

In both studies, other non-compartmental parameters, such as half-life ($t_{1/2}$), apparent clearance (CL/F), and apparent volume of distribution (Vz/F) were also determined. For Cohort B of Study B, $t_{1/2}$ for LY2456302 was not evaluated due to the alcohol interaction study design and sampling being restricted to 24 hours post-final dose.

Pharmacokinetic Statistical Inference

Dose proportionality. To delineate effects of dose proportionality for LY2456302, log-transformed $C_{\max,ss}$ and $AUC_{(0-\tau)}$ estimates were evaluated using a power model to estimate ratios of geometric means and the corresponding 90% confidence intervals (CIs). The approach of Smith et al.²⁵ was followed to assess dose proportionality by considering an (0.7, 1.43) acceptance range.

LY2456302 and ethanol interaction, Cohort B. To evaluate whether the PK of ethanol was altered in the presence of LY2456302, the PK of ethanol alone was compared to its PK when coadministered with LY2456302. The effect of ethanol on the PK of LY2456302 was also evaluated. The primary PK parameters for comparison were $C_{\max,ss}$ and $AUC_{(0-\tau)}$ of LY2456302, and C_{\max} and $AUC_{(0-t_{\text{last}})}$ of ethanol. A repeated-measures ANOVA was used to analyze the log-transformed PK parameters with appropriate covariance structure. The model included day as a fixed effect and subject as a random effect while day and dose/ethanol were confounded in this case. The ratio of least squares (LS) geometric means between the treatments (Period 2 Day 14 vs. Day 13 and Period 2 Day 14 vs. Period 1 Day 1) and corresponding 90% CI were estimated. Inclusion of the 90% CI within an equivalence region of 0.80–1.25 was used for interpretation of the PK parameter comparisons.

Statistics

Sample size. For Study A and Study B, the sample size was customary for Phase 1 studies evaluating safety and PK parameters, and not powered on the basis of statistical hypothesis testing.

Safety. Safety analyses conducted for all enrolled subjects included AEs, safety laboratory parameters, vital signs, and ECG parameters. The parameters were summarized using standard descriptive statistics.

Neurohormones. For analysis of cortisol, prolactin, LH, and ACTH, a repeated mixed model was used to evaluate the change from time 0 (baseline) to each post dose time point. Neurohormone concentrations were

log-transformed prior to analysis by a repeated-measures ANOVA model fitted with day, time, and day-by-time interaction as fixed effects and subject as a random effect. Additional analyses including gender as a fixed effect from the above model were performed. The LS mean differences to baseline (0 hour) at each scheduled time point was obtained with 90% CI and corresponding *P*-values. The mean difference and CI were back-transformed to obtain the mean ratio and corresponding CI.

Cognitive and Motor Assessments. For each cognitive parameter change from pre-dose scores was analyzed using a repeated-measures mixed-model analysis of covariance (ANCOVA). In the model, pre-dose score was a covariate with day, time, and day-by-time interaction as fixed effects, and subject as a random effect with an unstructured correlation matrix between measurements within a subject. The LS means were calculated for each treatment condition at each time point. Mean differences and 95% CI were calculated with time-matched comparisons of ethanol alone versus ethanol placebo coadministered with LY2456302 and ethanol coadministered with LY2456302. The data presented include subjects in Cohort B who received 10 mg LY2456302.

Results

Demographics and Disposition

Study A enrolled 21 subjects; 11 subjects in Cohort A and 10 subjects in Cohort B (Table 1). All 21 subjects received

at least one dose of LY2456302. Eighteen subjects completed the study. Reasons for early discontinuation included; lost to follow-up (*n* = 1), protocol violation (*n* = 1), and discontinuation due to an AE (*n* = 1) due to ventricular tachycardia after receiving 25 mg LY2456302 in Period 2.

