

Pharmacokinetics, Pharmacodynamics, and Safety of a Prostaglandin D₂ Receptor Antagonist

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Laropiprant is a selective antagonist of the prostaglandin D₂ (PGD₂) receptor subtype 1 (DP1). Three double-blind, randomized, placebo-controlled studies evaluated the safety, tolerability, pharmacokinetics, and pharmacodynamics of single and multiple oral doses of laropiprant in healthy male volunteers. Single doses up to 900 mg and multiple doses up to 450 mg were generally well tolerated. Laropiprant exhibited dose-proportional pharmacokinetics. Oral absorption is rapid ($T_{\max} = 0.8\text{--}2.0$ h) and the terminal half-life is approximately 12–18 h. The pharmacokinetics of laropiprant was not affected by food. Single doses of 6 mg and higher were effective in suppressing PGD₂-induced cyclic AMP accumulation in platelets, demonstrating laropiprant target engagement with DP1. Laropiprant has detectable off-target antagonist effects at the thromboxane A₂ receptor but no clinically significant effect on collagen-induced platelet aggregation or bleeding times with multiple doses up to 200 mg.

Cardiovascular disease is a leading cause of morbidity and mortality.^{1,2} Hypercholesterolemia, hypertriglyceridemia, and low high-density lipoprotein cholesterol are important risk factors.^{3,4} In patients with cardiovascular disease or risk factors, niacin (nicotinic acid) significantly improves the lipid profile and reduces cardiovascular events and mortality.⁵ Niacin is the most effective pharmacotherapy available for increasing high-density lipoprotein cholesterol, but its use has been limited by flushing (warmth, erythema, pruritus, tingling) of the face, neck, and upper torso.⁶ Niacin-induced flushing occurred in most patients who received niacin in clinical trials.⁷

Laropiprant (also known as MK-0524) is a selective antagonist of the prostaglandin D₂ (PGD₂) receptor subtype 1 (DP1). In animal models, PGD₂- and niacin-induced vasodilation was suppressed by laropiprant.⁸ In clinical studies, laropiprant was effective in reducing flushing symptoms induced by niacin.^{8,9} The objectives of this study were to (1) assess the safety and tolerability of single (1–900 mg) and multiple oral doses (30–450 mg) of laropiprant; (2) evaluate the pharmacokinetics of laropiprant and its

acyl glucuronide metabolite after single and multiple dosing; (3) determine the pharmacodynamic effects of laropiprant on DP1 receptors by measuring PGD₂-stimulated cyclic AMP (cAMP) generation in platelets and (4) monitor any off-target effects of laropiprant on TP receptors using U-46619-stimulated platelet aggregation and collagen-induced platelet aggregation assays and bleeding times (*in vitro* studies show that laropiprant has off-target activity at the thromboxane (thromboxane A₂ (TxA₂)) receptor (*i.e.*, TP receptor).

RESULTS

Patient characteristics

The mean age of study participants in study 1 was 30.1 years (range: 20–45). Their mean height was 181.0 cm (range: 171.5–188.0) and mean weight was 74.1 kg (range: 59.5–97.9). The mean age in study 2 was 28.3 years (range: 19–43). The mean height was 182.1 cm (range: 168.5–194.0) and mean weight was 75.7 kg (range: 60.9–96.3). The mean age in study 3 was 31.5 years (range: 19–45). The mean height was 176.8 cm (range: 165.0–185.0) and mean weight was 79.3 kg (range: 61.9–98.8).

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Safety and tolerability

Laropiprant was safe and generally well tolerated at single doses of 1–900 mg or at multiple doses of 30–450 mg administered once daily for 10 days. No subject discontinued because of an adverse event (AE).

No serious clinical or laboratory AEs were observed. Of six AEs noted from laboratory findings, none was considered to be related to study drug, and three involved subjects receiving placebo. Although some laboratory values were outside the normal range, the investigator and clinical monitor considered them to be of no clinical importance. There were no consistent, clinically meaningful dose–response relationships in changes from baseline to end point for any laboratory parameter.

Most AEs were transient and mild to moderate. The most frequently reported AEs, while subjects were on active study drug, were considered possibly or probably drug related by the investigator (in all three studies together), and included headache, heaviness of head, decreased appetite, stomach heaviness, dizziness upon standing, orthostatic hypotension, bitter taste, dry mouth/throat, and loose stools. There were no consistent treatment-related changes in electrocardiography findings or vital signs.

Pharmacokinetics

Single-dose evaluations. Pharmacokinetic parameters of single-dose laropiprant are depicted in **Table 1** and **Figure 1**. Laropiprant was rapidly absorbed (median Time of maximum concentration (T_{max}) = 0.8–2.0 h). Plasma levels declined from C_{max} (peak plasma concentration) in a biphasic manner, with a rapid decline from 2 to 4 h postdose followed by a slower decrease with an apparent terminal half-life ($t_{1/2}$) of 12.0–17.7 h. Laropiprant was excreted unchanged in the urine to a negligible extent (pooled f_e = 0.41% (95% confidence interval (CI) = 0.37–0.44)) and had a low renal clearance (pooled Cl_R = 0.68 ml/min (95% CI = 0.64–0.72)) that did not vary significantly across doses. Area under the curve (AUC) $_{0-\infty}$, C_{max} , and C_{24h} were

dose proportional over the range of doses with the available data (12–900 mg for $AUC_{0-\infty}$ and C_{24h} , and 3–900 mg for C_{max}).

