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ARTICLE

LY3127760, a Selective Prostaglandin E4 (EP4) Receptor Antagonist, and Celecoxib: A Comparison of Pharmacological Profiles

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Safety, tolerability, and pharmacology profiles of LY3127760, an EP4 antagonist, were explored in healthy subjects in a subject/investigator-blind, parallel-group, multiple-ascending dose study. Cohorts consisted of 13 patients randomized to LY3127760, celecoxib (400 mg), or placebo (9:2:2 ratio) for 28 days. LY3127760 was well tolerated; the most commonly observed adverse events were gastrointestinal, similar to celecoxib. LY3127760 increased release of *ex vivo* tumor necrosis factor alpha after lipopolysaccharide/prostaglandin E2 stimulation when compared with placebo, suggesting a dose-dependent blockade of the EP4 receptor. Compared with placebo, 24-h urinary excretion of prostaglandin E metabolite was modestly increased; prostacyclin metabolite was inhibited; and thromboxane A2 metabolite was unchanged. Effects on sodium and potassium excretion were similar to those of celecoxib. We conclude that LY3127760 demonstrated similar effects on prostacyclin synthesis and renal sodium retention as celecoxib. These data support exploration of LY3127760 at daily doses of 60 mg to 600 mg in phase II trials. This trial's registration number: NCT01968070.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ The EP4 receptor has been shown to be the main receptor that mediates pain and inflammatory signaling in animal studies. Grapiprant, a selective EP4 antagonist, has been approved for the treatment of osteoarthritis pain in dogs.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ That LY3127760, a selective EP4 antagonist, is safe and tolerable in healthy subjects during oral dosing for 28 days, and there is a pharmacological differentiation between LY3127760 and celecoxib.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE
✔ The selective EP4 antagonist LY3127760 demonstrated a safety profile that was very similar to that of celecoxib.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
✔ EP4 receptor antagonist, such as grapiprant, are being developed as alternatives to NSAIDs. The understanding of the PD profile of EP4 antagonists compared with NSAIDs enables proper monitoring of the safety and efficacy profiles of these agents.

Prostaglandin E2 (PGE2) is an important pro-inflammatory pain mediator. It is also essential for the homeostasis of many vital organs, including the maintenance of mucosal integrity of the gastrointestinal tract, regulation of bicarbonate secretion in the intestines, modulation of renal sodium and water excretion, and prevention of ischemic cardiomyopathy after acute ischemic events. The physiological activities of PGE2 are mediated by 4 G-protein–coupled receptors identified as E prostanoid receptors 1–4 (EP1–EP4).[1] EP4 has been shown to be the main receptor that mediates pain and inflammatory signaling in animal studies,[2] whereas many of the other activities of PGE2 on physiological homeostasis are mediated by EP1, EP2, and EP3.[3] These data suggest that an agent that selectively antagonizes the EP4 receptor has the potential to provide an attractive risk/benefit profile in the treatment of painful, inflammatory conditions, such as osteoarthritis.

Recently, grapiprant became the first approved EP4 receptor antagonist for the treatment of osteoarthritis in companion dogs.[4,5] However, the pharmacological differentiation between the EP4 antagonist vs. traditional or selective nonsteroidal anti-inflammatory drugs (NSAIDs) is unclear.

LY3127760 is a selective EP4 receptor antagonist that is structurally distinct from grapiprant.[6] It demonstrated analgesic and anti-inflammatory efficacy in a variety of preclinical models, including the rat monoiodoacetate model, an adjuvant arthritis model, and a rat plasma protein extravasation model for migraine headaches.[6] A combined single (unpublished data, Lilly and Company) and multiple-ascending dose study was conducted to evaluate the safety and tolerability of LY3127760 in healthy subjects. In order to test the pharmacological differences between an EP4 antagonist and a selective cyclooxygenase (COX)-2 inhibitor, celecoxib was

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chosen as the active pharmacodynamic (PD) comparator. The 400 mg daily dose was chosen because it has been shown to produce reliable changes in urinary prostaglandin metabolites.[7] In addition, it is the marketed dose strength for treatment of rheumatoid arthritis and acute pain and is twice the approved dose for osteoarthritis. This paper reports the safety and pharmacological differences between LY3127760 and celecoxib in healthy subjects who were evaluated after oral dosing for 28 days in the multiple-ascending dose portion of the study.

METHODS

Study design

This was a subject/investigator-blind, parallel-group, multiple-ascending dose study. Healthy subjects were assigned into cohorts I through IV, with each cohort consisting of 13 patients randomized to receive LY3127760, celecoxib (400 mg), or placebo in a 9:2:2 ratio, for 28 days.

Participants

Overtly healthy men and women of nonchildbearing potential between the ages of 18 and 60 years were included in the study. Subjects with supine systolic blood pressure (BP) $<$140 mmHg, diastolic BP $<$90 mmHg, and a body mass index between 18.5 and 32.0 kg/m^2 were eligible to participate. Subjects who had active or a recent history of cardiovascular, gastrointestinal, hepatic, renal, respiratory, or neoplastic illness, or a history of bleeding diathesis were excluded from the study. Subjects were to refrain from using NSAIDs, including celecoxib, aspirin, acetaminophen, or herbal supplements, within 14 days of the first study drug dosing were also advised not to change exercise or dietary habits for the duration of the study.