Study B enrolled 37 subjects; 12 each in Cohorts A and C (9 subjects on LY2456302 and 3 subjects on placebo in each Cohort), and 13 in Cohort B (10 subjects on LY2456302 and 3 subjects on placebo) (Table 1). All subjects in Cohort B were exposed to ethanol with and without the study drug, and received ≥ 1 dose of LY2456302. A total of 35 subjects completed the study. Discontinuations were due to subject (10-mg dose group) decision after receiving nine doses of LY2456302, and sponsor decision due to a non-treatment-emergent cardiac arrhythmia; the non-treatment-emergent AE occurred just prior to dosing the subject with a single dose of 35 mg LY2456302. Consequently the subject was withdrawn after the dosing with 35 mg LY2456302.

Safety and Tolerability

Study A. A total of 26 AEs were reported by 9 subjects (Table 2a). The most common AEs (≥ 2 incidences) occurring after dosing with LY2456302 were headache (*n* = 6), viral upper respiratory tract infection (*n* = 3), anxiety (*n* = 2), and diarrhea (*n* = 2) of which anxiety and diarrhea were considered LY2456302-related. All AEs were mild or moderate in severity. One subject was withdrawn from the study due to an AE. This subject was

Table 1. Demographics (Study A = Single Dose Study; Study B = Multiple Dose Study)

	Study A		Study B				
	Cohort A	Cohort B	Cohorts A and C			Cohort B	
	LY: 4, 25, 60 mg, Placebo	LY: 2, 10 mg, Placebo	Placebo	Cohort A	Cohort C	Placebo + Ethanol	LY: 10 mg + Ethanol
			Placebo	LY: 2 mg	LY: 35 mg		
Number	11	10	6	9	9	3	10
Median age (years)	47.0	56.0	36.5	30	34	29	42
Sex							
Male	7	6	5	8	7	3	9
Female	4	4	1	1	2	0	1
Race	8	9	4	8	5	3	9
White	1	1	2	0	2	0	1
Black	1	0	0	1	1	0	0
Asian	1	0	0	0	1	0	0
American Indian or Alaska Native							
Ethnicity							
Hispanic or Latino	0	0	0	0	0	2	1
Not Hispanic or Latino	11	10	6	9	9	1	9
Median weight (kg)	73.4	88.2	83.0	80.2	77.9	82.8	83.5
Median BMI (kg/m ²)	27.5	30.3	26.5	25.7	25.8	28.2	26.1

BMI = body mass index; LY = LY2456302.

Table 2a. Adverse Event Profile after Administration of Single Doses of LY2456302 in Study A; All Causality and Related to Study Drug, by Treatment in Order of Frequency

	Number of Adverse Events (Subjects) ^a													
	Placebo		LY2456302											
	(n = 13)		2 mg (n = 6)		4 mg (n = 8)		10 mg (n = 7)		25 mg (n = 7)		60 mg (n = 7)		All Doses	
	Total	Related	Total	Related	Total	Related	Total	Related	Total	Related	Total	Related	Total	Related
Headache	1 (1)	1 (1)	0	0	0	0	5 (2)	0	1 (1)	1 (1)	0	0	6 (4)	1 (1)
Nausea	1 (1)	1 (1)	0	0	0	0	0	0	0	0	0	0	0	0
Anxiety	0	0	0	0	1 (1)	1 (1)	0	0	1 (1)	1 (1)	0	0	2 (1)	2 (1)
Abdominal pain	0	0	0	0	0	0	1 (1)	1 (1)	0	0	0	0	1 (1)	1 (1)
Diarrhea	1 (1)	1 (1)	0	0	1 (1)	1 (1)	0	0	1 (1)	1 (1)	0	0	2 (1)	2 (1)
Somnolence	0	0	0	0	0	0	1 (1)	1 (1)	0	0	0	0	1 (1)	1 (1)
Viral upper respiratory tract infection	0	0	0	0	1 (1)	0	1 (1)	0	1 (1)	0	0	0	3 (3)	0
Paraesthesia	0	0	0	0	0	0	1 (1)	1 (1)	0	0	0	0	1 (1)	1 (1)
Flushing	1 (1)	1 (1)	0	0	0	0	0	0	0	0	0	0	0	0
Gingival infection	1 (1)	0	0	0	0	0	0	0	0	0	0	0	0	0
Arrhythmia	0	0	0	0	0	0	0	0	1 (1)	0	0	0	1 (1)	0
Ventricular tachycardia	0	0	0	0	0	0	0	0	1 (1)	1 (1)	0	0	1 (1)	1 (1)
Neck pain	1 (1)	1 (1)	0	0	0	0	0	0	0	0	0	0	0	0
Pain in extremity	0	0	0	0	0	0	1 (1)	1 (1)	0	0	0	0	1 (1)	1 (1)
Catheter site pain	0	0	0	0	0	0	0	0	0	0	1 (1)	0	1 (1)	0
Total	6 (4)	5 (3)	0	0	3 (3)	2 (2)	10 (4)	4 (2)	6 (3)	4 (3)	1 (1)	0	20 (8)	10 (5)