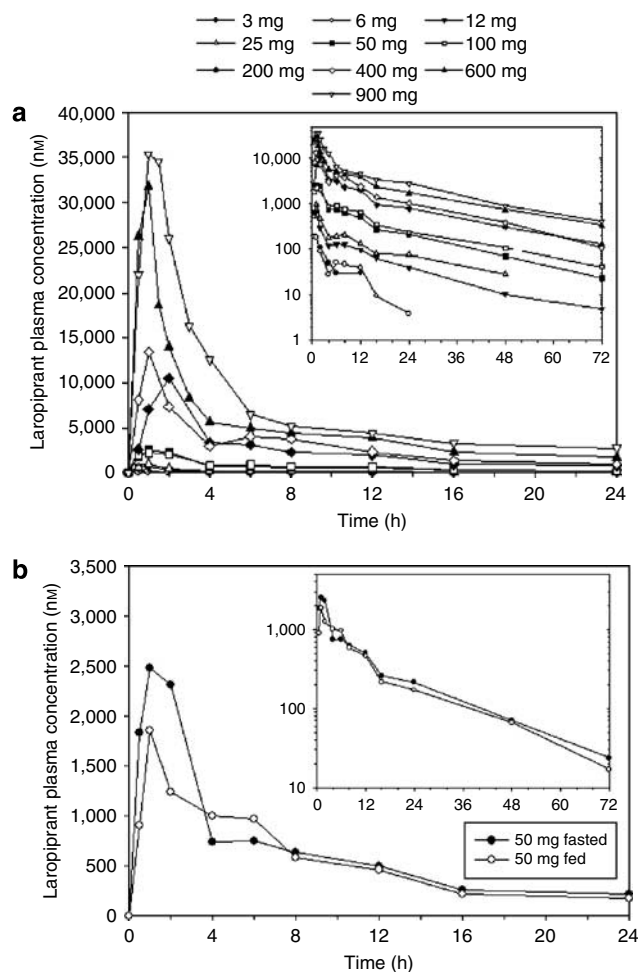


Figure 1 Plasma concentration-time profile for laropiprant. Mean laropiprant plasma concentrations (nM) following (a) single oral doses of 3–900 mg in fasted state, (b) single oral doses of 50 mg in the fed and fasted states. Inset: Semi-log scale.

Table 1 Laropiprant pharmacokinetics following single oral doses

Parameter	Laropiprant dose (mg)										
	3 ^a	6 ^b	12 ^a	25 ^b	50 ^a	50 ^a (fed)	100 ^b	200 ^a	400 ^b	600 ^c	900 ^c
$AUC_{0-\infty}$ ^d (μ Mh)	—	—	4.10	5.93	18.18	15.53	21.72	71.07	84.80	164	235
C_{max} ^d (μ M)	0.09	0.19	0.88	0.95	2.74	1.97	2.52	9.48	10.72	32.9	40.3
C_{24h} ^d (μ M)	—	—	0.045	0.068	0.190	0.145	0.228	0.687	0.926	1.412	2.462
T_{max} ^e (h)	2.0	0.8	0.8	1.0	1.0	1.0	1.0	2.0	1.0	—	—
Apparent terminal $t_{1/2}$ ^f (h)	—	—	12.0	15.7	13.1	13.1	16.8	17.7	14.1	—	—
Cl_R ^d (ml/min)	—	—	—	0.6	0.6	0.7	0.7	0.6	1.0	0.8	1.1
f_e ^g (%)	—	—	—	0.3	0.4	0.5	0.3	0.5	0.5	0.5	0.6

AUC , area under the curve; Cl_R , renal clearance; C_{max} , peak plasma concentration; LS, least square; $t_{1/2}$, half-life; —, data not calculated. ^aSubjects in panel B were given single doses of laropiprant 3, 12, 50, and 200 mg or placebo in the fasted state in periods 1–4; in period 5, panel B, subjects were given a repeat oral dose of laropiprant 50 mg or placebo following a standard high-fat breakfast. ^bSubjects in panel A were given single doses of laropiprant 1 (data not shown), 6, 25, 100, and 400 mg or placebo in the fasted state. ^cSubjects were given single doses of laropiprant of 600 and 900 mg in the fasted state. ^dLS geometric mean. ^eMedian. ^fHarmonic mean. ^gLS arithmetic mean.

Table 2 Laropiprant pharmacokinetics following once-daily multiple oral doses for 10 days

Parameter	Laropiprant dose (mg)											
	30		60		125		200		300		450	
	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10	Day 1	Day 10
AUC _{0–24} ^a ($\mu\text{M h}$)	6.54	8.30	18.45	24.84	32.05	43.88	49.72	56.95	58.5	75.9	85.3	111.0
C _{max} ^a (μM)	1.85	2.10	5.02	3.89	7.00	9.05	9.40	11.41	14.8	16.3	17.9	20.8
C _{24h} ^a (μM)	0.078	0.013	0.327	0.478	0.471	0.784	0.883	1.039	0.771	1.315	1.503	2.817
T _{max} ^b (h)	0.8	0.8	1.0	1.5	1.5	1.3	1.5	0.5	—	—	—	—
Apparent terminal t _{1/2} ^c (h)	—	15.6	—	15.0	—	18.4	—	16.0	—	—	—	—
f _e ^d (%)	—	0.7	—	0.5	—	0.4	—	0.6	0.6	0.6	0.5	0.5
Cl _R ^a (ml/min)	—	0.5	—	0.7	—	0.5	—	0.5	0.9	0.8	1.0	0.7

AUC, area under the curve; Cl_R, renal clearance; C_{max}, peak plasma concentration; LS, least squares; t_{1/2}, half-life. —, data not collected. ^aLS geometric mean. ^bMedian. ^cHarmonic mean. ^dLS arithmetic mean.

