ARTICLE

Pharmacokinetic and Pharmacodynamic Effects of Oral CXA-10, a Nitro Fatty Acid, After Single and Multiple Ascending Doses in Healthy and Obese Subjects

Rachel M. Garner[1], Diane R. Mould[2], Carla Chieffo[1] and Diane K. Jorkasky[1],*

10-nitro-9(E)-octadec-9-enoic acid (CXA-10), a novel nitro fatty acid compound, demonstrates potential as a therapeutic agent in multiple disease indications in which oxidative stress, inflammation, fibrosis, and/or direct tissue toxicity play significant roles. Phase I studies were conducted in healthy and obese subjects to evaluate the pharmacokinetics (PK), pharmacodynamics (PD), safety, and tolerability of oral CXA-10 after single and multiple doses in the fed and fasted states that would confirm the mechanisms of action of CXA-10. After single and multiple ascending doses, CXA-10 demonstrated dose-proportional increases in plasma exposure. CXA-10 decreased levels of biomarkers associated with altered inflammation and metabolic stress observed from nonclinical studies. In CXA-10-202, a consistent decrease from baseline was observed with CXA-10 150 mg dose, but not 25 or 450 mg doses, for biomarkers of altered inflammation and metabolic dysfunction, including leptin, triglycerides, cholesterol, MCP-1, and IL-6. In CXA-10-203, after coadministration with CXA-10, geometric mean peak plasma concentration (C_{max}) and area under the plasma concentration-time curve from time point 0 to the end of the dosing interval (AUC_{0-t}) decreased 20% and 25% for pravastatin, increased 10% and 25% for simvastatin, and decreased 20% and 5% for ezetimibe. These findings are consistent with the pharmacological effects of CXA-10. Adverse events (AEs) were dose-related, and the most frequently reported AEs (>10% of subjects) were diarrhea, abdominal pain, and nausea. CXA-10 was safe and well-tolerated with no clinically significant abnormalities reported on physical examination, vital signs, clinical laboratory evaluations, or electrocardiographic evaluation. Phase II studies are underway in patients with focal segmental glomerulosclerosis and pulmonary arterial hypertension to investigate the efficacy and tolerability of CXA-10 75–300 mg once daily.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Nonclinical studies with 10-nitro-9(E)-octadec-9-enoic acid (CXA-10), a nitro fatty acid, demonstrated potential therapeutic effects in disease states in which oxidative stress, inflammation, fibrosis, and/or direct tissue toxicity play significant roles.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ Phase I clinical studies were conducted in healthy and obese subjects as a surrogate for a population with inflammation to confirm the pharmacological effects of CXA-10 observed in nonclinical studies. In addition, these studies determined the pharmacokinetics, pharmacodynamics, safety, and tolerability of oral CXA-10 after single

and multiple dose administrations in the fed and fasted state to identify the optimal dose for phase II studies.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ The results from phase I studies demonstrate pharmacological actions of CXA-10 that underlie the improvements in renal and vascular diseases characterized in multiple animal models.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ Based on the results, phase II studies were initiated in patients with focal segmental glomerulosclerosis and pulmonary arterial hypertension to investigate the efficacy and tolerability of CXA-10 doses of 75, 150, or 300 mg once daily.

Pioneering work has identified nitro fatty acids (NFAs) as a novel signaling pathway and modulators of inflammation and metabolic stress across many body systems.[1] Nitro-oleic acid (OA-NO$_2$), an NFA that is an approximate 1:1 mixture of 9-nitro-oleic acid and 10-nitro-oleic acid, belongs

to a class of endogenous signaling agents that regulate a variety of cellular processes.[2] 10-nitro-9(E)-octadec-9-enoic acid (CXA-10) is a specific regioisomer of OA-NO$_2$, characterized by a nitro group on carbon 10 with effects similar to the mixed isomer. NFAs, in general, and CXA-10, in

particular, demonstrate potential as effective therapeutic agents in multiple disease indications in which metabolic and oxidative stress, inflammation, fibrosis, and/or direct tissue toxicity play significant roles.[3–9]

Results from preclinical studies demonstrate a novel mechanism of action for OA-NO[2] and CXA-10 that restores reparative pathways and modulates inflammation in body systems, including kidney, cardiovascular, cardiopulmonary, and central nervous systems.[1,6] CXA-10 upregulates the major anti-inflammatory and reparative pathway of the body, nuclear factor (erythroid-like)-like 2 (Nrf2) as well as the heat shock response (HSR),[10] whereas inhibiting the major pro-inflammatory pathway, nuclear factor κB as well as toll-like receptor 4.[2,11,12] CXA-10 also inhibits xanthine oxidoreductase, which is one of the major enzymes involved in the production of reactive oxygen species that cause much of the damage associated with oxidative stress.[11]

Increased expression of Nrf2-regulated genes and inhibition of nuclear factor κB and toll-like receptor 4 proinflammatory activity with CXA-10 has been observed *in vitro* in animals and in humans to prevent the elaboration of proinflammatory mediators, such as cytokines (for example, interleukin-6 (IL-6)) and chemokines (monocyte chemoattractant protein-1 (MCP-1)), profibrotic agents, and adhesion molecules.[2,6,9,10,12,13] CXA-10 also regulates gene expression that is important for modulation of inflammation, oxidant defense, metabolic stress, DNA and protein repair, and activity of cellular transporters.[10,14,15] CXA-10 seems to induce the activity of the Nrf2 pathway through posttranslation modification, which may affect Nrf2-related transporters and enzymes involved in drug metabolism, specifically MRP1-4, OATP2, OATP1B1, and glucuronosyltransferase (UGT). Drugs metabolized through these enzymes and transporters include statins and cholesterol absorption inhibitors.[14,15]

Recently, CXA-10 was shown to induce Nrf2-dependent and HSR-dependent gene expression in blood and kidneys after single i.v. doses in subjects with chronic kidney disease.[16] Based on its mechanism of action and pharmacological profile in nonclinical studies, CXA-10 is undergoing clinical development for focal segmental glomerulosclerosis (FSGS) and pulmonary arterial hypertension (PAH). We report results from three phase I clinical studies conducted in healthy and obese subjects to confirm the pharmacological effects of CXA-10 observed in nonclinical studies and to evaluate the pharmacokinetics (PK), pharmacodynamics (PD), safety, and tolerability of oral CXA-10 after single and multiple dose administrations in the fed and fasted states.

METHODS

These studies were conducted at Jasper Clinic, Kalamazoo, MI, in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines of the International Council for Harmonisation. Study protocols, amendments, and informed consent forms were approved by an institutional review board (IntegReview IRB, Austin, TX). Prior to any study procedures, each subject provided written informed consent to participate.

The objectives were to investigate the PK, safety, and tolerability of oral CXA-10 and its metabolites after single and multiple ascending doses in the fasted and fed states, the PD of CXA-10 on biomarkers of inflammation and metabolism in obese subjects as surrogates for the pharmacological effects of CXA-10, and to assess the pharmacological effect of CXA-10 on Nrf2-related metabolizing enzymes and transporters with concomitant administration of several cholesterol-lowering drugs.