^aAll events were mild or moderate in severity.

asymptomatic, however via telemetry monitoring, a 5-beat ventricular tachycardia occurring approximately 8 hours after receiving a single 25-mg dose of LY2456302 was observed. The AE was mild in severity and, in the absence of a robust baseline measurement, was judged by

the investigator to be related to study drug. A follow-up telemetry monitoring for 24 hours did not reveal any clinically significant findings. Aside from this AE, there were no other clinically significant changes in ECGs. There were no clinically significant changes in vital signs,

Table 2b. Adverse Event Profile (Events With ≥ 2 Occurrences) After Administration of Multiple Doses of LY2456302 in Study B; Adverse Events, All Causality and Related to Study Drug, by Treatment in Order of Frequency

	Number of Adverse Events (Subjects) ^a									
	Placebo		LY2456302							
	(n = 9)		Cohort A		Cohort B ^b		Cohort C		Cohorts A, B, and C	
	Total	Related	2 mg (n = 9)		10 mg (n = 10)		35 mg (n = 9)		Total (n = 28)	
		Total	Related	Total	Related	Total	Related	Total	Related	
Headache	2 (2)	2 (2)	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)	0	4 (4)	2 (2)
Dyspepsia	0	0	0	0	3 (3)	3 (3)	2 (1)	2 (1)	5 (4)	5 (4)
Pruritus generalized	0	0	0	0	0	0	3 (3)	3 (3)	3 (3)	3 (3)
Constipation	1 (1)	1 (1)	0	0	1 (1)	0	1 (1)	1 (1)	2 (2)	1 (1)
Diarrhea	1 (1)	1 (1)	0	0	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)	2 (2)
Nasal congestion	2 (2)	0	0	0	1 (1)	0	0	0	1 (1)	0
Abdominal pain	0	0	0	0	1 (1)	1 (1)	1 (1)	1 (1)	2 (2)	2 (2)
Pain in extremity	0	0	0	0	0	0	2 (1)	0	2 (1)	0

^aAll adverse events were mild in severity.

^bSubjects in Cohort B, which consisted of two treatment periods, were administered ethanol only in Period 1 (not included here) and ethanol with LY2456302 or placebo on Period 2 Day 14.

clinical laboratory data, or dose-related changes in the mean change from baseline in neurohormones (cortisol, prolactin, and LH) (data not shown).

Study B. Of the 28 subjects who received ≥ 1 dose LY2456302, 12 reported a total of 40 AEs, and 5 of the 9 subjects who received ≥ 1 dose of placebo reported a total of 12 AEs (Table 2b). The number of AEs reported tended to increase with increasing doses of LY2456302. Of note, 2 subjects in Cohort B (10-mg LY2456302) and 1 subject in Cohort C (35-mg LY2456302) experienced a total of 24 AEs. The most common AEs (≥ 2 incidences) after LY2456302 were dyspepsia ($n = 5$), headache ($n = 4$), pruritus generalized ($n = 3$), and with two instances each: constipation, diarrhea, abdominal pain, and pain in the extremity. Of these dyspepsia, pruritus generalized, diarrhea, abdominal pain, and two of the four headaches were considered LY2456302-related. All AEs were of mild severity, with the exception of 1 moderate event of hemorrhoids in Cohort B, which occurred after 10-mg LY2456302 and was not deemed study drug-related.