The extent and rate of absorption of laropiprant were not affected by food (**Figure 1b**). The profile of the metabolite was consistent with formation rate-limited pharmacokinetics and resembled the profile of the parent compound, with a median T_{max} of 1.0–2.0 h and an apparent terminal t_{1/2} of 10.1–17.8 h.

Multiple-dose evaluations. The pharmacokinetic profile of multiple-dose laropiprant and its metabolite was similar to that of single-dose laropiprant (**Table 2**), with a median T_{max} of 0.8–1.5 h, apparent terminal t_{1/2} of 15.0–18.4 h, negligible excretion of laropiprant unchanged in the urine, and low Cl_R. AUC_{0–24h} (**Figure 2**), C_{max}, and C_{24h} were dose proportional over the entire dose range studied. Steady-state C_{24h} levels were attained by day 2 at all dose levels. Accumulation is minimal, with an AUC accumulation ratio of approximately 1.3 and C_{max} accumulation ratio of approximately 1.1.

Pharmacodynamics

Effects of laropiprant on PGD₂-stimulated cAMP generation. Near-maximal (approximately 90%) inhibition of the PGD₂-stimulated cAMP response was achieved at 4 h after single doses of laropiprant at all doses tested (1–400 mg). Near-maximum (>80%) inhibition persisted for 48 h after laropiprant ≥ 6 mg. Because near-maximum inhibition was observed for laropiprant at all sampling time points with concentrations at or above the lower limit of quantitation, no pharmacokinetic–pharmacodynamic relationship between laropiprant plasma concentrations and inhibition of PGD₂-stimulated cAMP production could be defined, and it could not be determined if there was a time delay in this relationship. One possible interpretation of these data is that the EC₅₀ (the fixed effects of concentration that results in 50% response) for laropiprant-mediated inhibition of PGD₂-stimulated cAMP production was below the assay limit for detection of laropiprant (<10 ng/ml = 22.9 nM).

Findings from the multiple-dose study were similar, with near-maximal inhibition being achieved within 4 h at all tested doses and persisting 24 h after administration on day 10.

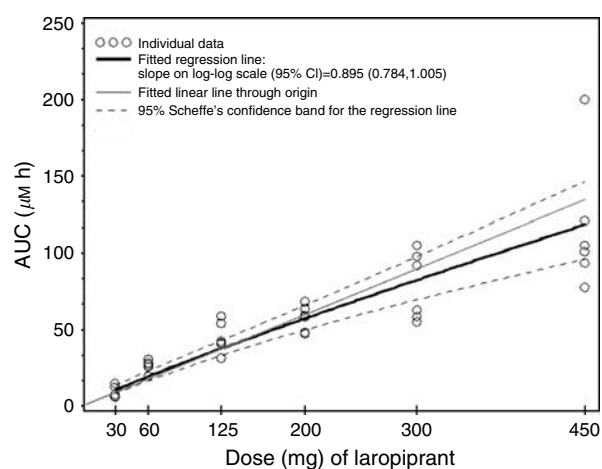


Figure 2 Dose proportionality of laropiprant AUC_{0–24h} following 10-day multiple once-daily oral doses of 30–450 mg.

Effects of laropiprant on TxA₂ agonist (U-46619)-stimulated platelet aggregation.

At single oral laropiprant doses up to 50 mg, there was no clinically significant difference from placebo in inhibition of TxA₂ agonist-stimulated platelet aggregation. At single doses of ≥ 100 mg, inhibition of aggregation was dose dependent. A pharmacokinetic–pharmacodynamic relationship was apparent for percent inhibition of TxA₂ agonist-stimulated platelet aggregation and laropiprant plasma concentrations. Data were fitted to a sigmoid Maximum response (effect) (E_{max}) model, with E_{max} being fixed at 100% inhibition. For percent inhibition measured as amplitude, the population estimate of EC₅₀ was 1.12 \pm 0.24 μM (typical value \pm SE) with an estimated interindividual variability (IIV) of 81%, and the population estimate of the Hill coefficient (γ) was 3.8 \pm 1.0. For percent inhibition measured as slope, the estimated EC₅₀ was 1.36 \pm 0.18 μM , IIV was 47%, and the estimated Hill coefficient was 2.9 \pm 0.8.

Pharmacodynamic results from multiple-dose laropiprant were similar, with differences in percent inhibition of TxA₂ agonist-stimulated platelet aggregation for laropiprant doses

and placebo at all time points throughout the 10-day dosing interval. Inhibition of approximately 70% or greater (vs placebo) was observed at daily laropiprant doses ≥ 60 mg on day 5, at 4 h after dosing on day 10, and at trough (24 h after dosing on day 10). A pharmacokinetic–pharmacodynamic relationship was observed between laropiprant plasma concentration and percent inhibition (Figure 3), with EC_{50} values of $0.762 \pm 0.125 \mu\text{M}$; IIV = 78% for inhibition measured as percent amplitude and $0.822 \pm 0.122 \mu\text{M}$; IIV = 67% for percent inhibition measured as slope.