The study protocol was approved by Schulman Associates, and was performed in compliance with the principles of good clinical practice and in accordance with provisions of the Declaration of Helsinki. All subjects provided written informed consent prior to receiving any study drug or undergoing any study procedure. The study took place at one center in the United States, was begun in October 2013 and was completed in April 2014.

Interventions

Subjects randomized to LY3127760 received ascending doses of 20 mg, 60 mg, and 200 mg given once daily and 300 mg given twice daily. The target engagement profile of LY3127760 was previously established using an *in vitro* human whole blood assay.[6] Assuming that: (1) the potency of LY3127760 in a similar *ex vivo* assay implemented in the present study was comparable to that in the earlier *in vitro* and *ex vivo* human whole blood tumor necrosis factor alpha (TNF-α) release assay[6]; and (2) that this potency could represent that *in vivo*, and then based on observed drug exposure from the single ascending dose portion of the study (unpublished data), the dose range of 20 mg to 600 mg daily was selected for the multiple-ascending dose period. The 20 mg q.d. dose was predicted to have drug concentration profiles above the EC80 at maximum concentration (C_{max}) and less than the EC10 at trough. The 600 mg daily dose (given as 300 mg b.i.d. for more stable concentration profiles) was predicted to achieve exposure over the EC90 at C_{max} and maintain exposure above the EC50 at trough. This dose range was expected to produce ~10–90% of the maximal EP4 antagonism in the *ex vivo* human whole blood assay, and that the PD responses from this dose range would provide sufficient data for exposure response analysis of the PD effect by pharmacokinetic/pharmacodynamic modeling. This dose range was also supported by the review of safety data from all subjects in the previous cohort(s) by the investigator and sponsor. Patients randomized to celecoxib (400 mg daily) or placebo received over-encapsulated tablets identical in appearance to LY3127760. Study medication was given by mouth in the morning, and twice daily dosing was given at 12-h intervals.

Subjects were admitted to the clinical research unit for an inpatient stay of ~31 days. Patients were admitted on day -2 and for baseline 24-h ambulatory blood pressure monitoring (APBM) and 24-h urine collection. From days 1–28, the patients received the study drug, safety was assessed, and vital signs, electrocardiogram (ECG) monitoring, and blood and urine samples were collected at intervals determined *a priori*. Postdose ABPM and a 24-h urine collection were obtained on day 27. Subjects were discharged from the clinical research unit on day 29 after the safety assessment and sample collections were completed.

Outcomes

The primary objective of this study was to explore the tolerability and safety features of LY3127760 after multiple doses, in order to define an appropriate dose range for further clinical research. The secondary objective was to evaluate the pharmacokinetics (PKs) of LY3127760 after multiple doses.

Exploratory objectives were to evaluate the effect of multiple doses of LY3127760 on *ex vivo* whole blood TNF-α release after lipopolysaccharide (LPS)/PGE2 stimulation; on sodium, potassium, and creatinine excretion; on eicosanoid biosynthesis; and on serum TXB2, compared with celecoxib.

ANALYTIC METHODS

Pharmacokinetic analysis

Quantification of LY3127760 in human plasma. The method for the determination of LY3127760 in human plasma was validated over the calibration range of 0.5–250 ng/mL at Covance Laboratories (Madison, WI). Plasma samples were collected with K_2EDTA anticoagulant and treated with phosphoric acid as a stabilizer. Samples above the limit of quantification were diluted to yield results within the calibrated range. After spiking with internal standard [$^2H_5$]-LY3127760, samples were subjected to protein precipitation followed by liquid chromatographic separation and tandem mass spectrometry/mass spectrometry detection. Interassay accuracy and precision of the method during validation were $<$8%.

TNF-α release

***Ex vivo* whole blood TNF-α release.** Whole blood samples were collected in Sodium Heparin BD Vacutainers (Becton, Dickinson and Company, Franklin Lakes, NJ) on day 1 just before LY3023703 dosing (baseline) and on days 1 and 28 after LY3127760 dosing. Samples were held at room temperature (~25°C) for 20–24 h prior to LPS stimulation to accommodate for shipping and minimize variability. The LPS

stimulation was as follows: PGE_2 (Cayman Chemicals, Ann Arbor, MI) was added to 1 mL of whole blood to a final concentration of 10 nM. Samples were incubated with gentle shaking for 30 min in a humid cell culture incubator with atmospheric conditions set to 37°C with 5% CO_2. The LPS (Enzo Life Sciences, Farmingdale, NY) was then added to incubated samples to a final concentration of 10 μg/mL LPS. Samples were then incubated as described above for 20–24 h. After incubation, samples were chilled gently to slow reaction before being centrifuged to collect the heparinized plasma.

The TNF-α concentrations were determined utilizing the Quantikine ELISA Human TNF-α Immunoassay (R&D Systems, Minneapolis, MN). Samples were diluted 50-fold using a sample diluent prior to analysis in the kit. Sample diluent was BupH Phosphate Buffered Saline (Thermo Fisher Scientific, Waltham, MA) containing 1% RIA-Grade Bovine Serum Albumin (Sigma Aldrich, St. Louis, MO) and 0.1 mg/mL HBR-1 (Scantibodies Laboratory, Santee, CA).