There were no clinically significant changes in vital signs or ECGs. There were no clinically significant changes in clinical laboratory data or dose-related changes in the mean change from baseline in neurohormones (cortisol, prolactin, LH, and ACTH) (data not shown). Statistically significant increases in the mean ratio of change over 24 hours for cortisol were observed on Day 1 in subjects who received 10- or 35-mg doses of LY2456302 compared to placebo. These increases in cortisol ratio occurred within a range of 2–8 hours after a single dose of LY2456302 on Day 1 but not after 14 days dosing (data not shown).

Cognitive and Motor Assessments: Study B

Ethanol impacted cognitive and motor performance compared with ethanol placebo during the first 2.5-hours post-ethanol dose (Figure 1a–e, first and second panels, respectively). The effect of ethanol was most pronounced for the digit vigilance task (Figure 1a). In contrast, there was no effect of LY2456302 alone on any of the five cognitive/motor measures (Figure 1a–e, second panel). Furthermore, there were no differences between ethanol coadministered with LY2456302 (Figure 1a–e, third panel) and ethanol alone (first panel) at any of the time points suggesting no interaction between LY2456302 and ethanol on these assessments. Time-matched *P*-values between ethanol and ethanol placebo and between ethanol and ethanol plus LY2456302 are presented in Supplementary Tables S1 and S2.

Pharmacokinetics

Study A. The concentration–time profile for LY2456302 was consistent across dose levels and exhibited a biexponential disposition (Figure 2a). LY2456302 was rapidly absorbed with peak plasma concentrations occurring