Study designs
CXA-10-201 and CXA-10-202 were single-center, randomized, double-blind, placebo-controlled, single ascending dose (CXA-10-201) and multiple ascending dose (CXA-10-202) studies. CXA-10-202 was conducted in obese male subjects who were restricted to an inpatient clinical unit, and caloric intake was adjusted to maintain body weight during confinement. CXA-10-203 was a single-center, open-label, exploratory study in healthy male subjects to explore the effect of CXA-10 on Nrf2-related transporters by assessing the PK of pravastatin and simvastatin/ezetimibe when administered with and without CXA-10. The projected therapeutic dose for the oral administration of CXA-10 (150 mg) was selected based on data from the first-in-human study with i.v. CXA-10, formal Good Laboratory Practices toxicology, and PK assessment of oral CXA-10 in 14 rat and dog studies, as well as quantitative modeling of the animal PK data to predict estimated exposures for pharmacologically active doses and to evaluate the maximally tolerated doses in humans.

Subject selection
In CXA-10-201, healthy male and female subjects between the ages of 18 and 50 years (inclusive), with a body mass index (BMI) between 18 and 30 kg/m^2 (inclusive) were eligible. In CXA-10-202, healthy, obese male subjects 18–60 years (inclusive) with BMI between 27 and 40 kg/m^2 (inclusive) were eligible. In CXA-10-203, selection criteria were more restrictive to achieve a homogeneous population of healthy male subjects between 19 and 30 years (inclusive) with a BMI between 19 and 26 kg/m^2 (inclusive).

For all three studies, eligible subjects were in good general health based on medical history, physical examination, 12-lead electrocardiogram (ECG), vital signs (blood pressure, heart rate, respiratory rate, and body temperature), and clinical laboratory testing (chemistry, hematology, and urinalysis). Women had to be of nonchildbearing potential or have a negative pregnancy test prior to dosing. Subjects were excluded for any clinically relevant medical condition that could interfere with the conduct of the study.

Study treatments
In CXA-10-201, subjects received single ascending doses of CXA-10 oral capsule (or placebo) given after an overnight fast at doses of 150, 300, 600, 1,200, or 1,800 mg (two 900 mg doses were administered separated by 6 hours).

In CXA-10-202, subjects received multiple ascending doses of CXA-10 oral capsules (or placebo) 25, 150, or 600/450 mg administered once daily after an overnight fast for 14 days. The initial 600 mg dose was reduced to 450 mg on day 2 due to gastrointestinal intolerance. On day 15, subjects in the 450 mg cohort could receive an additional 450 mg dose given within 30 minutes of a high-fat (50%) meal.

In CXA-10-203, subjects received CXA-10 oral capsules 150 mg daily on days 4–12. Pravastatin and ezetimibe/simvastatin were administered because they are substrate drugs for Nrf2-related transporters and enzymes, and these cholesterol-lowering agents are used by the target patient populations for CXA-10 clinical studies. A single dose of pravastatin 40 mg was administered on days 1 and 11, and a single dose of 20 mg simvastatin/10 mg ezetimibe (Vytorin) was administered on days 2 and 12. All doses were administered with a standard fat (30%) meal after an overnight fast.

Study assessments

Safety assessments included physical examinations; adverse events (AEs); vital signs (blood pressure, heart rate, and respiratory rate); clinical laboratory tests (hematology, biochemistry, and urinalysis); and 12-lead ECGs (CXA-10-201; CXA-10-202; and CXA-10-203). In CXA-10-202, continuous 24-hour Holter monitoring was performed on day -1 and on day 14 in all 3 cohorts. ECGs were extracted from the Holter monitor at specified time points on day -1 and day 14, which coincided with PK sampling times: 0 (predose), 1, 2, 3, 4, 6, 8, 10, 12, and 24 hours postdose.

Concentrations of CXA-10 and the inactive, major metabolites, 8,9 alkene and 10-nitrostearate, in human plasma were measured by MicroConstants (San Diego, CA) using a validated high-performance liquid chromatography/tandem mass spectrometry assay. In CXA-10-201, initial samples from the first four dose groups (150 mg through 1,200 mg) were assayed using the original and less sensitive assay. Subsequently, an updated method was used that measured concentrations of CXA-10 ranging from 0.0500–20.0 ng/mL, 8,9 alkene ranging from 0.400–200.0 ng/mL, and 10-nitrostearate ranging from 0.100–50.0 ng/mL using 400 μL of preserved human plasma for extraction. For PK assessments, maximum observed plasma drug concentration (C_{max}), terminal phase half-life ($t_{1/2}$), area under the plasma drug concentration vs. time curve (AUC), clearance, volume of distribution, and terminal elimination rate constant were determined, as data permitted, for CXA-10 and metabolites.

Assays for pravastatin, 3-alpha-hydroxy pravastatin, simvastatin, simvastatin hydroxy acid, and ezetimibe were performed by inVentiv Health (Québec, QC, Canada) using validated high-performance liquid chromatography/tandem mass spectrometry methodology.[17–19] The lower limit of quantitation for pravastatin and 3-alpha-hydroxy pravastatin was 0.25 ng/mL, for simvastatin and simvastatin hydroxy acid was 100 pg/mL, and for ezetimibe was 0.20 ng/mL.

In CXA-10-202, serum biomarkers related to the pharmacological action of CXA-10 were evaluated, including serum leptin (Leptin Human ELISA Kit, Life Technologies, Carlsbad, CA), triglycerides, total cholesterol, MCP-1 (Human CCL2/MCP-1 Quantikine SixPak ELISA Kits, R&D Systems), and IL-6 (SMC Human Interleukin 6 Immunoassay Kit, Millipore, Burlington, MA). Samples were collected at multiple time points for MCP-1 and IL-6 because of the known diurnal variability in blood levels,[20] and results were averaged across timepoints.

In CXA-10-203, 24-hour urine total creatinine excretion was examined prior to and following administration of CXA-10 to determine the effects on creatinine transporters or creatinine production. On days 2 and 12, 24-hour urine samples were collected, the urine volume was quantitated based on the net weight of urine, and a measured specific gravity was obtained on an aliquot of the full urine collection.

Study analysis

The PK population included all subjects who received at least 1 dose of CXA-10 and had a PK sample. Plasma concentration time data for CXA-10 were evaluated using standard noncompartmental analysis. Results were summarized using descriptive statistics (mean, median, and coefficient of variation). In CXA-10-203, the ratios of ln-transformed C_{max}, area under concentration-time profiles (AUC_{0-t}), and area under the concentration-time curve from zero to infinity (AUC_{0-inf}) and their associated 90% confidence intervals (CIs) were determined. Paired *t*-tests were performed to evaluate if the mean of the ln-transformed PK parameters (C_{max}, AUC_{0-t}, and AUC_{0-inf}) were different when pravastatin or simvastatin/ezetimibe were administered without CXA-10 vs. when these drugs were coadministered with CXA-10. PK data were analyzed with Phoenix WinNonlin version 6.3 (Pharsight Corporation, Mountain View, CA), study 201; Phoenix (Phoenix Professional version 3.2, PharSight Corp.), study 202; and Phoenix Professional version 6.4 (PharSight Corp.), study 203.