Urinary sodium and potassium

During inpatient days, subjects received a consistent amount of sodium in their diets based on individual 3-day dietary diaries. Twenty-four-hour urine collection was conducted the day before the first dose (day -1), and after first and 27th dose (days 1 and 27). Total urine volume was recorded, and an aliquot of urine sample was stored, and analyzed for sodium, potassium, and creatinine at a central clinical laboratory. Plasma creatinine concentrations were measured on days -1, 1, and 28 before study drug administration. Urinary sodium or potassium excretion was calculated as: urinary sodium (or potassium) excretion = urine sodium (or potassium) concentration $\times$ urine volume.

Creatinine clearance was calculated as: Creatinine clearance = urinary creatinine concentration $\times$ urine volume/(plasma creatinine $\times$ 24 $\times$ 60) (mL/min).

Urinary prostanoid analysis

Twenty-four-hour urine samples were collected from each subject on days -1 (baseline), 1, and 27. Samples were refrigerated after void, urine volume was recorded, and aliquots of urine samples were obtained and stored in a –80°C freezer until time of analysis for urinary prostanoid metabolites or creatinine concentrations. Gas chromatography-tandem mass spectrometry was used to analyze three prostanoids in human urine over the indicated ranges: prostacyclin metabolite (PGIM; 20–500 pg/mL), thromboxane metabolite (TXM; 40–2,000 pg/mL), and PGE metabolite (PGEM; 200–10,000 pg/mL) using a validated method.[8] The mean percentage of coefficient of variation values recorded across all validation samples were PGIM (11.0%), TXM (5.6%), and tetranor PGEM (7.0%).

Serum thromboxane B2

Serum thromboxane B2 (TXB2) levels were determined using a liquid chromatographic separation and tandem mass spectrometry/mass spectrometry method validated over a range of 0.2–250 ng/mL using a 50-μL sample volume.[8]

Ambulatory blood pressure monitoring

Twenty-four-hour ABPM was conducted before study drug dosing (day -1 to 1) and on the 27th day of dosing (days 27–28). Systolic and diastolic BP and pulse were recorded every 20 min during the period when subjects were awake and every 30 min while they were asleep.

Statistical analysis

The sample size was customary for phase I studies evaluating safety, PK, and/or PD parameters and was not based on formal hypothesis tests. Confidence intervals (CIs) were reported at 90%. All PD parameters (*ex vivo* whole blood TNF-α release, 24-h urinary excretion of PGIM, PGEM, TXM, sodium excretion, potassium excretion, creatinine clearance, and serum TXB2) were log-transformed prior to analysis. The ratio to baseline of PD parameters was evaluated by mixed-effect model repeated measures with treatment and the relevant time, day, and treatment-by-day-by-time interactions as fixed effects and baseline as a covariate. For *ex vivo* whole blood TNF-α release, baseline white blood cell counts were also included as a covariate. An unstructured variance-covariance matrix was used for all models to allow for repeated measures within a subject.

The weighted mean ambulatory systolic BP, diastolic BP, and pulse rate over 24-h postdose were calculated, in which the weighted mean was derived as the area under the curve (AUC) divided by time interval (ie., 24-h). Change from baseline was evaluated by analysis of covariance with treatment as a fixed effect and baseline as a covariate. All adverse events (AEs) were listed and summarized using descriptive methodology. The incidence of symptoms for each treatment was presented by severity and by association with investigational product, as perceived by the investigator. Symptoms reported to occur prior to study enrollment were distinguished from those reported as new or increased in severity during the study. Each symptom was classified by the most suitable term from the Medical Dictionary for Regulatory Activities (MedDRA, version 16.0). Additional safety parameters (laboratory parameters, vital signs, and ECG) were listed and summarized using standard descriptive statistics.

The PK parameters for LY3023703 were calculated by noncompartmental methods using Phoenix WinNonlin, version 6.3.0.

RESULTS

Demographics

In this phase I investigator-blinded and subject-blinded, placebo-controlled, multiple-ascending dose study of LY3127760, a total of 53 healthy subjects, aged 20–61 years, were enrolled in three treatment arms: LY3127760 (n = 37), celecoxib (n = 8), or placebo (n = 8). Within the LY3127760 arm, subjects were enrolled sequentially into four dose levels: 20 mg daily (n = 9), 60 mg daily (n = 10, included one replacement subject), 200 mg daily (n = 9), and 300 mg b.i.d. (n = 9). Of the 53 subjects, 9 discontinued from the study before its completion: 1 due to an AE, 4 due to subject decision, 2 due to protocol violation, and 1 each due to physician decision and lost to follow-up. The remaining 39 subjects completed the study. The majority of subjects were men (79.2%); 54.7% were white, and 41.5% were black. The mean body mass

index was 27.5 kg/m^2 (SD 3.0). Demographic characteristics of subjects were similar among all treatment arms.

PK evaluation

After a light breakfast, patients given multiple LY3127760 doses had peak plasma concentrations of LY3127760 evaluated between 1 and 4 h, with a median of ~2 h across the 20 mg q.d. to 300 mg b.i.d. dose range. As shown in **Figure 1**, during the distribution phase (the first 8–12 h postdose), the LY3127760 concentration dropped at least ~10-fold from the maximum observed drug concentration. Hence, after daily or b.i.d. dosing, limited drug accumulation was observed. For most dose levels, the average terminal half-life was between 8 and 16 h.