Effects of laropiprant on collagen-stimulated platelet aggregation. No significant differences were observed between single doses of laropiprant 6–400 mg and placebo in inhibition of collagen-induced platelet aggregation 4 h after laropiprant dosing. Similarly, at 4 h after multiple-dose laropiprant administration on day 5 or at 4 and 24 h after laropiprant dosing on day 10, there were no differences from placebo for doses of 30–200 mg. Effects of laropiprant became apparent with higher doses of 300 and 450 mg given once daily for 10 days. Results from these assays were fitted to a sigmoid E_{max} model resulting in a population EC_{50} estimate

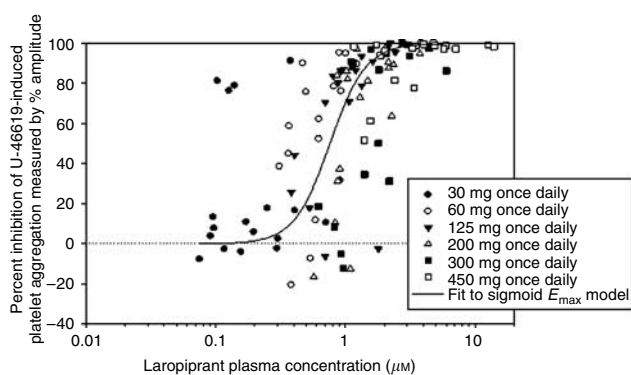


Figure 3 Inhibition of TxA_2 agonist (U-46619)-induced platelet aggregation (amplitude) vs plasma concentration for subjects administered multiple oral doses of 30, 60, 125, 300, or 450 mg laropiprant once daily for 10 days. Data are from days 5 and 10.

of $5.29 \pm 1.28 \mu\text{M}$; IIV = 76% for inhibition measured as percent amplitude (Figure 4).

Effects of laropiprant on bleeding times. No significant differences in bleeding time between laropiprant and placebo were observed following single doses up to 400 mg or multiple doses up to 450 mg ($P > 0.05$) in the fold change from baseline of bleeding time at 4 h postdose after 10 days of multiple dosing. In contrast, aspirin 325 mg displayed a statistically significant increase in bleeding time compared with placebo (approximately 44% increase; $P = 0.004$) at 24 h after once-daily doses for 7 days (Table 3).

DISCUSSION

Laropiprant administered as single doses up to 900 mg and multiple doses up to 450 mg is generally well tolerated. There were no serious AEs or discontinuations due to an AE. There were no clinically significant, treatment-related effects of laropiprant observed upon analysis of laboratory, vital sign, or electrocardiography parameters.

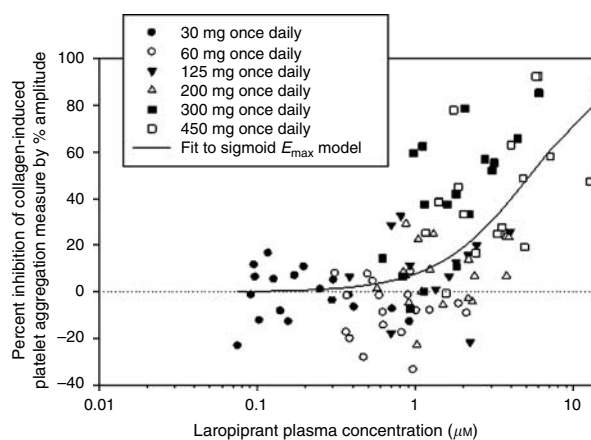


Figure 4 Inhibition of collagen-induced platelet aggregation (amplitude) vs plasma concentration for subjects administered multiple oral doses of 30, 60, 125, 300, or 450 mg laropiprant once daily for 10 days. Data are from days 5 and 10.

Table 3 Fold increase from baseline in bleeding time following multiple once-daily oral dosing of laropiprant 300 and 450 mg and aspirin 325 mg

Treatment	N	Min	Median	Max	Between-subject SD ^a	LS geometric mean (95% CI) ^{a,b}	GMR (active vs placebo) (90% CI)	P-value
<i>4 h postdose on day 14 (last day of 10-day multiple dosing) (panels A and B)</i>								
Placebo	6	0.73	1.14	1.85	0.41	1.21 (0.90, 1.64)		
Laropiprant 300 mg	6	0.75	1.05	2.64	0.66	1.37 (1.01, 1.85)	1.12 (0.80, 1.58)	> 0.200
Laropiprant 450 mg	6	1.00	1.46	3.00	0.66	1.20 (0.86, 1.67)	0.99 (0.67, 1.45)	> 0.200
<i>24 h postdose on day 7 (last day of 7-day multiple dosing) (panel C)</i>								
Placebo	4	1.17	1.75	2.50	0.62	1.68 (1.43, 1.97)		
Aspirin 325 mg	6	1.91	2.34	3.20	0.52	2.42 (2.12, 2.76)	1.44 (1.22, 1.71)	0.004

CI, confidence interval; GMR, geometric mean ratio; LS, least squares. ^aTransformed back from log scale. ^bGMR.

The DP1 antagonist is rapidly absorbed and exhibits dose-proportional pharmacokinetics over a dose range of 12–900 mg. Plasma levels decline from C_{\max} in a biphasic manner, with an apparent terminal $t_{1/2}$ of 12–18 h. Absorption is not affected by high-fat food. Laropiprant exhibits a low Cl_R that is consistent with a highly (>99% *in vitro*) protein-bound compound. The major circulating metabolite of laropiprant is an inactive acyl glucuronide metabolite, which is likely formed by several UDP glucuronyl transferase (UGT) isoenzymes (1A1, 1A3, 1A9, and 2B7),¹⁰ and which is excreted into bile and urine.¹¹ Plasma levels of this metabolite displayed formation rate-limited pharmacokinetics, with a profile similar to the parent compound. Steady-state pharmacokinetic data are generally consistent with single-dose pharmacokinetic data, with no evidence of time-dependent changes in clearance.

