

ARTICLE

Phase I Studies of Acebilustat: Pharmacokinetics, Pharmacodynamics, Food Effect, and CYP3A Induction

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Acebilustat is a new once-daily oral antiinflammatory drug in development for treatment of cystic fibrosis (CF) and other diseases. It is an inhibitor of leukotriene A4 hydrolase; therefore, production of leukotriene B4 (LTB4) in biological fluids provides a direct measure of the pharmacodynamic (PD) response to acebilustat treatment. Here we compare the pharmacokinetics (PK) and PD between CF patients and healthy volunteers, and investigate the food effect and CYP3A4 induction in healthy volunteers. No significant differences between study populations were observed for peak plasma level (C_{max}) or exposure (AUC). In healthy volunteers, a shift in time to C_{max} (T_{max}) was observed after a high-fat meal, but there was no change in AUC. LTB4 production was reduced in the blood of both populations and in sputum from CF patients. Acebilustat did not induce CYP3A4. These results support continued clinical study of once-daily oral acebilustat in CF at doses of 50 and 100 mg.

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Study Highlights

✔ WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Persistent neutrophilic lung inflammation is a major cause of morbidity and mortality in cystic fibrosis. Safe and effective antiinflammatory treatment remains a significant unmet need. Acebilustat is a potential new antiinflammatory treatment intended to target the excessive influx of neutrophils into the lungs of cystic fibrosis patients.

✔ WHAT QUESTION DID THIS STUDY ADDRESS?

Whether once-daily oral acebilustat is suitable for further clinical development as a treatment for cystic fibrosis patients when combined with current standard of care. We address this question by evaluating the pharmacokinetic and pharmacodynamic data from two clinical studies in healthy volunteers and one clinical study in cystic fibrosis patients.

✔ WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

We found that the pharmacokinetics and pharmacodynamics of once-daily oral acebilustat were comparable between cystic fibrosis patients and healthy volunteers, that consumption of a high-fat meal is not expected to significantly alter drug exposure, and that acebilustat does not induce CYP3A4 enzyme.

✔ HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

These results demonstrate that once-daily oral acebilustat is suitable for further development for the treatment of cystic fibrosis and can be used in combination with current standard of care, including therapies that are sensitive to clearance by CYP3A4 such as those containing ivacaftor.

Cystic fibrosis (CF) lung pathology is characterized by a cycle of airway obstruction, infection, and inflammation. Despite intensive treatment efforts to combat airway obstruction and infection, airway inflammation is a major cause of pulmonary exacerbations, hospitalizations, and death for CF patients. Inflammation in the lung is initiated by infection and sustained predominately by a neutrophilic response.^{1,2} There are currently no generally accepted treatments directly addressing pulmonary inflammation in CF patients. Thus, antiinflammatory therapy represents an area of great unmet need for CF patients, and has been set as an area of research priority for the Cystic Fibrosis Foundation.³

Acebilustat is an antiinflammatory drug in development for the treatment of CF and other diseases. It is a potent inhibitor of the enzyme leukotriene A4 hydrolase (LTA4H), which catalyzes the rate-limiting step in the formation of leukotriene B4 (LTB4), a potent chemoattractant and activator of inflammatory immune cells including neutrophils.^{4–6} Once activated, neutrophils are prodigious producers of LTB4.^{7,8} This overproduction of LTB4 can lead to rapid amplification of a self-promoting cycle of neutrophil swarming as well as inciting a downstream cascade of cytokine and chemokine overproduction.^{9–11} When this condition persists, it can lead to deleterious immune overreaction, as seen in skin diseases

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and gout flares.^{12–15} In particular, many inflammatory lung diseases, such as chronic obstructive pulmonary disease, bronchiectasis, and CF, are characterized by significant neutrophilic infiltrates, and LTB₄ is found to be elevated in the airways.^{16–18} In such airway diseases, products of neutrophil activation, including elastase and other proteases, contribute to airway dysfunction and disease progression.^{19–21} Thus, reducing production of LTB₄ via inhibition of LTA₄H has the potential to reduce excessive neutrophil migration and activation in a number of important human inflammatory conditions, including CF.^{22–24}

Here we compare the pharmacokinetics (PK) and pharmacodynamics (PD) of once-daily oral acebilustat between CF patients and healthy volunteers. We also report the effect of a high-fat meal on acebilustat PK and the effect of steady-state acebilustat on midazolam PK in healthy volunteers. These studies support continued development of acebilustat for treatment of CF using once-daily oral doses of 50 mg and 100 mg.