PD EVALUATION

Ex vivo *whole blood TNF-α release*

Ex vivo whole blood TNF-α release after PGE2 and LPS stimulation has been used as a target engagement marker for EP4 antagonism (**Figure 2a,b**).[9] From day 1–28, LY3127760 increased *ex vivo* TNF-α release in whole blood in an approximate dose-dependent manner, with statistically significant increases relative to placebo observed for all doses of LY3127760 >20 mg. Greater than twofold increases were observed for all doses ≥60 mg and were sustained for at least 8 h postdose, with effects beginning to subside by 24 h. There were no notable changes in TNF-α observed for celecoxib. The variability of *ex vivo* whole blood assay for TNF-α levels was moderate to high, which precluded a precise estimation of a monotonic dose response in this study.[10]

Urinary sodium, potassium excretion, and creatinine clearance

Urinary sodium and potassium excretion and creatinine clearance were assessed on days -1, 1, and 27 (**Figure 3a–c**). LY3127760 was observed to have similar effects to celecoxib on renal sodium excretion. Sodium excretion was reduced by LY3127760 up to 40%, relative to placebo (90% CI = 23–54%) for the 200 mg dose across the 24-h collection on day 1 and followed an approximate dose-related trend. Effects were no longer present on day 27, with the exception of the highest dose, LY3127760 300 mg b.i.d., for which sodium excretion remained inhibited by 42% (90% CI = 23–57%) relative to placebo. Celecoxib inhibited the 24-h sodium excretion on average by 23% (90% CI = 0–41%) on day 1 and 15% (90% CI = -11 to 36%) on day 27 relative to placebo.

LY3127760 was observed to have effects on renal potassium excretion similar to those of celecoxib. Potassium excretion was reduced up to 28% by LY3127760, relative to placebo (90% CI = 18–36%) across the 24-h collection period on day 1 and followed an approximate dose-related trend. Effects were no longer present on day 27, with the exception of the highest dose, LY3127760 300 mg b.i.d., for which potassium excretion remained inhibited by 27% (90% CI = 11–40%) relative to placebo. Celecoxib inhibited the 24-h potassium excretion on average by 18% (90% CI = 6–28%) on day 1 and 20% (90% CI = 3–33%) on day 27 relative to placebo.

On day 1 of dosing, there were no clinically significant changes in creatinine clearance for LY3127760 or celecoxib relative to placebo. On day 27 of dosing, there was a signal of reduced creatinine clearance only in the highest LY3127760 dose, 300 mg b.i.d. Creatinine clearance was reduced by 37% (90% CI = 24–48%) relative to placebo across the 24-h collection period, which was a statistically greater inhibition than that observed for all other treatment groups, including celecoxib.

Urinary prostaglandin metabolites

Continuous 24-h collections of urinary PGEM, prostacyclin metabolite (PGIM), and thromboxane A2 metabolite (TXAM) excretion were obtained on days -1, 1, and 27 (**Figure 4a–c**). The excretion of urinary prostaglandin metabolites was corrected with urine creatinine concentration.[7] Because of the reduction in urinary creatinine excretion 27 days after LY3127760 300 mg b.i.d., the correction of the renal filtration rate for urinary prostanoid metabolite excretion was biased for that dose, which exaggerated the differences in urinary prostanoid metabolite excretion between this dose and placebo or celecoxib on day 27.

Celecoxib inhibited PGEM excretion by ~37% on day 1 and 66% on day 27 relative to placebo, which was consistent with previous observations.[8] In contrast, LY3127760 increased the excretion of PGEM in an approximately dose-dependent manner up to 68% (90% CI = 24–128%) for the 300 mg b.i.d. dose relative to placebo on day 1. Trends were less consistent on day 27, except for the 300 mg b.i.d. dose.

Celecoxib inhibited PGIM excretion by ~44% on day 1 and 44% on day 27 relative to placebo. LY3127760 was observed to inhibit urinary PGIM excretion relative to placebo across the entire 24-h period, although the magnitude of inhibition was not as large as that observed for celecoxib. Inhibition did not seem to follow a clear dose-dependent trend, with maximal inhibition of 31% (90% CI = 12–46%) observed in the 20-mg dose relative to placebo on day 1. Findings were similar on day 27, with the exception of the 300 mg b.i.d. dose, which did not demonstrate PGIM inhibition relative to placebo.

There were no consistent changes in TXAM excretion for LY3127760 or celecoxib relative to placebo. Slight (24%; 90% CI = 9–40%) increases in TXAM for LY3127760 were observed only on day 1 after the 300 mg b.i.d. dose relative to placebo. There was no clear evidence of increased TXAM after all other LY3127760 doses on day 1 or on day 27 compared with placebo. Celecoxib did not significantly alter TXAM excretion compared with placebo on days 1 or 27.