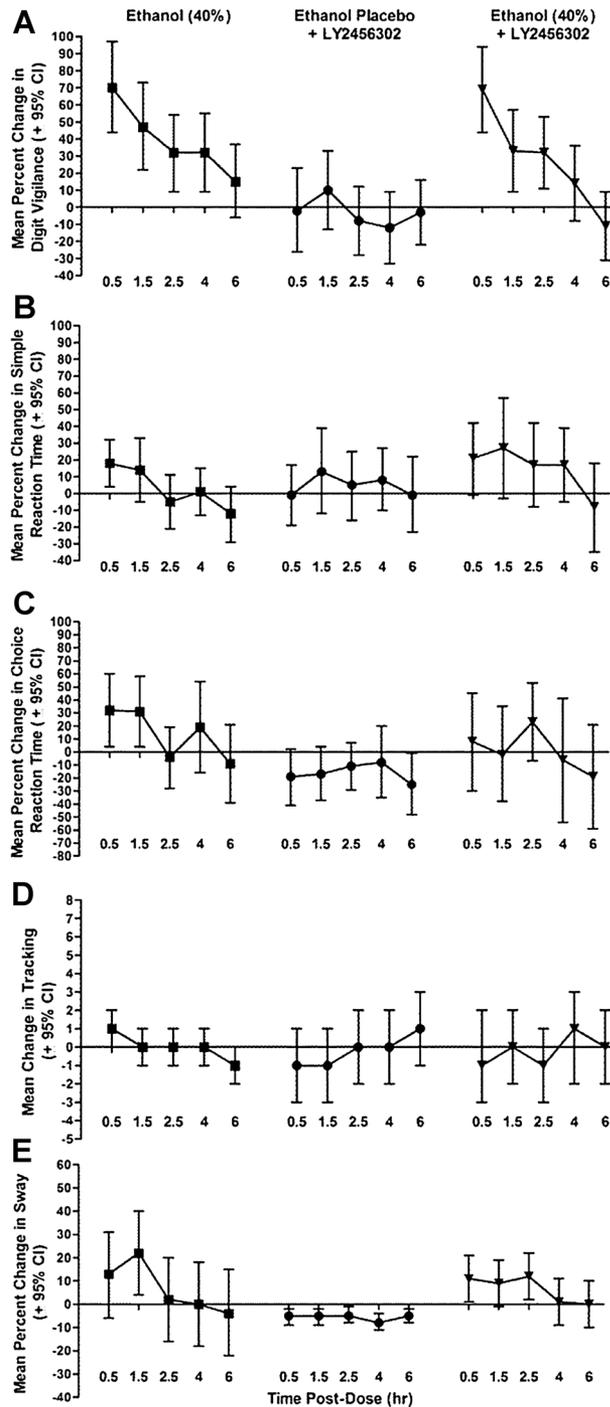


Figure 1. Effect of LY2456302 on ethanol induced deficits in cognitive and motor performance. The data presented include those subjects in Cohort B who received 10 mg LY2456302 ($n = 10$) and are the mean change from the pre-ethanol dose over 6 hours ($\pm 90\%$ CI) for the cognitive measures. The first panel of each figure shows the effect of ethanol alone; the second shows the effect of ethanol placebo plus LY2456302 at steady state; the third shows the effect of ethanol plus LY2456302 at steady state. (A) Mean change from pre-ethanol dose for digit vigilance. (B) Mean change from pre-ethanol dose for simple reaction time. (C) Mean change from pre-ethanol dose for choice reaction time. (D) Mean change from pre-ethanol dose for tracking. (E) Mean change from pre-ethanol dose for postural stability (body sway).

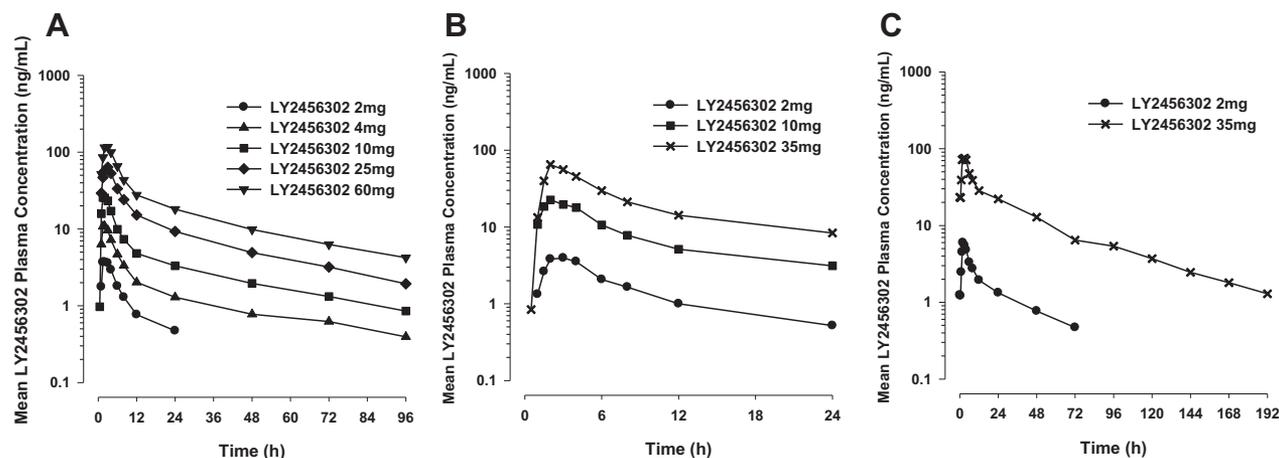


Figure 2. LY2456302 mean plasma concentration-time profiles following (A) a single dose of 2, 4, 10, 25, and 60 mg LY2456302 in healthy subjects in Study A, (B) single dose of 2, 10, and 35 mg on Day 1 of Study B, and (C) 14 days of 2 and 35 mg once-daily dosing in Study B.

at 1.5–2.4 hours postdose. Mean values of $t_{1/2}$ ranged from approximately 21 to 39 hours (Table 3). Overall, these results showed proportional increases in exposure with increasing dose.