For biomarkers (CXA-10-202), change from baseline or, where appropriate, percent change from baseline, was summarized with a repeated measures mixed effects model. The least squares (LS) mean difference and 95% CIs between each active treatment and placebo for each assessment time and across all assessment times were obtained from the model.

RESULTS

Baseline characteristics and disposition

In CXA-10-201, 40 subjects were enrolled and were evaluated for safety. Two subjects discontinued the study after the dosing evaluation was completed. One was lost to follow-up and one failed to complete the final study visit. Twenty-eight subjects who received CXA-10 were included in the PK analysis. Subjects were predominantly men, non-Hispanic and white ranging in age from 19–48 years, with a median BMI of 26.8 kg/m^2 in CXA-10 cohorts and 28.4 kg/m^2 in the placebo cohort. Baseline characteristics were similar across the CXA-10 and placebo cohorts.

In CXA-10-202, 43 subjects were enrolled, and 42 completed the study and were included in safety and PD populations; 1 subject in the placebo group discontinued for family reasons and was replaced. Thirty subjects were included in the PK analysis. Ages ranged from 19–57 years, all subjects were men, 20 were white, 23 were black or African American, and median BMI ranged from 27.9–30.7 kg/m^2 for active and placebo groups.

In CXA-10-203, 10 subjects were enrolled and completed the study for PK and safety evaluations. All subjects were men, the majority (80%) were white, mean age was 25.8 years, and median BMI was 23.6 kg/m^2.

Pharmacokinetics

In CXA-10-201, low plasma CXA-10 concentrations were observed after single oral CXA-10 doses of 150–1,800 mg. Bioanalytical assay sensitivity was updated during this study, and the calibration range of the assay was reduced from 0.500–1,000 ng/mL to 0.0500–20.0 ng/mL. Samples from the first four dose groups (150–1,200 mg) were assayed using the original but less-sensitive assay, and samples for the 1,800 mg dose group were assayed using the more sensitive assay. Therefore, interpretation of data from the first four dose groups is limited, and comparisons across the full dose range should be interpreted with caution.

Across all CXA-10 dose levels, time of maximum plasma concentration (T_{max}) ranged from 0.5–12 hours (**Table 1**). The wide range for T_{max} values was likely an artifact of multiple peak plasma concentrations (**Figure 1a**). Mean CXA-10 $AUC_{0–last}$ increased in a dose-proportional manner across the 150–1,200 mg dose range, but the increase in C_{max} was less than dose proportional. C_{max} and AUC were highest at the 1,800 mg dose but could not be compared with the lower doses due to assay differences. The $t_{1/2}$ could not be calculated for the lower dose groups but the mean terminal elimination half-life of CXA-10 was 18 hours for the 1,800 mg dose.

For the 8,9 alkene and 10-nitrostearate metabolite concentrations, which were only analyzed at the CXA-10 doses of 1,200 and 1,800 mg, C_{max} and $AUC_{0–t}$ exhibited a greater than dose-proportional increase (data not shown). For the 8,9 alkene metabolite, geometric mean (%CV) C_{max}, and $AUC_{0–last}$ at the 1,800 mg dose were 179 (58.3) ng/mL and 19,500 (47.3) hour ng/mL, respectively. For the 10-nitrostearate metabolite, geometric mean (%CV), C_{max}, and $AUC_{0–last}$ at 1,800 mg were 205 (73.7) ng/mL and 2,870 (64.5) hour ng/mL, respectively.

In CXA-10-202, quantifiable CXA-10 concentrations were limited at the 25 mg dose, but at 150 and 450 mg doses resulted in quantifiable plasma concentrations for 12–24 hours postdose. After reaching C_{max}, plasma CXA-10 concentrations decreased in a monoexponential manner (**Figure 1b**). Mean C_{max} was less than dose proportional at doses of 25, 150, and 450 mg and showed minimal or no accumulation with repeat dosing (**Table 2**). Mean 0–24-hour area under the concentration-time curve ($AUC_{0–24}$) was approximately dose proportional and showed minimal or no accumulation with repeat dosing. Administration of a single 450 mg dose of CXA-10 with a high-fat meal increased C_{max} by 120% and $AUC_{0–24}$ by 60% and prolonged T_{max} by 3 hours compared with administration of multiple doses of CXA-10 under fasted conditions.

For 8,9 alkene and 10-nitrostearate metabolites, where quantifiable, concentration-time profiles exhibited multiple peaks. After reaching C_{max}, plasma concentration decreased in a monoexponential manner (data not shown). Mean C_{max} was greater than dose proportional at 150 and 450 mg doses for both metabolites with accumulation on repeat dosing for 8,9 alkene and no accumulation for 10-nitrostearate. Mean $AUC_{0–24}$ was greater than dose proportional across 150 and 450 mg dose groups for both metabolites with accumulation on repeat dosing for 8,9 alkene and no accumulation for 10-nitrostearate.

In CXA-10-203, after coadministration with CXA-10, geometric mean C_{max} and $AUC_{0–t}$ decreased 20% and 25% for pravastatin, increased 10% and 25% for simvastatin, and decreased 20% and 5% for ezetimibe (**Table S1**). Evidence for a clinically relevant interaction among CXA-10 and pravastatin, simvastatin, and ezetimibe was not apparent; the point estimates for PK parameters were generally within the range of 75–100% (**Table 3**). However, simvastatin hydroxy acid showed a greater than twofold increase (point estimates were 237% and 223% for C_{max} and $AUC_{0–t}$, respectively). The effect on simvastatin hydroxy acid was considered of possible clinical relevance. Therefore, the dose of

Table 1 Geometric mean (%CV) for PK parameters following a single oral dose of CXA-10 (study CXA-10-201)

Parameter	CXA-10 oral dose				
	150 mg $n = 5$	300 mg $n = 5$	600 mg $n = 6$	1,200 mg $n = 6$	1,800 mg (900 mg at 0 & 6 hour) $n = 4$[a]
C_{max}, ng/mL	3.4 (48.9)	5.2 (49.6)	5.8 (40.4)	8.3 (24.0)	9.7[b] (31.0)
$AUC_{0–last}$, hour × ng/mL	5.65[c] (102)	13.4 (98.2)	25.8 (60.0)	55.3 (37.0)	131 (24.3)
$AUC_{0–inf}$, hour × ng/mL	NC	NC	NC	69.6[c] (10.1)	143 (27.5)
AUC% extrap	NC	NC	NC	9.31[c] (193)	7.00 (80.3)
T_{max}, hour[d]	1.5 (1.5–3.0)	3.0 (1.5–10.0)	6.0 (0.5–10.0)	3.0 (1.0–12.0)	5.0[b] (4.0–6.0)
$t_{1/2}$ hour	NC	NC	NC	4.55[e] (33.0)	18.0 (114)
CL/F, L/hour	NC	NC	NC	17,246[c] (10.1)	12,612 (27.5)
V_z/F, L	NC	NC	NC	107,696[c] (47.4)	327,488 (72.1)