Serum thromboxane B2

LY3127760 showed signals of serum inhibition relative to placebo, although trends did not follow a clear dose-dependent or time-dependent pattern (**Figure 5**). Maximal inhibition of serum TXB2 was observed for the 300 mg b.i.d. dose at 1-h postdose on day 28, with a reduction of 77% (90% CI = 52–88%) relative to placebo. However, such trends were not observed consistently, probably due to the high variability of serum TXB2 concentrations of this assay. Celecoxib was observed to reduce serum TXB2 concentrations, particularly on day 28,

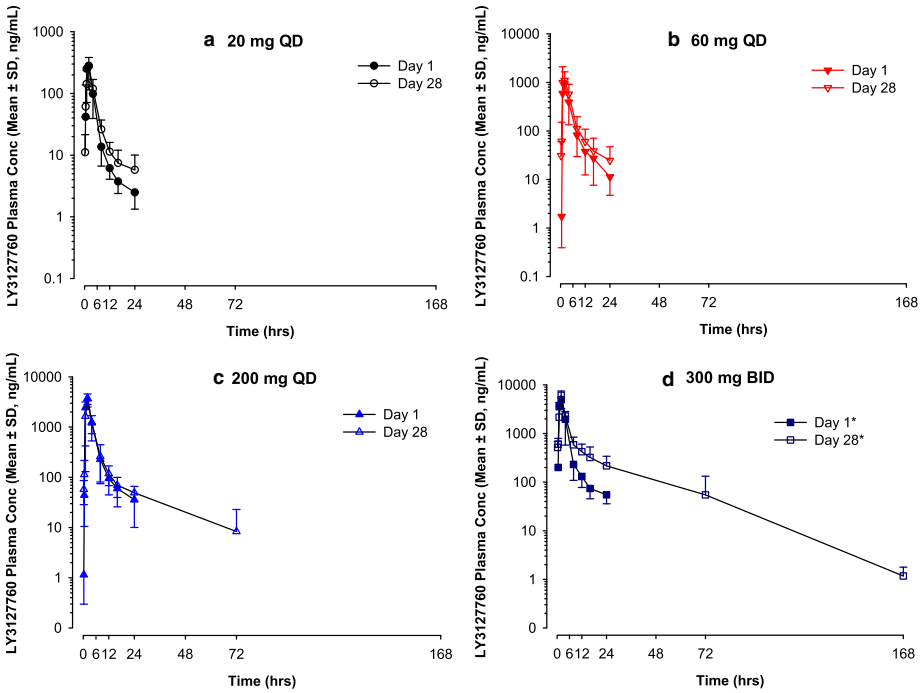

Figure 1 Time course of LY3127760 (LY) plasma concentrations (arithmetic mean ± SD) following administration on days 1 and 28. Doses were 20 mg q.d. (**a**), 60 mg q.d. (**b**), 200 mg q.d. (**c**), and 300 mg b.i.d. (**d**). *For the 300 mg b.i.d. dose, subjects received only a single dose on day 1 and day 28. Conc, concentration ; QD, once daily; SD, standard deviation

with maximal mean inhibition relative to placebo of 61% (90% CI = 20–81%).

Safety

LY3127760 was well tolerated by healthy subjects, and there were no deaths or serious AEs during the study or in the follow-up period. Only one subject was discontinued due to an AE before study completion. This subject, randomized to receive LY312770 60 mg daily, developed upper abdominal pain that was mild and improved with food on day 11. By day 14, the pain was reported as moderate to severe and the study drug was discontinued. The subject was empirically treated with omeprazole 20 mg and the pain improved within 2 h, completely resolving within 16 h.

Of the 53 subjects who participated in this study, 37 received at least 1 dose of LY3127760, 8 received celecoxib, and 8 received placebo and were evaluated for safety. Nineteen of the 37 subjects randomized to LY3127760 reported 46 treatment-emergent AEs (TEAEs) of all causality; three of eight subjects randomized to celecoxib reported four TEAEs, and two of eight subjects randomized to placebo reported three TEAEs. All AEs were of mild or moderate severity. Seventeen TEAEs were considered to be related to the study drug by the investigators. There was a trend for the incidence of TEAEs to increase as the dose of LY3127760 increased. Gastrointestinal AEs were the most frequently reported AEs in subjects randomized to receive LY3127760 (**Supplementary Table S1** online). One subject randomized to celecoxib also reported intermittent upper abdominal pain from days 4–13. In addition, one subject randomized to LY3127760 200 mg q.d. reported maculopapular rash on both upper extremities from days 16–28. The rash resolved 6 days after the last dose of the study drug.

During the course of the study, there were no clinically significant alterations in laboratory values, including no clinically significant changes in hematology and liver tests. Modest increases in serum creatinine were observed in the study: two subjects during placebo treatment, one subject during celecoxib treatment, one subject each during 20 mg and 200 mg LY3127760 treatment, and three subjects during 300 mg b.i.d. LY3127760 treatment. All creatinine concentrations returned to normal range after study drug dosing was completed. Regarding vital signs, systolic BP >140 mmHg was observed in two subjects during the 28-day dosing period (one after dosing with LY3127760 200 mg daily and one after celecoxib dosing), but these measurements normalized at the time of discharge. No significant increase in diastolic BP or pulse was observed. During the course of the study, there were no clinically significant changes in ECG parameters, including corrected QT Fridericia's formula. Regarding ABPM, statistical analysis of the change from baseline in 24-h mean systolic BP, diastolic BP, and pulse rate on day 27 did not reveal any dose-dependent or concentration-dependent increases (**Supplementary Table S2** online).