METHODS

Clinical trials and study cohorts

Two phase I dose-ranging clinical studies were conducted over 14 days of treatment in healthy volunteers and 15 days of treatment in CF patients.^{25,26} In both studies, acebilustat was administered orally once daily in doses of 50 mg or 100 mg as a powder blend in a hard gelatin capsule with 250 ml water. Additionally, doses of 150 mg and 200 mg were administered for 14 days in the healthy volunteer study. A matching placebo capsule was given in parallel in each study. For the 14-day healthy volunteer study, planned cohorts consisted of nine acebilustat-treated and three placebo subjects at each dose level. For the 15-day CF patient study, planned cohorts consisted of six acebilustat treated and three placebo subjects at each dose level. One CF patient receiving placebo in the first cohort terminated early due to an adverse event. The second cohort in the 15-day CF study was reduced by one patient due to lack of enrollment, which resulted in an actual cohort consisting of six patients treated with acebilustat and two patients treated with placebo. A single-dose food effect substudy was included in the 14-day healthy volunteer study.²⁵ In this substudy, six subjects were treated with 50 mg or 100 mg acebilustat and three with matching placebo after fasting for 12 h or immediately after consuming a high-fat meal consistent with US Food and Drug Administration (FDA) guidance, “Food-Effect Bioavailability and Fed Bioequivalence Studies” 2002. One healthy volunteer in the food effect substudy did not return for the second course of treatment (fed state) at the 100 mg dose. For the CYP3A4 study, 20 healthy volunteers were enrolled for an in-clinic drug interaction study conducted over the course of 10 days.²⁷ Midazolam was supplied as 10 mg/mL midazolam hydrochloride (HCL) oral solution. Acebilustat was supplied as 100 mg capsules.

For the 14-day healthy volunteer study, which was conducted in Australia, the protocol and informed consent documents were reviewed by a Human Research Ethics Committee (HREC), Bellberry, and all patients provided informed consent. For the phase I study in CF patients and CYP3A4

induction study, which were conducted in the UK, the protocol and informed consent documents were reviewed by the Office for Research Ethics Committees Northern Ireland (ORECNI) and all patients provided informed consent. Additional details for the phase I CF study, including study design, biomarker outcomes, and study-specific safety data are reported separately.

Pharmacokinetics samples

PK samples were collected on days 1 (baseline), 7 (mid-course), and 14 (end of treatment) for healthy volunteers and on days 1 (baseline), 8 (mid-course), and 15 (end of treatment) for CF patients. The exception is that no mid-course point was collected for the healthy volunteers treated with 50 mg acebilustat. On days 1 and 14 of the 14-day healthy volunteer study, PK samples were collected predose (0) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h postdose. On day 7 of the 14-day healthy volunteer study, PK samples were collected predose (0) and 3, 6, 12, and 24 h postdose. In the CF study, PK samples were collected at the same times on each day, consisting of predose (0) and at 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h. PK samples for the food effect study were collected predose (0) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 h postdose in both the fasting state and fed state.

For the CYP3A4 induction study, on day –2, a single oral dose of midazolam, nominally 2 mg (0.2 mL x 10 mg/mL solution), was administered at hour 0 following an overnight fast. Samples assessing the PK of midazolam and 1-OH-midazolam were collected at the following timepoints: predose (0) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h postdose. Following a 2-day washout period, oral doses of 100 mg acebilustat were administered once daily on days 1 through 7 (within ± 1 h of dosing time on day 1). A single PK sample assessing trough plasma levels of acebilustat was collected on each days 1 through 8 prior to administration of acebilustat. On day 8, a single oral dose of midazolam nominally 2 mg (0.2 mL x 10 mg/mL solution) was administered at hour 0, following an overnight fast. Samples assessing the PK of midazolam and 1-OH-midazolam were collected at the following timepoints: predose (0) and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 h postdose.