%CV, percent coefficient of variation (geometric mean); $AUC_{0–inf}$, area under the concentration-time curve from zero to infinity; $AUC_{0–last}$, area under the concentration-time curve from time of administration up to the time of the last quantifiable concentration; CL/F, total apparent clearance; C_{max}, peak plasma concentration; CXA-10, 10-nitro-9(E)-octadec-9-enoic acid; NC, not calculated (data are missing for more than half of the subjects due to lack of quantifiable concentrations); PK, pharmacokinetic; $t_{1/2}$, terminal half-life; T_{max}, time of maximum plasma concentration; V_z/F, volume of distribution based on the terminal phase.
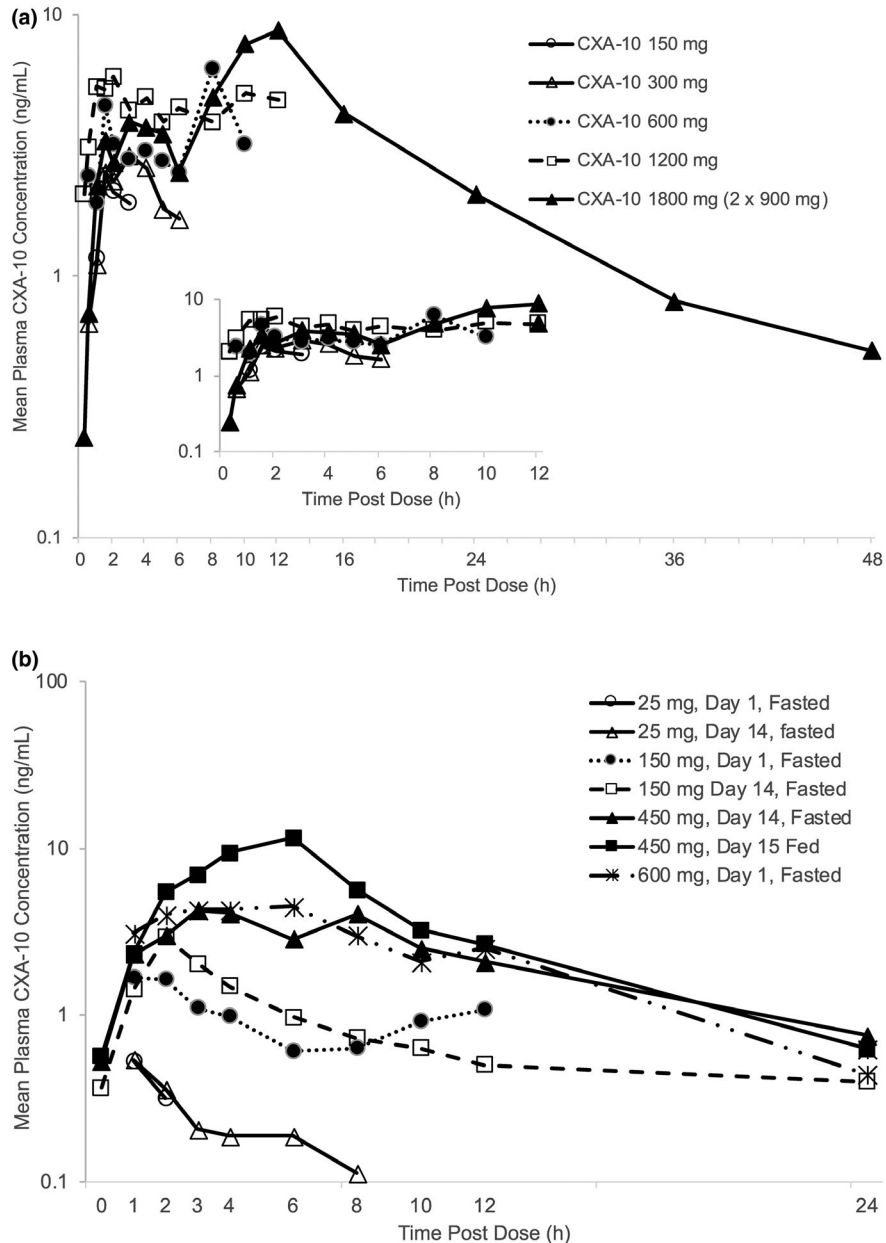[a]Two subjects did not receive the full 1,800 mg dose, and are not included.
[b]C_{max} and T_{max} reported for the 1,800 mg dose level is following the second 900 mg dose given at 6 hours.
[c]$n = 3$.
[d]Median (range).
[e]$n = 4$.

Figure 1 Mean plasma CXA-10 plasma concentrations (study CXA-10-201) following (**a**) single oral doses of CXA-10 and (study CXA-10-202) on days 1 and 14 after (**b**) multiple oral doses. (i) Only time points with quantifiable concentrations in ≥ 50% of subjects per dose level are shown. (ii) $n = 6$ for the 150 mg, 300 mg, 600 mg, and 1,200 mg dose groups; $n = 4$ for 1,800 mg dose group. (iii) Individual lower limit of quantitation values were calculated based on a dilution factor and the subject's day -1 hematocrit, ranging from 1.61–1.80 ng/mL for the first four dose groups (150–1,200 mg) and from 0.163–0.177 ng/mL for the 1,800 mg dose group. (iv) Below the quantifiable limit values reported prior to peak plasma concentration (C_{max}) were changed to 0.0 for the purposes of calculating summary statistics at each time point. CXA-10, 10-nitro-9(E)-octadec-9-enoic acid.

simvastatin will be limited to 20 mg daily in future protocols until a definitive drug–drug interaction is conducted.

Pharmacodynamics
In CXA-10-202, a consistent decrease from baseline was observed with CXA-10 150 mg dose, but not 25 or 450 mg doses, for biomarkers of altered inflammation and metabolic dysfunction, including leptin, triglycerides, cholesterol, MCP-1, and IL-6 (**Figure 2**). The change from baseline

LS mean difference from placebo was significant ($P < 0.05$) for MCP-1 on day 14 (**Table 4** and **Figure 2**). In the 150 mg group, MCP-1 change from baseline LS mean difference from placebo was consistently decreased on days 7 and 14 (**Table 4** and **Figure 2**). For triglycerides, the change from baseline LS mean difference for CXA-10 vs. placebo was significant ($P < 0.05$) for the 150 mg dose on day 8 (–45.4 mg/dL (95% CI: –87.4 to –3.5 mg/dL)) and on day 15 (–59.6 mg/dL (95% CI: –102.3 to –16.9 mg/dL)). Decreases