Persistent antagonist activity on platelet DP1 over 24 h at all tested doses is consistent with once-daily dosing. At all dose levels examined, steady state was reached by day 2 during the 10-day dosing period and slight to moderate accumulation was observed (on average across all examined doses, approximately 30% for AUC, 10% for C_{\max} , and 60% for C_{24h}).

Inhibition of PGD₂-stimulated cAMP on platelets *ex vivo* was assessed to gauge the pharmacologic activity of laropiprant on platelet DP1. Maximum inhibition of cAMP was achieved for 24 h postdose across all laropiprant dose levels tested. These results demonstrate that laropiprant is a potent antagonist of DP 1.

The off-target activity of laropiprant on TP 1 was monitored with two different assays of platelet aggregation and bleeding time. The sensitivity of the U-46619 platelet aggregation assay to the perturbation of TP signaling is dependent on the concentration of agonist. For these studies, the assay was designed to be highly sensitive to any interaction of laropiprant with TP. Because the concentration of the agonist U-46619 used in this study is just above or at the threshold concentration required to trigger platelet aggregation, it was expected that a small to modest degree of blockade of the TP receptor would be sufficient to inhibit U-46619-induced platelet aggregation. Therefore, inhibition of U-46619-induced platelet aggregation does not necessarily predict a clinically meaningful effect on platelet function.

Collagen induces aggregation of platelets by binding and activating two glycoprotein receptors. This triggers multiple pathways that result in platelet aggregation. One of these pathways is the generation of thromboxane by the platelet that activates TP. Because multiple pathways are activated by collagen, it has been observed that inhibition of collagen-induced platelet aggregation requires a high degree of blockade of the thromboxane pathway. Increases in bleeding time on the order of >50%, such as those observed with aspirin, are associated with a small but measurable increase in bleeding risk. In general, there is a correlation between increases in bleeding time and inhibition of collagen-induced platelet aggregation (with concentrations of agonist similar to those used in this study).

Studies with aspirin indicate that >95% inhibition of thromboxane synthesis is associated with >50% inhibition of collagen-induced platelet aggregation, whereas approximately 50% inhibition of thromboxane synthesis does not have a significant effect.^{12–14} A similar degree of inhibition of the thromboxane pathway through receptor blockade is associated with prolongation of bleeding time. A study with a TP antagonist demonstrates that >94% blockade of the TP receptor results in an increased bleeding time, whereas lower levels of receptor blockade results in measurable effects on collagen-induced platelet aggregation or bleeding time.¹⁵

For the U-46619-induced platelet aggregation assay, differences between laropiprant and placebo were observed for the multiple doses tested (30–450 mg). With data pooled from the multiple-dose studies, a sigmoid E_{\max} model was used to estimate the EC_{50} for inhibition of U-46619-induced platelet aggregation (amplitude) as $0.762 \pm 0.125 \mu\text{M}$ (typical value \pm SE). These results are in contrast to those obtained with the collagen-induced platelet aggregation assay and bleeding time.

There was no clinically significant difference between the laropiprant doses up to 200 mg once daily for 10 days and placebo in the inhibition of collagen-induced platelet aggregation or the fold increase of bleeding time at 4 h postdose. There was also no statistically significant prolongation of bleeding time with multiple doses of laropiprant at 300 and 450 mg. However, collagen-induced platelet aggregation was inhibited at 4 h following multiple doses of laropiprant at 300 and 450 mg. At 24 h postdose, inhibition of collagen-induced platelet aggregation is observed with the 450 mg dose but not with the 300 mg dose. With data pooled from the multiple-dose studies, a sigmoid E_{\max} model was used to estimate the EC_{50} for inhibition of collagen-induced platelet aggregation (amplitude) as $5.29 \pm 1.28 \mu\text{M}$ (typical value \pm SE).

By comparison, 325 mg aspirin has a more pronounced and more persistent effect on platelet function than either 300 or 450 mg of laropiprant. At 24 h, following the last dose of a 7-day treatment, aspirin significantly inhibited collagen-induced platelet aggregation and prolonged bleeding time. These results suggest that laropiprant at multiple doses up to 200 mg does not inhibit platelet function to a clinically meaningful degree. At doses of 300 and 450 mg, laropiprant interferes with platelet function, but this effect is transient and less robust compared to aspirin.

Limitations of this study include the small sample sizes and relatively short exposure times. Further investigation should include a wider population segment, including women and elderly patients, who bear a disproportionate burden of morbidity and mortality secondary to cardiovascular disease.

Laropiprant (MK-0524) holds promise as an adjunctive treatment to enhance the tolerability of and compliance with niacin regimens for patients with cardiovascular disease and/or dyslipidemia.

METHODS

Subjects. Study candidates were healthy non-smoking male subjects aged 18–45 years. Subjects were required to be within 25% of their ideal body weight. Other eligibility criteria included normal arachidonate- and collagen-stimulated platelet aggregation. Participants were required to refrain from strenuous exercise, excess alcohol and caffeine, and use of prescription/non-prescription medications.

Study designs. Three randomized, double-blind, placebo-controlled studies were conducted to evaluate laropiprant at single and multiple doses. Study 1 included 16 men, study 2 included 32 men, and study 3 included 18 men (plus an aspirin panel).