Blood pharmacodynamics samples

For PD assessment of LTB₄ production in *ex vivo* stimulated whole blood, samples were collected predose (0) and 3, 6, 12, and 24 h postdose in conjunction with PK baseline, mid-course, and end of treatment samples. Kits containing stimulation and quench vials were prepared by CPR Pharma Services (Thebarton, South Australia, AU) and provided frozen to the clinical sites. The method for *ex vivo* stimulation is described in the **Supplementary Material**.

Sputum pharmacodynamics samples

For assessment of sputum LTB₄ in the CF study, sputum was induced according to previously described methods.^{28–30} In brief, patients were administered nebulized saline to aid in airway hydration and then asked to expectorate. Samples

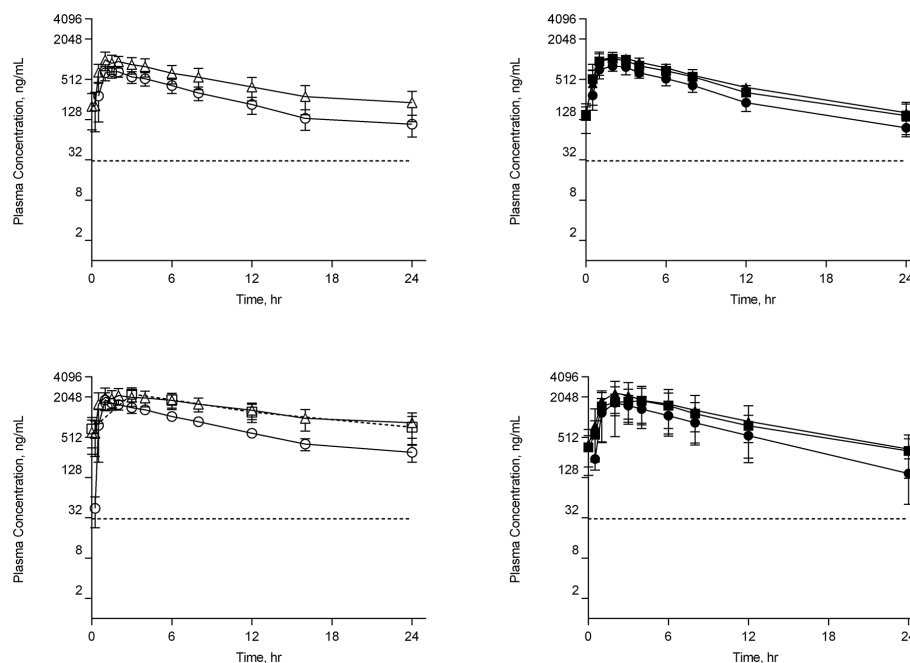


Figure 1 Plasma concentration as ng/ml (mean \pm SD) vs. time curves over the 24 h after a 50 mg daily dose (top panels) or 100 mg daily dose (bottom panels) for healthy volunteers (HV, left panels, open symbols) or CF patients (CF, right panels, closed symbols). Each graph shows a curve for first dose (circles), mid-course dose on day 7 for HV and day 8 for CF (squares), and last dose on day 14 for HV and day 15 for CF (triangles). IC_{50} for inhibition of LTB₄ production in human whole blood by acebilustat is 31 ng/ml, represented as a dashed line.

were collected on day -1 (baseline) and postdose on day 9 (steady state). Specimens were processed at the clinical sites by gentle mechanical homogenization according to previously reported methods and stored frozen at -70°C until analyzed.³¹

Analysis of LTB₄ in blood and sputum samples was carried out at Celerion (Lincoln, NE) using EIA kits from Cayman Chemical Company (Ann Arbor, MI; Cat no. 520111).