Table 2 PK parameters after multiple oral doses of CXA-10 (study CXA-10-202)

| | C_{max}, ng/mL (%CV)[b] | | T_{max}, hour [a] | | AUC_{0-24}, hour×ng/mL (%CV)[b] | | AUC_{0-t}, hour×ng/mL (%CV)[b] | |
	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14	Day 1	Day 14
25 mg	0.5 ± 0.3 (57.5)	0.6 ± 0.2 (29.9)	1 1–4	2 1–4	1.9 ± 0.8 (42.7)	2.1 ± 0.8 (38.2)	ND	ND
150 mg	2.5 ± 1.2 (49.5)	3.3 ± 1.3 (40.7)	2 1–10	2 1–4	10.6 ± 5.2 (48.6)	19.0 ± 5.7 (30.2)	10.6 ± 5.2 (48.6)	95.9 ± 63.8 (66.5)
600/450 mg fasting	7.1 ± 3.2 (45.0)	5.9 ± 1.7 (28.8)	6 1–12	3 1–12	50.7 ± 23.6 (46.5)	51.6 ± 8.5 (16.4)	50.7 ± 23.6 (46.5)	55.7 ± 13.8 (24.7)
450 mg fed (day 15)	ND	13.1 ± 6.4 (48.5)	ND	6 3–6	ND	87.0 ± 19.9 (22.9)	ND	211.0 ± 51.0 (24.2)

%CV, percent coefficient of variation (geometric mean); AUC_{0-24}, area under the concentration-time curve from zero to 24 hours; AUC_{0-t}, area under concentration-time profiles; C_{max}, peak plasma concentration; CXA-10, 10-nitro-9(E)-octadec-9-enoic acid; ND, not determined; PK, pharmacokinetic; T_{max}, time of maximum plasma concentration.
[a]T_{max} is summarized using median and range.
[b]Mean ± SD.

Table 3 Statistical comparison of CXA-10 with pravastatin or ezetimibe/simvastatin; to pravastatin or ezetimibe/simvastatin alone (study CXA-10-203)

Analyte	Parameter	Number	Point estimate (%)	95% CI	*P* value
Pravastatin	C_{max}	10	74.2	(47.4–116)	0.1654
	AUC_{0-t}	10	74.2	(47.4–116)	0.1654
	AUC_{0-inf}	9	67.3	(43.8–103)	0.0659
3-alpha-hydroxy pravastatin	C_{max}	10	80.3	(41.2–156)	0.4757
	AUC_{0-t}	10	76.6	(43.2–136)	0.3208
	AUC_{0-inf}	5	215	(33.3–1,390)	0.3178
Ezetimibe	C_{max}	9	76.5	(47.7–123)	0.2267
	AUC_{0-t}	9	89.9	(75.6–107)	0.1948
	AUC_{0-inf}	9	96.2	(69.5–133)	0.7925
Simvastatin	C_{max}	9	108	(45.9–254)	0.8435
	AUC_{0-t}	9	121	(65.8–222)	0.4944
	AUC_{0-inf}	9	118	(65.6–214)	0.5291
Simvastatin hydroxy acid	C_{max}	9	237	(118–478)	0.0218
	AUC_{0-t}	9	223	(117–425)	0.0213

AUC_{0-inf}, area under the concentration-time curve from zero to infinity; AUC_{0-t}, area under concentration-time profiles; CI, confidence interval; C_{max}, peak plasma concentration; CXA-10, 10-nitro-9(E)-octadec-9-enoic acid.
Point estimate calculated with Phoenix WinNonlin software uses geometric mean ratio.
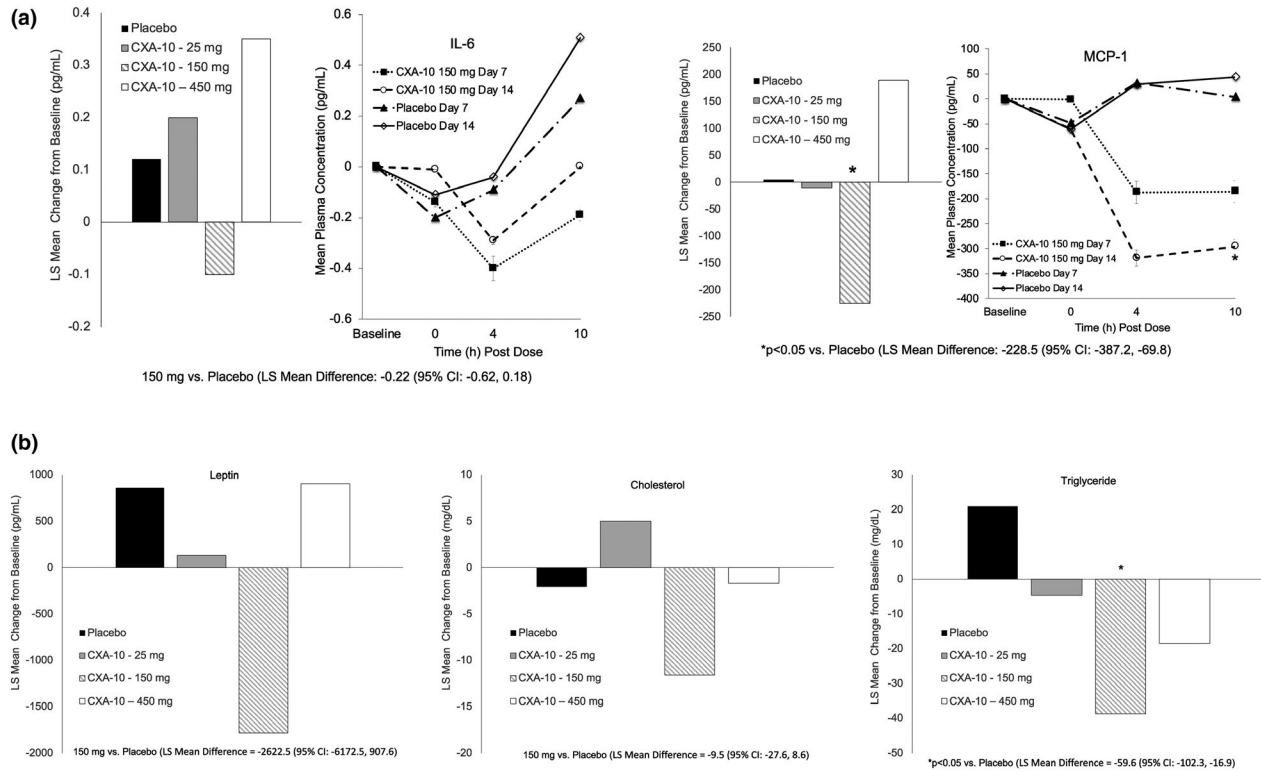
from baseline were also observed in the 150 mg dose group for leptin, total cholesterol, and IL-6 (**Table 4** and **Figure 2**). For leptin on day 14, mean change from baseline for LS mean difference was –2,640 pg/mL for CXA-10 150 mg vs. placebo. For IL-6 in the 150 mg dose group, mean change from baseline was greater vs. placebo at days 7 and 14 (**Figure 2**). In the 150 mg group, mean change from baseline was consistently greater vs. placebo at day 14 (**Table 4**). The day 15 change from baseline LS mean difference demonstrated no significant effect of CXA-10 on total cholesterol.