Treatments. *Study 1:* Subjects were assigned to one of two treatment panels (A and B), which alternately received single-rising oral doses of laropiprant over five treatment periods. For each panel, six subjects were administered laropiprant and two subjects received placebo. Subjects in panel A were given single doses of 1, 6, 25, 100, and 400 mg of laropiprant or placebo in the fasted state. Subjects in panel B were given single doses of 3, 12, 50, and 200 mg of laropiprant or placebo in the fasted state (Table 4). In period 5, panel B, subjects were given a repeat oral dose of 50 mg laropiprant or placebo following a standard high-fat breakfast to assess the effect of food on the plasma concentration profile of laropiprant. There was at least a 6-day washout between treatment periods for any individual subject.

Study 2: Four panels (panels A–D) of eight subjects received 30, 60, 125, and 200 mg of laropiprant or placebo, in the fasted state, once daily for 10 consecutive days. For each panel, six subjects were administered laropiprant and two subjects received placebo.

Study 3: Subjects were assigned to one of two treatment panels (panels A and B), with six subjects in each panel being administered laropiprant and three subjects in each panel being administered matching placebo. Subjects in panel A received a single dose of 600 mg laropiprant followed 96 h later with once-daily doses for 10 days (days 5–14) of 300 mg laropiprant. Subjects in panel B received a single dose of 900 mg laropiprant followed 96 h later with once-daily doses for 10 days (days 5–14) of 450 mg laropiprant. Day 1, 5, and 14 doses were administered in the fasted state. In panel C, six subjects received 325 mg aspirin daily for 7 days (days 1–7), and four subjects received placebo to aspirin daily for 7 days (days 1–7).

Ethics. Studies were conducted in accordance with the Declaration of Helsinki Principles and were consistent with the International

Conference on Harmonization guidelines for Good Clinical Practices. Informed consent was obtained from each subject.

Studies 1 and 2 were conducted in Belgium and both were reviewed and approved by the Ethical Review Committee of the University Hospital of Leuven (Leuven, Belgium). Study 3 was conducted in the United States and was reviewed and approved by the Division of Human Subjects Protection (Institutional Review Board), Office of Scientific Affairs, Thomas Jefferson University.

Assessments. *Safety:* A medical history was obtained during the screening period. Safety assessments included the following:

- physical examination
- vital signs
- 12-lead electrocardiography
- laboratory evaluations.

Tolerability (AEs): All AEs were documented, coded by the investigator using the Medical Dictionary for Regulatory Activities (MedDRA), and classified as mild, moderate, or severe, and as definitely, probably, or possibly related (or definitely or probably unrelated) to study drug. Serious AEs are those that were fatal, life threatening, disabling, or resulted in (or prolonged) hospitalization.

Analytical methods and pharmacokinetics: Plasma samples were assayed at predose, specified time points postdose, and poststudy.

Plasma and urine levels of laropiprant were determined using validated assays. The lower limit of quantitation of both the plasma and urine assays was 10 ng/ml (22.9 nM). Plasma samples from studies 1 and 2 were also analyzed for concentrations of the acyl glucuronide metabolite of laropiprant. The lower limit of quantitation for the metabolite assay was 10 ng/ml (16.3 nM).¹⁶

All calculations of pharmacokinetic parameters were performed using WinNonlin version 5.0.1. Half-life ($t_{1/2}$) was calculated as the quotient of $\ln(2)$ and the apparent terminal rate constant (λ). AUCs were calculated using the linear trapezoidal method for ascending concentrations and the log trapezoidal method for descending concentrations. $AUC_{0-\infty}$ was estimated as the sum of AUC_{0-last} and the extrapolated area given by the quotient of the last measured concentration and λ . C_{max} , T_{max} , and C_{24h} were obtained by inspection of the plasma concentration data.

The percent of the dose excreted unchanged in urine over the collection interval ($f_{e0-\tau}$) was determined by the quotient of the sum of laropiprant collected over all intervals and the dose administered, with the result multiplied by 100. Following each dose, $AUC_{0-\tau}$ was calculated, where τ represents the nominal stop time of the final urine collection interval. Cl_R was determined as the quotient of $f_{e0-\tau}$ and $AUC_{0-\tau}$.

Pharmacodynamics: The pharmacodynamic effects of laropiprant were assessed at screening and the following time points using the following assays:

- blood PGD_2 -stimulated cAMP response assay performed at screening, predose, and 4, 8, 24, and 48 h after laropiprant administration (days 1 and 2) in study 1; and predose and 4 and 24 h after dosing (days 10 and 11) in study 2;
- platelet aggregation assays predose, 4 h after laropiprant administration on day 1 in study 1; predose on days 1 and 5, and 4 and 24 h after dosing (days 10 and 11) in study 2; and predose on days 1 and 10, and 4 and 24 h after laropiprant administration on day 14 in study 3; additional assays were performed on subsequent days if >50% inhibition was observed.
- bleeding time assays (± 30 min from designated time points) predose and 4 h after laropiprant administration on day 1 in study 1; 4 h after dosing on day 10 (with additional measurement on day 11 if day 1 4-h value is two times higher than baseline and outside normal range) in study 2; and 4 h after dosing on day 14 in study 3; additional Bleeding time

Table 4 Study 1: rising single-dose treatments administered

Panel	Subjects (N)	Period 1	Period 2	Period 3	Period 4	Period 5
A ^a	2	1	PBO	25	100	400
	2	1	6	PBO	100	400
	2	1	6	25	PBO	400
	2	PBO	6	25	100	PBO
B ^a	2	3	PBO	50	200	50 ^a
	2	3	12	PBO	200	PBO ^a
	2	3	12	50	PBO	50 ^a
	2	PBO	12	50	200	50 ^a

^aPBO, placebo. Within each treatment period, six subjects were randomized to receive laropiprant and two subjects to receive placebo for laropiprant according to a computer-generated allocation schedule. Subjects in period 5, panel B, had identical randomization assignments as in period 3, panel B, *i.e.*, subjects who received laropiprant in period 3, panel B, also received laropiprant in period 5, panel B.