DISCUSSION

The EP4 receptor antagonists, such as grapiprant, are being developed for the treatment of inflammatory pain[4] and solid cancers.[11] They hold the promise that selective antagonism of the EP4 receptor retains the therapeutic benefits of NSAIDs and COX-2 inhibitors without the

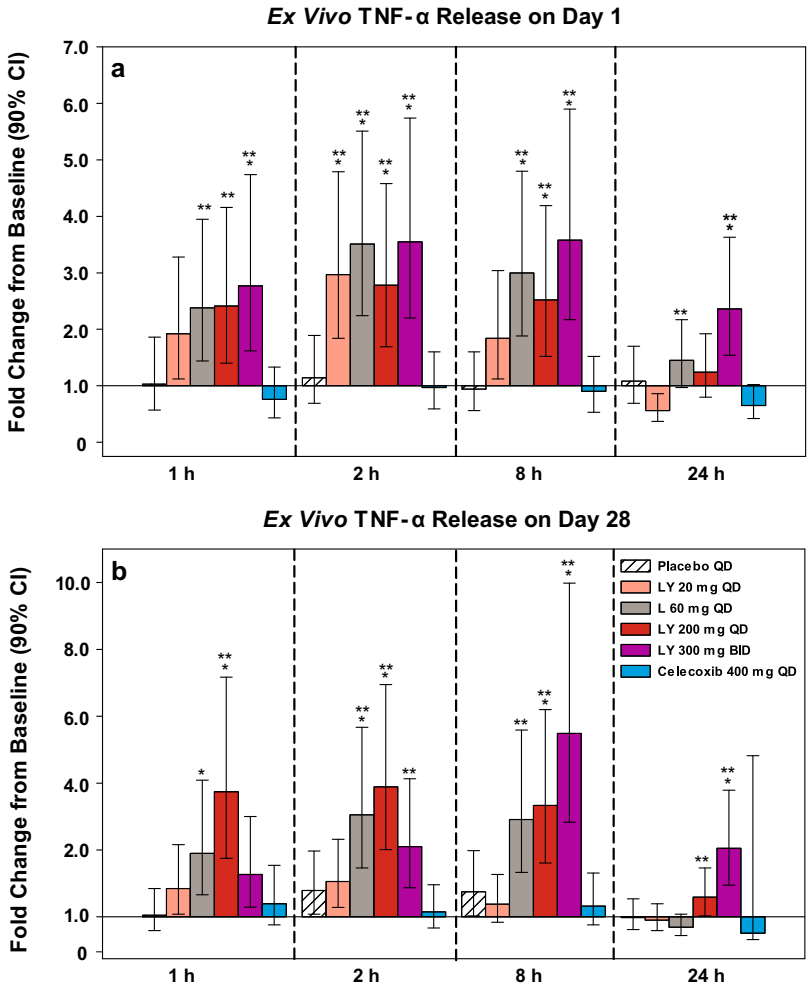

Figure 2 Percent change from baseline (day -1) in *ex vivo* tumor necrosis factor alpha (TNF-α) release on day 1 (**a**) and day 28 (**b**). Bars represent geometric LY3127760 (LS) means; error bars represent 90% confidence interval (CI). *$P < 0.05$ compared with placebo; **$P < 0.05$ compared with celecoxib BID, twice daily; CI, confidence interval; LY, LY3127760; QD, once daily.

gastrointestinal, cardiovascular, and renal AEs. However, their potential for such differentiation from NSAIDs has not been tested in humans. In this study, the pharmacological profile and safety of LY3127760, an EP4 receptor antagonist, was compared against that of celecoxib, a COX-2 inhibitor.

LY3127760 was well tolerated in healthy subjects after multiple doses ranging from 20 mg q.d. to 300 mg b.i.d. Gastrointestinal AEs were the most commonly observed AEs in this study, with abdominal pain, nausea, and dyspepsia observed after multiple doses of LY3127760. This AE profile was consistent with results of a nonclinical toxicology study suggesting that gastrointestinal mucosa was a target organ of toxicity for this molecule (unpublished data).

No dose-dependent changes from baseline were observed in 24-h mean systolic BP, diastolic BP, or pulse rate. Significant increases in systolic BP as measured through ABPM were observed in the 60-mg cohort only. As patients in this cohort were in the first dosing period, they may have been more anxious than subsequent cohorts due to period effects.[12] No clear trend of BP increase was observed in subjects randomized to receive celecoxib. As NSAID-induced hypertension is both dose-dependent and time-dependent, 4-week treatment may not completely abrogate risks for iatrogenic hypertension by LY3127760.

Ex vivo TNF-α release has been used as the biomarker of target engagement for EP4 antagonism. Clinical data from grapiprant suggested that efficacy was observed for osteoarthritis at exposures approximately equivalent to the EC50 in this assay.[4,6] Consistent with these findings, LY3127760 clearly increased TNF-α release, and confirmed target engagement at doses >60 mg once daily.[6] Unfortunately, due to high variability for this assay,[10] a clear dose/concentration-response relationship could not be defined. Nonetheless, these findings, taken together with the acceptable safety and tolerability profile, support exploration of doses of 60 mg daily to 300 mg twice daily in phase II trials.