Safety and tolerability
In CXA-10-201, a dose-related increase in the incidence of AEs occurred with single-dose administration of CXA-10 and 25 subjects (62.5%) experienced 48 AEs, mostly treatment related. The most frequently reported AEs (>10% of

subjects who received CXA-10) were diarrhea, abdominal pain, and nausea (**Table 5**); these common AEs were only reported by subjects receiving ≥600 mg CXA-10. All diarrhea events were grade 1 (*n* = 2, increase of <4 stools/day over baseline) or grade 2 (*n* = 11, increase of 4–6 stools/day over baseline) in severity and began as loose stools that became watery over time. Diarrhea occurred within 0.5–3 hours after dosing and resolved within 4–12 hours. No serious or severe AEs or discontinuation for AEs were reported. No clinically significant changes in clinical laboratory values or ECG findings were reported.

In CXA-10-202, CXA-10 daily for 14 days was safe and well tolerated with no clinically significant abnormalities reported on physical examination, vital signs, clinical laboratory evaluations, or routine ECG evaluation. The 24-hour Holter monitoring demonstrated that CXA-10 had no clinically meaningful effect on the correct QT interval at

(a)

150 mg vs. Placebo (LS Mean Difference: -0.22 (95% CI: -0.62, 0.18)

*p<0.05 vs. Placebo (LS Mean Difference: -228.5 (95% CI: -387.2, -69.8)

(b)

150 mg vs. Placebo (LS Mean Difference = -2622.5 (95% CI: -6172.5, 907.6)

150 mg vs. Placebo (LS Mean Difference = -9.5 (95% CI: -27.6, 8.6)

*p<0.05 vs. Placebo (LS Mean Difference = -59.6 (95% CI: -102.3, -16.9)

Figure 2 LS mean change from baseline at day 14 and mean plasma concentrations for (**a**) IL-6 and MCP-1, and LS mean change from baseline for (**b**) leptin (day 14), triglycerides (day 15), and cholesterol (day 15) after repeated administration CXA-10 in obese subjects (study CXA-10-202). The analysis is based on a repeated measures analysis of variance model. CI, confidence interval; CXA-10, 10-nitro-9(E)-octadec-9-enoic acid; IL, interleukin; LS, least squares; MCP-1, monocyte chemoattractant protein-1.

plasma concentrations up to ~8 ng/mL. At least one AE was reported by 26 of 43 subjects overall (60.5%), and over half were treatment related. Most common were diarrhea, nausea, back pain, and abdominal pain, and the incidence was highest in the 450 mg group (**Table 5**). Presyncope ($n = 3$) occurred with the 450 mg dose but was considered vasovagal and occurred in subjects who were also experiencing gastrointestinal AEs, such as nausea, vomiting,

Table 4 Mean baseline and change from baseline LS mean difference from placebo for key biomarkers with administration of oral CXA-10 to obese subjects (study CXA-10-202)

CXA-10 dose	Leptin (pg/mL) day 14	Triglyceride (mg/dL) day 15	Cholesterol (mg/dL) day 15	MCP-1 (pg/mL) day 7	MCP-1 (pg/mL) day 14	IL-6 (pg/mL) day 7	IL-6 (pg/mL) day 14
25 mg							
Mean baseline ± SD	5,858 ± 2,721	113.2 ± 61.3	184.2 ± 25.3	447.9 ± 183.4	447.9 ± 183.4	0.40 ± 0.15	0.40 ± 0.15
LS mean difference (95% CI)	−723.7 (−4,253.7, 2,806.3)	−25.5 (−68.2, 17.2)	7.1 (−11.0, 25.2)	−23.0 (−181.7, 135.74)	−14.2 (−172.9, 144.5)	−0.01 (−0.42, 0.39)	0.08 (−0.32, 0.48)
150 mg							
Mean baseline ± SD	6,764 ± 5,206	160.8 ± 145.5	187.6 ± 45.2	567.5 ± 646.0	567.5 ± 646.0	0.65 ± 0.40	0.65 ± 0.40
LS mean difference (95% CI)	−2,640.0 (−6,170.0, 890.0)	−59.6 (−102.3, −16.9)	−9.5 (−27.6, 8.6)	−120.9 (−279.1, 37.3)	−228.5 (−387.2, −69.8)	−0.24 −0.64, 0.16)	−0.22 (−0.62, 0.18)
450 mg							
Mean baseline ± SD	6,479 ± 4,604	116.3 ± 32.7	182.2 ± 23.3	423.1 ± 153.0	423.1 ± 153.0	0.88 ± 0.46	0.88 ± 0.46
LS mean difference (95% CI)	45.5 (−3,484.5, 3,575.5)	−39.5 (−82.2, 3.2)	0.38 (−17.7, 18.5)	77.6 (−80.6, 235.8)	185.6 (27.4, 343.7)	−0.06 (−0.46, 0.34)	0.23 (−0.17, 0.63)

CI, confidence interval; CXA-10, 10-nitro-9(E)-octadec-9-enoic acid; IL, interleukin; LS, least squares; MCP-1, monocyte chemoattractant protein-1; SD, standard deviation.

Table 5 Incidence of AEs occurring in >1 subject with CXA-10

Study CXA-10-201

	CXA-10 dose group					
Preferred term	150 mg, *n* = 6	300 mg, *n* = 6	600 mg, *n* = 6	1,200 mg, *n* = 6	1,800 mg, *n* = 6	Placebo, *n* = 10
Number (%) with AE	3 (50.0)	3 (50.0)	5 (83.3)	6 (100.0)	6 (100.0)	3 (30.0)
Abdominal pain	0	0	1 (16.7)	2 (33.3)	2 (33.3)	0
Diarrhea	0	0	3 (50.0)	5 (83.3)	5 (83.3)	0
Dysgeusia	0	0	0	0	3 (50.0)	0
Headache	0	1 (16.7)	1 (16.7)	0	1 (16.7)	1 (10.0)
Nasopharyngitis	0	1 (16.7)	1 (16.7)	0	0	0
Nausea	0	0	2 (33.3)	0	3 (50.0)	0
Vomiting	0	0	0	0	2 (33.3)	0

Study CXA-10-202

	CXA-10 dose group			
	25 mg, *n* = 10	150 mg, *n* = 10	450 mg, *n* = 10	Placebo, *n* = 13
Number (%) with AE	5 (50.0)	5 (50.0)	10 (100.0)	6 (46.2)
Abdominal pain	1 (10.0)	1 (10.0)	2 (20.0)	0
Abdominal pain upper	1 (10.0)	0	1 (10.0)	0
Back pain	2 (20.0)	0	2 (20.0)	0
Constipation	1 (10.0)	1 (10.0)	0	0
Diarrhea	0	5 (50.0)	9 (90.0)	0
Fatigue	0	0	3 (30.0)	0
Headache	0	1 (10.0%)	1 (10.0)	1 (7.7)
Nausea	1 (10.0)	0	5 (50.0)	1 (7.7)
Presyncope	0	0	3 (30.0)	1 (7.7)

AE, adverse event; CXA-10, 10-nitro-9(E)-octadec-9-enoic acid.

and diarrhea. All events resolved without sequelae. In the 600/450 mg group, 9 of 10 subjects (90%) experienced diarrhea intermittently during the 14 days of dosing in the fasted state. When subjects received a single 450 mg dose of CXA-10 in the fed state on day 15, 3 of 9 subjects (33%) experienced diarrhea. All diarrhea events were grade 1 or grade 2, and the severity did not worsen with increasing CXA-10 dose. No action was taken to treat the diarrhea, and all events resolved spontaneously without sequelae. No serious or severe AEs or discontinuation for AEs occurred.