(BT) measurements were obtained on subsequent days if BT was found to be twofold greater than baseline and above the normal range.

PGD₂-stimulated cAMP response assay: For this assay, approximately 3–4.5 ml of blood was collected per subject (one sample per subject). After equilibration to 37°C for ≥10 min, aliquots of 200 μl platelet-rich plasma (PRP) were introduced to two separate tubes containing premeasured amounts of 3-isobutyl-1-methylxanthine at a final concentration of 2 mM and PGD₂ at 200 nM.

Following incubation, the reaction was stopped by adding 20 μl of 1 N hydrochloric acid, the sample was flash-frozen, and 224 μl aliquots were capped and stored at ≤−70°C until shipping for cAMP analysis.

TxA₂ agonist (U-46619)- and collagen-stimulated platelet aggregation assays: Platelet aggregation in PRP was assessed by changes in light transmission using an aggregometer (chronolog 4-channel). Platelet aggregation was initiated by addition of arachidonic acid (1.0–1.6 mM) in the screening period, as well as U-46619 (Sigma) at a range of concentrations during screening and at a final concentration of 1.3 μM subsequently in all three studies. Collagen (2 μg/ml) was added during both screening and treatment periods in studies 2 and 3. A 4.5-ml aliquot of blood was collected by fresh venipuncture with a 19- to 21-G needle into buffered 0.105 M sodium citrate vacutainer tubes and mixed by inverting. For PRP determinations, blood was centrifuged at 150 g for 15 min at room temperature, the PRP removed, and the remaining volume centrifuged at 2,000 g for 15 min to obtain platelet-poor plasma. The platelet count of PRP was adjusted to approximately 200,000–300,000 platelets/ml by dilution with platelet-poor plasma. Aliquots of 0.3 ml adjusted PRP or platelet-poor plasma were added to separate aggregometer cuvettes and warmed to 37°C. The cuvettes were placed in separate wells with the platelet-poor plasma serving as a blank for light transmission. Once the baseline had stabilized, the aggregometer was calibrated, and the increase in light transmission was followed for 5 min after addition of agonist. The maximum percent of light transmission (extent of aggregation) and the instrument-calibrated slope (rate of aggregation) were reported. Post-treatment platelet aggregation was expressed as a percent of each subject's pretreatment level. Inhibition of platelet aggregation was computed as the percent change from pretreatment baseline in the extent of aggregation.

Bleeding times: Bleeding time was measured prestudy and 4 h after laropiprant administration on day 1 (study 1), day 10 (study 2), and day 14 (study 3). A small incision wound was made with a sterile disposable spring-loaded device (Simplate Pediatric).

Outcome measures. Primary end point: For all three studies, the primary outcome measure was on safety parameters (laboratory findings, electrocardiography results, and vital signs). An additional primary end point in the two multiple-dose studies was the plasma concentration of laropiprant at 24 h postdose (C_{24h}) on the final day of dosing.

Secondary end points: Secondary end points included other pharmacokinetic parameters such as $AUC_{0-\infty}$, or AUC_{0-24h} , C_{max} , T_{max} , apparent terminal $t_{1/2}$, and the percent of dose excreted unchanged in urine and Cl_R (f_e).

Pharmacodynamic parameters included percent inhibition from baseline of PGD₂-stimulated cAMP generation (peak and time-weighted average percent inhibition over 48 h postdose in study 1 and percent inhibition at 4 and 24 h at steady state in study 2); percent change from baseline for TxA₂ agonist- and collagen-induced platelet aggregation (at 4 h following single doses and at 4 and 24 h at steady state following multiple doses); and fold change from baseline of bleeding times (at 4 h following single doses and at steady state following multiple doses).

Statistical methods. Safety and tolerability: Safety was evaluated by clinical assessment of AEs.

Pharmacokinetics: (a) Single-dose pharmacokinetics. (i) Study 1: For each available laropiprant pharmacokinetic parameter ($AUC_{0-\infty}$, C_{max} , C_{24h} , T_{max} , apparent terminal $t_{1/2}$, Cl_R , and f_e), the least-squares estimates and 95% CIs were determined separately from a linear mixed-effect model that included panel and treatment-within-panel as fixed effects and subject-within-panel as a random effect (see Table 4 for treatments). The primary pharmacokinetic hypothesis that C_{24h} exceeded 0.100 μM was tested in a stepwise manner using the linear mixed model. (ii) Study 3: Model-based least-squares means and 90% CIs were obtained for available pharmacokinetic parameters through a one-way analysis of variance model (study 3 was a parallel-group design). (iii) Single-dose proportionality (studies 1 and 3): Dose proportionality for $AUC_{0-\infty}$ was assessed with pooled single-dose data from studies 1 and 3 in an exploratory manner. A mixed linear model was fitted on the log scale on $\ln(AUC_{0-\infty})$ with $\ln(\text{dose})$ as a covariate and subject as a random effect. An estimate of the slope on $\ln(\text{dose})$ and the corresponding 95% CI was obtained from the model. A slope of 1 represents the exact dose proportionality, whereas a large deviation from 1 indicates the lack of dose proportionality. The mean regression line of $AUC_{0-\infty}$ vs dose was plotted on the linear scale (after back-transformation from log-scale), with 95% Scheffé confidence bands drawn around the regression line. Similar methods were applied to assess dose proportionality for C_{max} and C_{24h} following single doses of laropiprant.