Blockade of PGE pathways without inhibition of the biosynthesis of other eicosanoids is a desirable pharmacological differentiation of EP4 antagonist from NSAIDs. Nonclinical study data suggested that, unlike NSAIDs or selective COX-2 inhibitors, LY3127760 would lead to a modest increase in PGE2 synthesis *in vivo* without changing

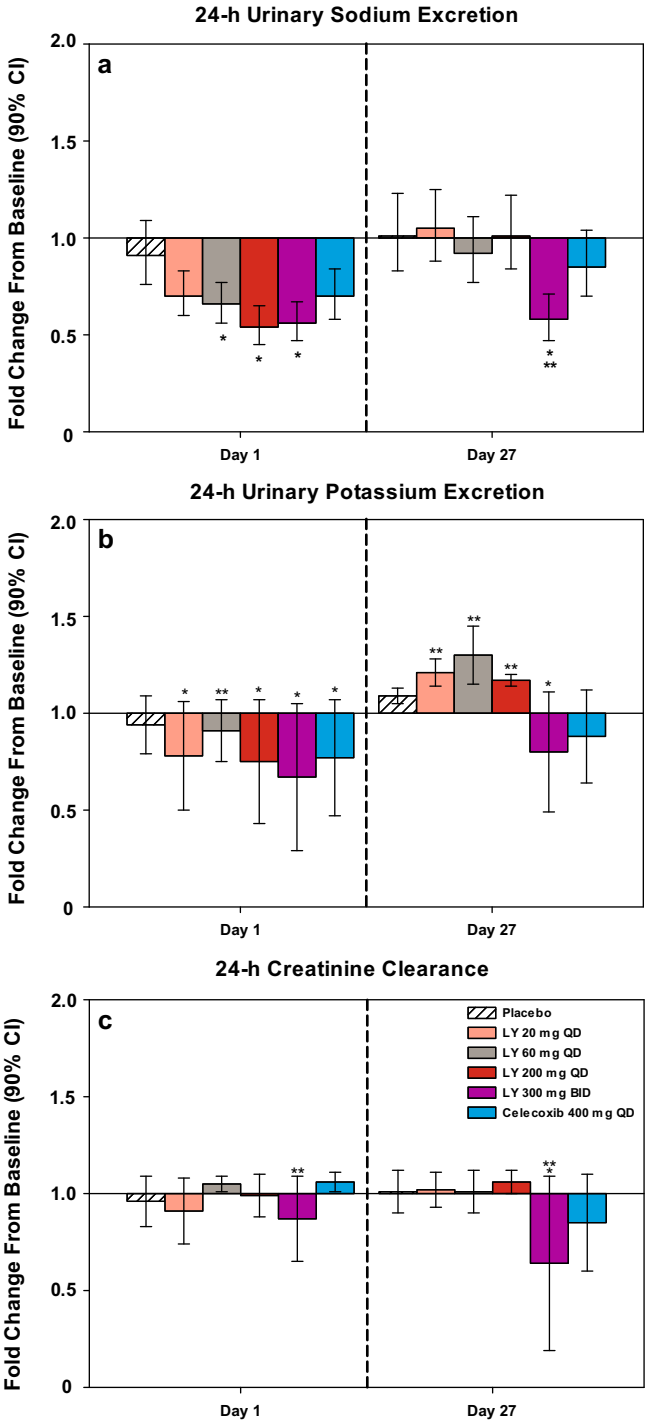

Figure 3 Changes in 24-h urinary sodium excretion (**a**), urinary potassium excretion (**b**), and creatinine clearance (**c**) in response to LY3127760 (LY) and celecoxib dosing on days 1 and 27. Bars represent geometric least squares means; error bars represent 90% confidence interval (CI). *$P < 0.05$ compared with placebo; **$P < 0.05$ compared with celecoxib BID, twice daily; CI, confidence interval; LY, LY3127760; QD, once daily.