Study B. Peak plasma concentrations of LY2456302 were observed approximately 2–3 hours postdose (Table 3; Figure 2b,c). Mean apparent oral clearance at steady state after multiple doses ranged from 34.9 to 41.3 L/h between doses of 2 and 35 mg, with no evidence of dose dependency. Mean $t_{1/2}$ was 28.3 hours after multiple doses of 2 mg and 39.7 hours after multiple doses of 35 mg (Table 3).

Steady state was achieved after approximately 6–8 days of dosing based on visual inspection of trough concentrations (data not shown). Following multiple oral doses of 2–35 mg of LY2456302, accumulation ratios (RA) were between 1.8 and 2.1 for AUC, and between 1.3 and 1.7 for C_{max} (Table 3), indicating moderate accumulation upon multiple dosing. AUC for LY2456302 increased in a dose proportional manner with a proportionality ratio of 1.09 (90% CI: 0.72, 1.66) for $AUC_{(0-t_{last})p}$ and 0.84 (90% CI: 0.66, 1.08) for $AUC_{(0-\tau)p}$ (Supplementary Table S3). The increase in C_{max} was approximately dose proportional although the proportionality ratio may indicate a slight tendency towards being less than dose-proportional (0.78; 90% CI: 0.61, 0.99).

Evaluation of Ethanol Interaction

The PK profile of ethanol alone or with LY2456302 was well characterized through the 4-hour time point, after which there were an insufficient number of quantifiable samples to display the 6-hour timepoint graphically. Visual inspection of the concentration–time profile of ethanol indicates no effect of LY2456302 on the PK of ethanol (Figure 3). The C_{max} of ethanol in the presence of LY2456302 compared with placebo showed equivalence

(ratio [90% CI] of 1.09 [0.97, 1.23]) since the CI was contained within 0.80–1.25 (Supplementary Table S4). Similarly, ethanol $AUC_{(0-6)}$ was equivalent, based on the ratio and 90% CIs of 1.12 (1.02, 1.23). The ethanol $AUC_{(0-t_{last})}$ parameter showed a minor increase when coadministered with LY2456302 (ratio of 1.22 [1.08, 1.39]) (Supplementary Table S4), but the increase was not considered clinically significant.

Statistical analysis also confirmed no effect of ethanol on the exposure to LY2456302 (Supplementary Table S5). C_{max} for LY2456302 showed no change in the presence of ethanol (ratio of 1.09 [0.99, 1.20]). LY2456302 AUC parameters also showed no change in presence of ethanol ($AUC_{[0-\tau]}$ ratio of 1.08 [1.03, 1.13]; $AUC_{(0-t_{last})}$ ratio of 1.01 [0.90, 1.14]).

Discussion

The combined results from Study A and Study B show that LY2456302 was generally well tolerated by the healthy volunteers administered up to 60 mg as a single dose and up to 35 mg as multiple doses administered once daily for 14 days. There were no serious adverse events observed in either study and no dose-limiting adverse events or other safety variables. Therefore, the dose escalations in both studies were not limited by any safety findings. Rather, the maximum dose in both studies was limited by a requirement to maintain a suitable margin of safety to pre-clinical findings of convulsions, using plasma concentration as a method of maintaining margin of safety. Importantly, there were no clinical signs of hyperactivity and no convulsions observed in the clinical studies. Aside from the single AE of ventricular tachycardia, there were no clinically significant changes in ECGs, including QTc prolongation.