Data analysis

Analysis of PK samples for the healthy volunteer study was conducted at CPR Pharma Services. PK sample analysis for CYP3A4 induction and CF studies were conducted at Celerion. PK data were fit and PK parameters calculated using a noncompartmental model in Phoenix WinNonlin v. 6.3.

For statistical comparison of all PK and PD results, an unpaired parametric one-tailed *t*-test with 95% confidence level was performed. For statistical comparison of acebilustat dose groups to placebo or comparison of postdose to predose timepoints, a one-way analysis of variance (ANOVA) was carried out using Dunnett's multiple comparison test with a 90% confidence level. Data for all analyses included in this study were graphed and statistical analyses were performed using GraphPad Prism v. 5.00 for Windows (GraphPad Software, La Jolla, CA; www.graphpad.com). Analysis of approach to acebilustat steady state was carried out by calculating Helmert contrasts for the day-to-day changes in acebilustat trough plasma levels.³²

Additional details for bioanalysis and statistics are provided as **Supplementary Material**.

RESULTS

Tabulated data used for generation of **Figure 3** are provided as **Supplementary Material**.

Pharmacokinetics

In healthy volunteers and CF patients at both dose levels, the concentration vs. time curves show an increase in exposure from baseline to the end of treatment (**Figure 1**). In the cases where mid-course samples were collected, healthy volunteers at the 100 mg dose level and CF patients at both dose levels, there is little or no change from mid-course to end of treatment. This suggests that steady state had been reached by the mid-course collection, day 7 for healthy volunteers and day 8 for CF patients. It is notable that for each dose and in each population, all measured plasma concentrations after the first dose remain substantially greater than the IC_{50} of acebilustat for inhibition of LTB₄ production in human whole blood (31 ng/ml, $\sim 0.064 \mu\text{M}$). Since the whole blood-to-plasma ratio for acebilustat is 1.0, plasma concentration can be used as a direct surrogate for whole-blood concentration.

No clear difference was observed in C_{max} or AUC_{0-24} between healthy volunteers and CF patients (**Figure 3**). At the end of treatment, C_{max} (mean \pm SD) was $1,100 \pm 280$ ng/ml ($n = 9$) for healthy volunteers and $1,100 \pm 190$ ng/ml ($n = 6$) for CF patients given the 50 mg dose and $2,300 \pm 520$ ng/ml ($n = 8$) for healthy volunteers and $2,400 \pm 1,300$ ng/ml ($n = 6$) for CF patients given the 100 mg dose. AUC_{0-24} was $11,000 \pm 3,700$ ng-hr/ml ($n = 9$) for healthy volunteers

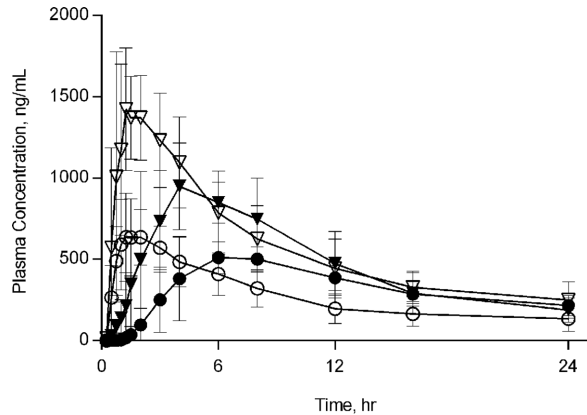


Figure 2 Plasma concentration as ng/ml (mean \pm SD) vs. time curves over the 24 h for healthy volunteers administered 50 mg (circles) or 100 mg acebilustat (inverted triangles) while fasting (open symbols) or after being fed a high-fat meal (closed symbols).

and $12,000 \pm 1,700$ ng-hr/ml ($n = 6$) for CF patients given the 50 mg dose and $32,000 \pm 9,300$ ng-hr/ml ($n = 8$) for healthy volunteers and $26,000 \pm 16,000$ ng-hr/ml ($n = 6$) for CF patients given the 100 mg dose.