(b) Multiple-dose pharmacokinetics. (i) Study 2: Model-based estimates of AUC_{0-24h} , C_{max} , and C_{24h} at different doses of laropiprant were obtained from a linear mixed-effect model that included dose, day (days 1 and 10) as fixed effect, and subject-within-dose as a random effect for each of the listed pharmacokinetic parameters; model-based estimates of Cl_R and f_e , where only day 10 data were available, a one-way analysis of variance model was employed with a factor for dose. For T_{max} and apparent terminal $t_{1/2}$, the sample median and harmonic mean were respectively provided by dose. The primary pharmacokinetic hypothesis for C_{24h} on day 10 was tested in a stepwise manner. Steady state was evaluated in an exploratory manner by stepwise testing using a linear mixed-effect model, with day as a fixed effect and subject as a random effect. Linear contrasts on C_{24h} were constructed over successive ranges of days until one was found to be no longer significantly different from 0 (the steady state). The accumulation ratio of AUC_{0-24h} , C_{max} , and C_{24h} was estimated separately by the geometric mean ratio of day 10/day 1 from a linear mixed model with dose and time (days 1 and 10) as fixed effects and subject-with-dose as a random effect. (ii) Study 3: Model-based least-squares means and 90% CIs were obtained for the available pharmacokinetic parameters using a one-way analysis of variance model with a factor for dose. Accumulation ratios of AUC_{0-24h} , C_{max} , and C_{24h} (day 14/day 5) were assessed using similar models as described for accumulation ratio evaluation in study 2.

(iii) Multiple-dose proportionality: Dose proportionality was assessed for AUC_{0-24h} , C_{max} , and C_{24h} at steady state following 10-day multiple dosing of laropiprant. Methods were similar to those used to evaluate single-dose proportionality, except that the model fitted to a log-transformed pharmacokinetic parameter was a simple linear regression model with covariate $\ln(\text{dose})$ only.

Pharmacodynamics: In study 1, the effect of a single dose of laropiprant, as compared to placebo, on the pharmacodynamic end points examined in this study was analyzed using a linear mixed-effect model on log-transformed data ($\log(\text{postdose measurement}/\text{baseline})$). The model included panel and treatment-within-panel as fixed effects, subject-within-panel as a random effect, and $\log(\text{baseline})$ as a covariate. For PGD₂-stimulated cAMP, inhibition (%) of cAMP at baseline (predose) and 4, 8, 24, and 48 h postdose, time-weighted average inhibition (%), and peak inhibition (%) over the

48-h postdose interval were evaluated separately. For percent inhibition of platelet aggregation from baseline (predose) and the fold change of bleeding time from baseline (predose), the comparisons between laropiprant treatments vs placebo were obtained at 4 h postdose where data were collected.

In study 2, the effect of multiple doses of laropiprant, as compared to placebo, on the percent inhibition of PGD₂-stimulated cAMP production (day 1 measurement as baseline) was analyzed using a linear mixed-effect model on log-transformed data (log(postdose measurement/baseline)). The model included dose and time (4 and 24 h on day 10) as fixed effects, subject-within-dose as a random effect, and log(baseline) as a covariate. The effect of multiple doses of laropiprant, as compared to placebo, on the percent inhibition in U-46619- or collagen-induced platelet aggregation (day 1 measurement as baseline) was analyzed using a linear mixed-effect model that included dose and time (day 5, 4 and 24 h on day 10) as fixed effects and subject-within-dose as a random effect; the effect of 10-day dosing of laropiprant, as compared to placebo, on the fold increase in bleeding time over baseline (prestudy) was analyzed using a one-way analysis of variance model with factor for dose.

In study 3, the percent inhibition in U-46619- or collagen-induced platelet aggregation (day 1 measurement as the baseline) was analyzed separately using a linear mixed-effect model on log-transformed data (log(postdose measurement/baseline)). The model included dose, time (predose on day 10, 4 and 24 h on day 14), dose-time interaction as fixed effects, subject-within-dose as a random effect, and log(baseline) as a covariate. The effect at 4 h after 10-day dosing of laropiprant on the fold change in bleeding time over baseline (prestudy), as compared to placebo, was assessed using a analysis of covariance model that included dose as a factor and baseline as a covariate. The effect of aspirin 324 mg at 24 h after 7-day dosing, as compared to placebo, was evaluated in a similar manner to laropiprant.

Pharmacokinetics/pharmacodynamics: Values for pharmacodynamic parameters were plotted against laropiprant plasma concentrations. For the platelet aggregation response parameters, data were fitted to a sigmoid E_{\max} model using a nonlinear mixed effects approach. The model used was as follows:

$$\text{Response} = \frac{E_{\max} C^{\gamma \exp(\eta_2)}}{(EC_{50} \exp(\eta_1))^{\gamma \exp(\eta_2)} + C^{\gamma \exp(\eta_2)}} + \varepsilon$$

where C is the plasma concentration, EC_{50} and γ are the fixed effects of concentration that results in 50% response and the Hill coefficient, respectively, η_1 and η_2 the random effects representing the IIV on EC_{50} and γ , respectively, and ε is the residual error. E_{\max} , which represents the maximum response, was fixed to 100% inhibition. η_2 could not be estimated for all data sets, and so for the analyses of single-dose data and the multiple-dose TxA₂ agonist-stimulated platelet aggregation data measured as slope, η_2 was fixed to 0. These analyses were performed using NONMEM V via the PDxPOP version 2.1a interface.

SAS version 8.02 (SAS Institute, Cary, NC) was used for statistical analyses.

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CONFLICT OF INTEREST

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