Non-selective opioid antagonists alter normal endogenous regulation of the HPA axis and elevate levels of cortisol, ACTH, and LH in humans. Specifically,

Table 3. Pharmacokinetics of LY2456302 After a Single Dose and at Steady State

	Geometric Mean (CV%)															
	Study A: Single Dose							Study B: Multiple Ascending Dose								
	2 mg		4 mg		10 mg		25 mg		60 mg		2 mg		10 mg		35 mg	
	6	8	8	8	7	7	7	7	7	9	9	9	9	9	9	8
	4.2 (25)	11.6 (26)	27.6 (26)	72.2 (33)	2.00 (1.33-3.00)	2.42 (1.50-3.08)	2.03 (2.00-4.00)	2.00 (2.00-3.10)	2.03 (1.5-3.05)	3.00 (2.00-3.00)	3.13 (1.22-4.20)	2.00 (2.00-2.08)	2.50 (1.50-4.05)	39.7 (31)	848 (28)	1860 (49)
$t_{1/2}$ (hour)	21.3 (62)	32.3 (42)	38.5 (22)	34.1 (32)	35.1 (27)	35.1 (27)	35.1 (27)	35.1 (27)	35.1 (27)	35.1 (27)	35.1 (27)	35.1 (27)	35.1 (27)	35.1 (27)	35.1 (27)	35.1 (27)
$AUC_{(0-\infty)}$ (ng hr/mL)	38.5 (45)	120 (38)	302 (32)	814 (32)	1530 (43)	1530 (43)	1530 (43)	1530 (43)	1530 (43)	1530 (43)	1530 (43)	1530 (43)	1530 (43)	1530 (43)	1530 (43)	1530 (43)
$AUC_{(0-24)}$ (ng hr/mL)	45.6 (46)	139 (37)	348 (36)	924 (36)	1730 (48)	1730 (48)	1730 (48)	1730 (48)	1730 (48)	1730 (48)	1730 (48)	1730 (48)	1730 (48)	1730 (48)	1730 (48)	1730 (48)
CL/F (L/hr)	43.9 (46)	28.7 (37)	28.7 (36)	27.1 (36)	34.8 (48)	34.8 (48)	34.8 (48)	34.8 (48)	34.8 (48)	34.8 (48)	34.8 (48)	34.8 (48)	34.8 (48)	34.8 (48)	34.8 (48)	34.8 (48)
V_z/F (L)	1,350 (16)	1,340 (42)	1,590 (34)	1,330 (42)	1,760 (35)	1,760 (35)	1,760 (35)	1,760 (35)	1,760 (35)	1,760 (35)	1,760 (35)	1,760 (35)	1,760 (35)	1,760 (35)	1,760 (35)	1,760 (35)
$R_A(C_{max})$																
$R_A(AUC)$																

$AUC_{(0-t_{last})}$ = area under the concentration versus time curve from time zero to time t, where t is the last time point with a measurable concentration; $AUC_{(0-24)}$ = area under the concentration versus time curve from zero to 24 hours; $AUC_{(0-\infty)}$ = area under concentration versus time curve from zero to infinity; CL/F = apparent total body clearance of drug calculated after extra-vascular administration; C_{max} = maximum observed drug concentration; CV% = coefficient of variation; hr = hour; N = sample size; RA = accumulation ratio; t_{max} = time to maximum observed drug concentration; $t_{1/2}$ = half-life associated with the terminal rate constant (λ_z) in non-compartmental analysis; V_z/F = apparent volume of distribution during the terminal phase after extra-vascular administration.

^aMedian (range).
^bParameter not estimated due to sampling restrictions (up to 24 hours only).
^cIn study A this is $AUC_{(0-t_{last})}$, in Study B on Day 1 after a single dose it is $AUC_{(0-24)}$, and at steady state after multiple doses on Days 13 and 14 it is $AUC_{(0-\infty)}$ or area under the concentration versus time curve during one dosing interval.