Food effect

For healthy volunteers administered oral acebilustat after eating a standard high-fat meal, there was a shift of 3 to 4 h in T_{max} , a modest reduction in C_{max} , and no change in the area under the curve (AUC_{0-24}) (**Figure 2**). In the fed state T_{max} ranged from 4.4 to 5.7 h, whereas in the fasting state T_{max} ranged from 1.4 to 1.7 h. At the 50 mg acebilustat dose level, C_{max} (mean \pm SD) was 710 ± 230 ng/ml ($n = 6$) and AUC_{0-24} was $8,600 \pm 3,400$ ng-hr/ml ($n = 6$) in the fasting state and 550 ± 120 ng/ml ($n = 6$) and $11,000 \pm 2,500$ ng-hr/ml ($n = 6$) in the fed state, respectively. At the 100 mg dose level of acebilustat, C_{max} (mean \pm SD) was $1,700 \pm 350$ ng/ml ($n = 6$) and AUC_{0-24} was $17,000 \pm 5,600$ ng-hr/ml ($n = 6$) in the fasting state and 1100 ± 280 ng/ml ($n = 6$) and $14,000 \pm 3,100$ ng-hr/ml ($n = 6$) in the fed state, respectively.

In the food effect study, an apparent reduction in C_{max} but no clear difference in AUC_{0-24} was observed in the fed state vs. fasting (**Figure 3**). C_{max} (mean \pm SD) at the 50 mg dose level was 550 ± 120 ng/ml ($n = 6$) after consumption of a high-fat meal and 710 ± 230 ng/ml ($n = 6$) while fasting and, at the 100 mg dose level, $1,100 \pm 280$ ng/ml ($n = 6$) in the fed state and $1,700 \pm 350$ ng/ml ($n = 6$) in the fasting state. In contrast, AUC_{0-24} was $11,000 \pm 2,500$ ng-hr/ml ($n = 6$) in the fed state and $8,600 \pm 3,400$ ng-hr/ml ($n = 6$) when fasting in subjects given the 50 mg dose and $14,000 \pm 3,100$ ng-hr/ml