In CXA-10-203, three subjects (30%) experienced nine AEs, but abdominal discomfort in two subjects (20%) was the only AE to occur in more than one subject. None of the AEs were considered related to CXA-10, and none were severe. All AEs resolved, and no serious AEs or discontinuations for AEs occurred. No effects were observed on ECG or clinical laboratory findings, and no changes from baseline were observed for 24-hour total urine creatinine excretion, serum creatinine, and creatinine clearance.

DISCUSSION

These studies were designed to confirm the pharmacological effects of oral CXA-10 to upregulate prometabolic and reparative pathways and inhibit proinflammatory pathways that are central to the pathophysiologic processes underlying both FSGS and PAH demonstrated in

nonclinical studies, to identify the potential dose range of CXA-10 for phase II studies of patients with FSGS and PAH, and to characterize PK, safety, and tolerability. An earlier study demonstrated that a single i.v. dose of CXA-10 activated Nrf2 and HSR-related genes in peripheral blood mononuclear cells and in the kidneys, as determined by urinary exosomes, in patients with chronic kidney disease.[16] The combined nonclinical and phase I results provide evidence that CXA-10 exerts activity on multiple mechanisms to reduce inflammation and modulate metabolic dysfunction as a novel approach for treating both FSGS and PAH.

Rodent absorption, distribution, metabolism, and excretion studies with radiolabeled CXA-10 suggest oral bioavailability up to 35%. However, plasma concentrations of CXA-10 in both animals and humans are in the low ng/mL range due to the bioanalytical assay measuring only free and albumin bound drug. It has been demonstrated that CXA-10 also circulates in blood to a large extent esterified to triglycerides associated with lipoproteins.[21]

In the single ascending dose study (CXA-10-201), oral CXA-10 demonstrated a dose proportional increase in exposure (AUC) across a dose range from 150–1,200 mg. The lack of dose proportionality at the 1,800 mg dose is due to different dosing regimens (once daily vs. twice daily), and the lower limit of quantitation was 10-fold lower for plasma samples from this dose group. At single doses of ≥600 mg,

grades 1 to 2 diarrhea occurred that was of limited duration, but otherwise CXA-10 was safe and well tolerated. This study identified CXA-10 doses ≤600 mg daily for further investigation.

In the multiple ascending dose study (CXA-10-202), healthy obese subjects were chosen for this study as a surrogate for examining the effects of a drug on inflammatory and metabolic pathways. After 14 days of dosing at 25 mg, 150 mg, and 600/450 mg daily, CXA-10 exhibited dose proportional (AUC) exposure with minimal accumulation, although metabolite exposures were greater than dose proportional at 150 and 450 mg doses. Although CXA-10 was safe and well tolerated at 25 and 150 mg, a higher incidence of gastrointestinal AEs occurred at 450 mg in the fasted state, which ameliorated with food. A slight increase in exposure (AUC) occurred with CXA-10 when administered with a high-fat meal.

CXA-10 produced clinically relevant effects at 150 mg daily on serum biomarkers, which were previously validated *in vitro* and in animal models as surrogate markers for normalized or improved inflammatory (MCP-1, IL-6, and leptin) and metabolic (leptin, triglycerides, and cholesterol) effects. These biomarkers have been reported to be elevated in FSGS (MCP-1, IL-6, triglycerides, and cholesterol) and in PAH (MCP-1, IL-6, and leptin). The primary observation of this study was the directionally correct change with CXA-10 150 mg on each of the 5 biomarkers with significant effects observed for MCP-1 and triglycerides. The ~20% reduction in leptin concentrations, although not statistically significant, was clinically meaningful. The decrease observed with CXA-10 was comparable to the decrease observed with a 30-lb weight loss.[22] Because there was no significant effect of CXA-10 150 mg on weight/BMI or insulin, the decrease observed in leptin concentrations in the 150 mg dose group was likely due to the mechanism of action of CXA-10. Changes in MCP-1 and triglyceride levels were statistically significant and clinically relevant and consistent with the magnitude of changes associated with a reduction in inflammation.[23] The lack of effect with the 25 and 450 mg doses may have been due to hormesis (i.e., an inverted U-shaped dose response curve). In animal models, CXA-10 has been shown to demonstrate a hormetic response, which is commonly observed with signaling agents.[24,25] This study identified CXA-10 doses <450 mg daily for further investigation.

The effect of CXA-10 on the PK of substrate drugs whose metabolism could be altered by CXA-10's effects on Nrf2 was explored in study CXA-10-203. Pravastatin served as an MRP2 and OATP2 substrate, simvastatin served as an OATP1B1 substrate, and ezetimibe served as a UGT substrate, although other enzymes or transporters have been recognized to be involved in the metabolism of pravastatin and simvastatin. As this was an exploratory study investigating the effect of CXA-10 on Nrf2 activation using PK end points, definitive conclusions could not be made about drug-drug interaction potential of CXA-10. However, the change in exposure (C_{max} and AUC), as indicated by the point estimates of cholesterol-lowering drugs following co-administration with CXA-10, suggests an effect of Nrf2 activation on these transporters at a clinically relevant dose of 150 mg daily. A potentially clinically relevant interaction was observed based on simvastatin-beta-hydroxyl acid, the active metabolite of simvastatin, which had an approximate twofold increase in exposure after concomitant administration with CXA-10. Importantly, the maximum effect of CXA-10 on Nrf2 activation may not have been achieved with the 7-day dosing regimen; 14 days of dosing may be required to achieve full induction.[26,27] Nevertheless, the results demonstrated that a pharmacological effect of a 150 mg dose of CXA-10 in humans consistent with the pharmacological actions observed in CXA-10-202. In this study, oral CXA-10 150 mg daily at steady-state concentrations was safe and well-tolerated when administered with single doses of ezetimibe/simvastatin or pravastatin, and no effects were observed on serum creatinine concentrations or 24-hour urinary creatinine excretion confirming lack of effect on creatinine transporters at clinically relevant dose.