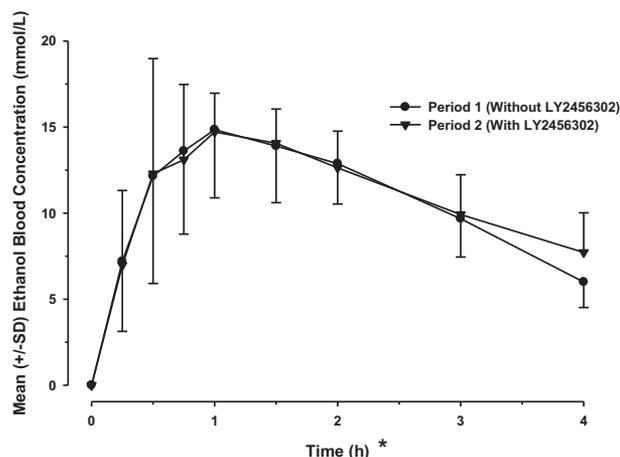


Figure 3. Ethanol mean blood concentration–time profiles following a single dose of ethanol alone or in combination with LY2456302 (at steady state).

naltrexone, a mu-preferring antagonist with activity against the KOR, increases levels of ACTH, cortisol, and LH in humans.^{26,27} The KOR system is also involved in HPA axis regulation, and KORs and their endogenous ligand dynorphin are localized in the hypothalamus.^{28,29} Therefore, potential effects of LY2456302 on HPA axis regulation were evaluated in both studies. There were no clinically significant changes in neurohormones, including cortisol, prolactin, ACTH, and LH in either study, consistent with pre-clinical pharmacology and toxicology studies that revealed no evidence of hypothalamic or HPA-related toxicities.

The estimated PK parameters after single doses of LY2456302 were reasonably consistent across both studies. The exposure of LY2456302 when administered as single doses over the range 2–60 mg, and as multiple doses for 14 days over the range 2–35 mg, appeared to increase generally dose-proportionally, with rapid absorption followed by a half-life of approximately 30–40 hours. The half-life estimated at the 2 mg dose appeared shorter, but this is likely due to limited detectable concentrations at the low dose and, hence, unreliable estimation of the slope of the terminal elimination phase. Steady state was attained after 6–8 days of once-daily dosing, as would be expected based on the reported half-life. Furthermore, the accumulation ratio of between 1.8 and 2.1 for AUC and between 1.3 and 1.7 for C_{max} , would be expected based on the reported $t_{1/2}$ and dosing interval, indicating that the PK of LY2456302 are time independent and approximately linear with dose.

Considering coadministration of ethanol and LY2456302 may likely occur in the target patient population, one concern would be an untoward interaction between LY2456302 and ethanol, and specifically, a

potential exacerbation of ethanol-induced cognitive impairment by LY2456302. To address this, the potential interaction between LY2456302 and ethanol was assessed in Study B, which indicated that LY2456302 did not alter the PK of ethanol. Ethanol alone was shown, as expected, to impair attention and reaction time, as assessed using a battery of cognitive/motor tests. Consistent with its lack of effect on the PK of ethanol, LY2456302 had no effect on ethanol-induced cognitive/motor impairment. Therefore, the results provide preliminary evidence that LY2456302 will not exacerbate cognitive impairment in patients who consume ethanol during treatment.

Conclusions

The overall safety and PK profile of LY2456302 suggests that it is well tolerated with a favorable PK profile, supporting its further development for depression, or other psychiatric disorders, including anxiety and substance use disorders for which pre-clinical evidence indicates potential therapeutic benefit of KOR antagonists.^{6,17,30,31}

Declaration of Conflicting Interests

Stephen L. Lowe, Conrad J. Wong, Jennifer Witcher, Robert L. Bell, Gemma L. Dickinson, Celedon R. Gonzales, Sitra Tauscher-Wisniewski, and Linda M. Rorick-Kehn are employees of and stockholders in Eli Lilly and Co. MaryAnn Weller, Randall R. Stoltz, and Jane Royalty have no financial conflicts.

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Supporting Information

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