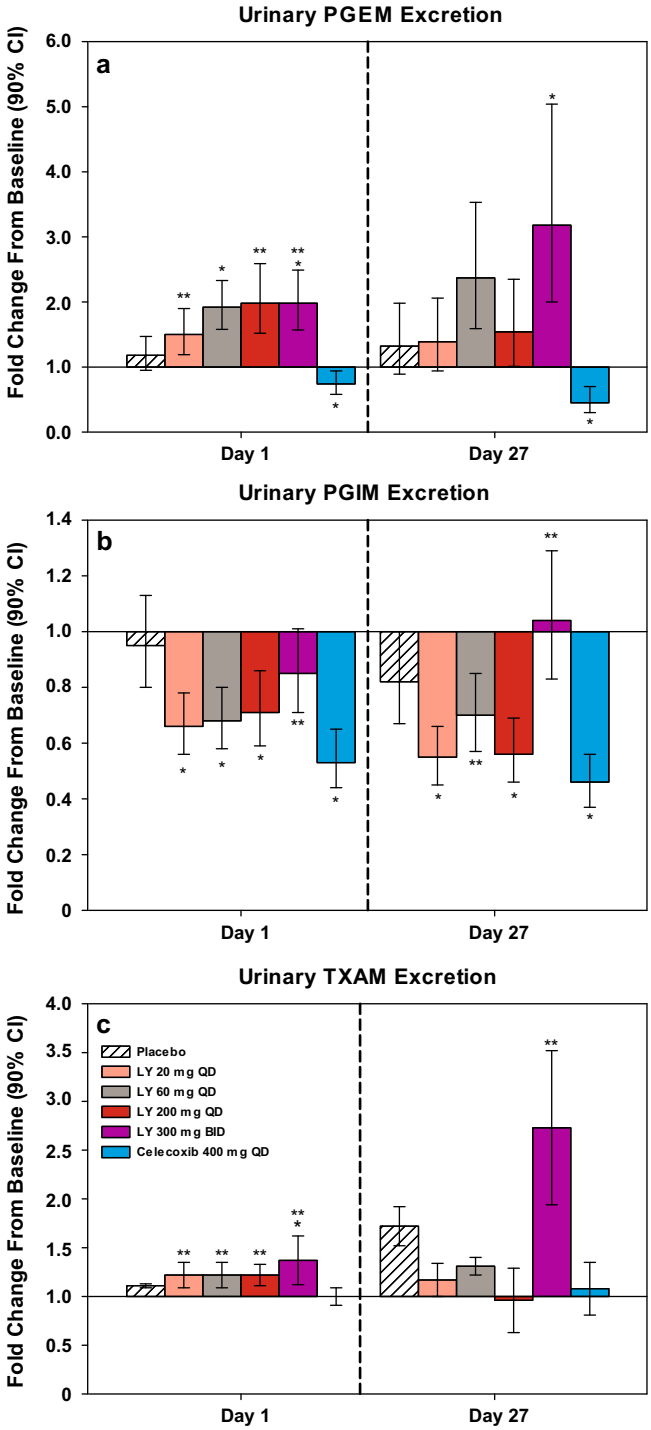

Figure 4 Changes in 24-h urinary excretion of prostaglandin E metabolite (PGEM) (**a**), prostacyclin metabolite (PGIM) (**b**), and thromboxane A2 metabolite (TXAM) (**c**) in response to LY3127760 (LY) and celecoxib dosing on days 1 and 27. Bars represent geometric least squares means; error bars represent 90% confidence interval (CI). *$P < 0.05$ compared with placebo; **$P < 0.05$ compared with celecoxib.

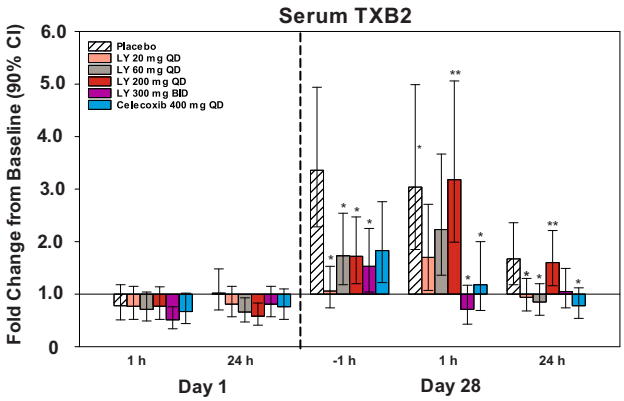

Figure 5 Changes in 24-h serum thromboxane B2 (TXB2) in response to LY3127760 (LY) and celecoxib dosing on days 1 and 28. Bars represent geometric least square means; error bars represent 90% confidence interval (CI). *P $<$ 0.05 compared with placebo; **P $<$ 0.05 compared with celecoxib.

either prostacyclin (PGI) or thromboxane synthesis (unpublished data). Surprisingly, modest PGI suppression was observed. The exact mechanism by which LY3127760 suppressed PGI synthesis in healthy subjects remains unknown. However, the extent of such inhibition is less than that observed after celecoxib.

Renal prostaglandins, especially PGE and PGI, have been implicated in the regulation of urinary sodium and potassium excretion. Sodium and potassium retention by NSAIDs has been attributed to their renal inhibition of these eicosanoids.[13] Surprisingly, LY3127760 led to a similar degree of sodium and potassium retention as celecoxib in this study. Unfortunately, kidney-derived PGI and PGE2 were not measured in this study, which makes it difficult to speculate whether the sodium/potassium retention observed in this study was mediated by a direct antagonistic effect of EP4 or indirectly due to the changes in renal eicosanoid synthesis. Better understanding of the effects of LY3127760 on renal PGE and PGI synthesis may clarify the mechanism by which LY3127760 decreased sodium and potassium excretion in these healthy subjects.

In conclusion, in this multiple-ascending dose study, LY3127760 demonstrated similar patterns of gastrointestinal AEs, PGI synthesis, and renal sodium excretion as those seen with celecoxib. The safety/tolerability profile and PD results support exploration of daily doses of 60 mg to 600 mg in phase II trials. The mechanisms by which an EP4 receptor antagonist regulated expression of other eicosanoids and renal sodium or potassium clearance in humans remain to be elucidated.

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Conflict of Interest. Y.J., C.L.S., L.H., D.E.C., K.P., T.A.M., X.Y.Y., B.L.A., T.G.P., and W.L. are employees of and minor stockholders in Eli Lilly and Company. K.W. is employed by Covance. Eli Lilly and Company contracted with Covance for the provision of services.

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