Among the limitations of these studies are the short-term treatments of up to 15 days. Although the PD and pharmacological effects of CXA-10 were demonstrated as early as 7 days in these short-term studies, longer-term studies are needed to determine the full extent and durability of response to CXA-10. However, the results with the 150 mg dose were consistent with findings from preclinical studies of CXA-10. The results were also limited in that the studies were not specifically powered to demonstrate a statistically significant effect. Nevertheless, clinically relevant and statistically significant effects of CXA-10 were observed on biomarkers of inflammation and metabolic stress. Of interest, the results from the study in obese subjects indicated that the optimal effect of CXA-10 occurred at the 150 mg daily (the predicted therapeutic dose in humans) rather than at 450 mg daily. It is possible that CXA-10, like many signaling agents, exhibits hormesis (inverted U-shaped dose response curve), which has been observed with CXA-10 in animal models.[13] Inverted U-shaped dose response curves are common with drugs that regulate signaling pathways and may protect against overexposure by simply "turning off" functions at high concentrations.[28,29]

Results from these three phase I studies in healthy and obese subjects confirmed the pharmacological actions and will help to identify an appropriate dose range of CXA-10. Phase II clinical studies were initiated in patients with FSGS and PAH to investigate the efficacy and tolerability of CXA-10 at doses of 75, 150, or 300 mg once daily.

676

1. Freeman, B.A., O'Donnell, V.B. & Schopfer, F.J. The discovery of nitro-fatty acids as products of metabolic and inflammatory reactions and mediators of adaptive cell signaling. *Nitric Oxide* **77**, 106–111 (2018).
2. Cui, T. *et al.* Nitrated fatty acids: endogenous anti-inflammatory signaling mediators. *J. Biol. Chem.* **281**, 35686–35698 (2006).
3. Beckel, J.M. & de Groat, W.C. The effect of the electrophilic fatty acid nitro-oleic acid on TRP channel function in sensory neurons. *Nitric Oxide.* https://doi.org/10.1016/j.niox.2018.03.015.
4. Deen, A.J., Sihvola, V., Härkönen, J., Patinen, T., Adinolfi, S. & Levonen, A.L. Regulation of stress signaling pathways by nitro-fatty acids. *Nitric Oxide.* https://doi.org/10.1016/j.niox.2018.03.012.
5. Jobbagy, S. & Tan, R.J. Nitrolipids in kidney physiology and disease. *Nitric Oxide.* https://doi.org/10.1016/j.niox.2018.03.021.
6. Kelley, E.E. *et al.* Fatty acid nitroalkenes ameliorate glucose intolerance and pulmonary hypertension in high-fat diet-induced obesity. *Cardiovasc. Res.* **101**, 352–363 (2014).
7. Mollenhauer, M., Mehrkens, D. & Rudolph, V. Nitrated fatty acids in cardiovascular diseases. *Nitric Oxide.* https://doi.org/10.1016/j.niox.2018.03.016.
8. Rom, O., Khoo, N.K.H., Chen, Y.E. & Villacorta, L. Inflammatory signaling and metabolic regulation by nitro-fatty acids. *Nitric Oxide.* https://doi.org/10.1016/j.niox.2018.03.017.
9. Woodcock, C.C. *et al.* Nitro-fatty acid inhibition of triple negative breast cancer cell viability, migration, invasion and tumor growth. *J. Biol. Chem.* **293**, 1120–1137 (2018).
10. Kansanen, E. *et al.* Nrf2-dependent and –independent responses to nitro-fatty acids in human endothelial cells: identification of heat shock response as the major pathway activated by nitro-oleic acid. *J. Biol. Chem.* **284**, 33233–33241 (2009).
11. Kelley, E.E. *et al.* Nitro-oleic acid, a novel and irreversible inhibitor of xanthine oxidoreductase. *J. Biol. Chem.* **283**, 36176–36184 (2008).
12. Villacorta, L. *et al.* Electrophilic nitro-fatty acids inhibit vascular inflammation by disrupting LPS-dependent TLR4 signaling in lipid rafts. *Cardiovasc. Res.* **98**, 116–124 (2013).
13. Arbeeny, C. *et al.* CXA-10, a nitrated fatty acid, is renoprotective in deoxycorticosterone acetate-salt nephropathy. *J. Pharmacol. Exp. Ther.* **369**, 503–510 (2019).
14. Kalliokoski, A. & Niemi, M. Impact of OATP transporters on pharmacokinetics. *Br. J. Pharmacol.* **158**, 693–705 (2009).
15. König, J., Müller, F. & Fromm, M.F. Transporters and drug-drug interactions: important determinants of drug disposition and effects. *Pharmacol. Rev.* **65**, 944–966 (2013).
16. Garner, R.M., Levonen, A.-L., Chieffo, C., Debouck, C., Schopfer, F. & Jorkasky, D.K. Use of urinary exosomes to confirm pharmacological activity of CXA-10 on Nrf2 and heat shock response gene expression in the kidney of patients with chronic kidney disease. San Diego, CA (2018).
17. Danafar, H. High performance liquid chromatographic method for determination of ezetimibe in pharmaceutical formulation tablets. *Pharm. Biomed. Res.* **2**, 38–46 (2016).
18. Ashour, S., Nakshbandi, H. & Omar, S. Quantitative determination of pravastatin in pharmaceutical dosage forms by high-performance liquid chromatography with ultraviolet detection. *Int. J. Biomed. Sci.* **4**, 135–139 (2008).
19. Wang, J., Luzum, J.A., Phelps, M.A. & Kitzmiller, J.P. Liquid chromatography-tandem mass spectrometry assay for the simultaneous quantification of simvastatin, lovastatin, atorvastatin, and their major metabolites in human plasma. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **983–984**, 18–25 (2015).
20. Geiger, S.S., Fagundes, C.T. & Siegel, R.M. Chrono-immunology: progress and challenges in understanding links between the circadian and immune systems. *Immunology* **146**, 349–358 (2015).
21. Fazzari, M., Vitturi, D.A., Woodcock, S.R., Salvatore, S.R., Freeman, B.A. & Schopfer, F.J. Electrophilic fatty acid nitroalkenes are systemically transported and distributed upon esterification to complex lipids. *J. Lipid Res.* **60**, 388–399 (2019).
22. Christiansen, T., Richelsen, B. & Bruun, J.M. Monocyte chemoattractant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. *Int. J. Obes.* **29**, 146–150 (2005).
23. Eardley, K.S. *et al.* The relationship between albuminuria, MCP-1/CCL2, and interstitial macrophages in chronic kidney disease. *Kidney Int.* **69**, 1189–1197 (2006).
24. Calabrese, E.J. Hormetic mechanisms. *Crit. Rev. Toxicol.* **43**, 580–606 (2013).
25. Wetzker, R. & Rubio, I. Hormetic signaling patterns. *Dose Response* **10**, 83–90 (2012).
26. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). FDA Guidance for Industry. Clinical Drug Interaction Studies — Study Design, Data Analysis, and Clinical Implications. October 2017
27. Horn, J.R. & Hansten, P.D. Time course for enzyme induction and deinduction. *Pharm Times* **77**, 10–12 (2011).
28. Calabrese, E.J. Hormesis: path and progression to significance. *Int. J. Mol. Sci.* **19**, 2871 (2018).
29. Ludovico, P. & Burhans, W.C. Reactive oxygen species, ageing and the hormesis police. *FEMS Yeast Res.* **14**, 33–39 (2014).