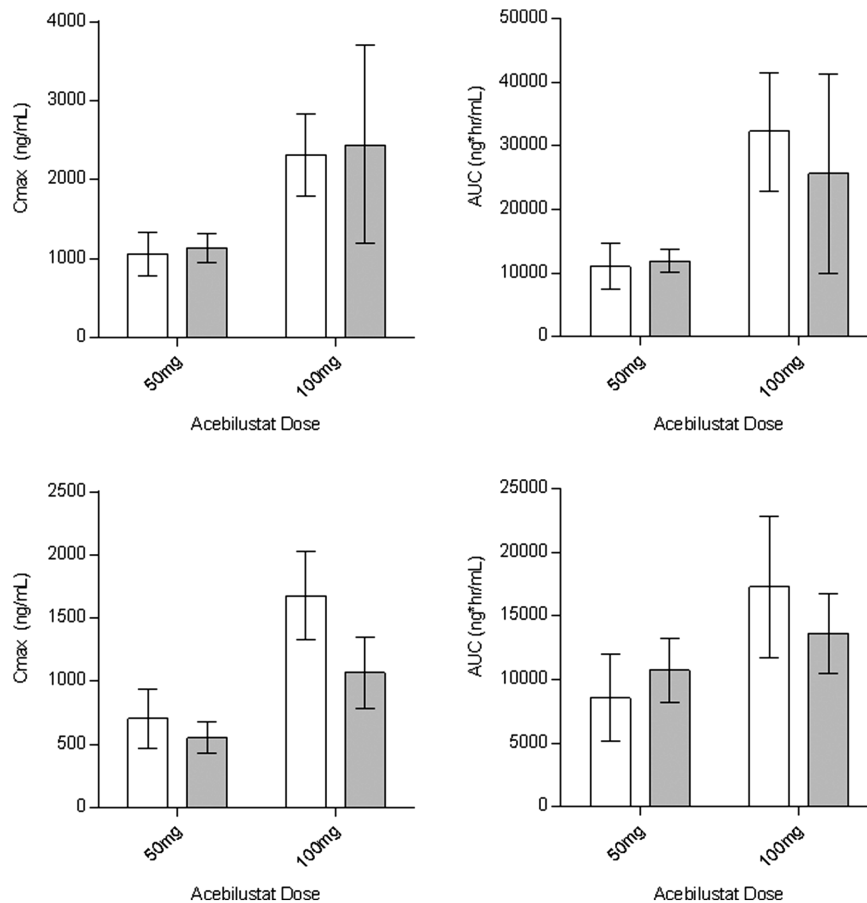


Figure 3 Comparison of exposures in terms of C_{max} as ng/ml (mean \pm SD) (left panels) and AUC_{0-24} as ng \cdot h/ml (mean \pm SD) (right panels). Top panels: steady-state exposures for healthy volunteers (open bars) and CF patients (shaded bars). Bottom panels: Exposures in healthy volunteers when given a single dose while fasting (open bars) and after being fed a high-fat meal (shaded bars).

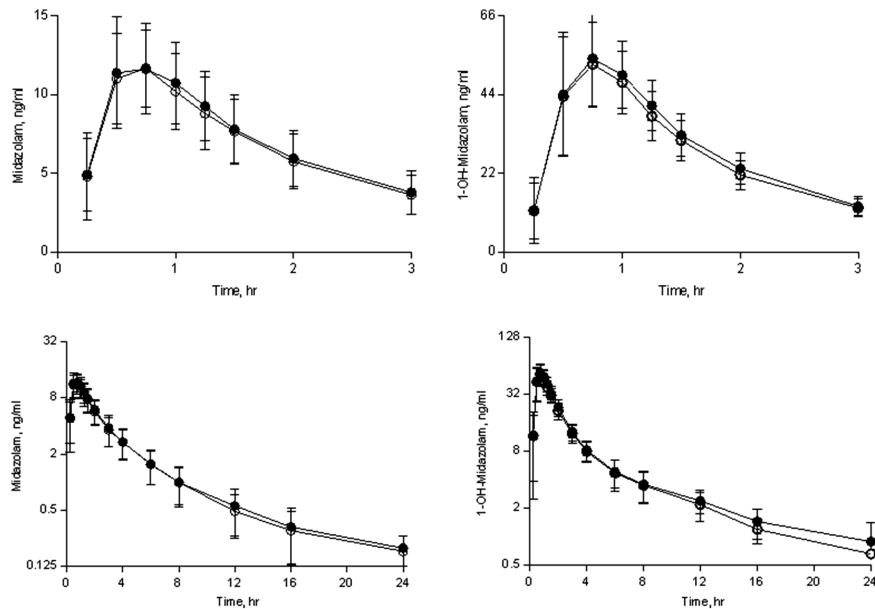


Figure 4 Plasma concentration as ng/ml (mean \pm SD) vs. time curves over the first 3 h (top panels) and over 24 h (bottom panels) after oral administration of midazolam. Left panels: midazolam profile PK before (open circles) and after (closed circles) acebilustat administration. Right panels: 1-OH-midazolam PK profile before (open circles) and after (closed circles) acebilustat administration.

($n = 6$) after a high-fat meal and $17,000 \pm 5,600$ ng-hr/ml ($n = 6$) when fasting at the 100 mg dose level.

CYP3A4 induction study

Based on an examination of Helmert contrasts for the trough levels of acebilustat from study day 5 to day 8, it was determined that 100 mg once-daily oral acebilustat reached steady state by study day 8 (day after the 7th treatment). After reaching steady state over 7 days of once-daily treatment with 100 mg oral acebilustat, there was no induction of CYP3A4 when assessed by the PK profile of either midazolam itself or 1-OH-midazolam, the primary CYP3A4-derived metabolite of midazolam before and after acebilustat treatment (**Figure 4**).

Blood LTB4 pharmacodynamics

At all dose levels in both healthy and CF populations, there was a rapid decrease in LTB4 in the blood, which was evident in the first sample collected 3 h after the first oral dose of acebilustat (**Figure 5**). LTB4 levels in blood (mean \pm SD of % predose) remained significantly decreased during the 24-h interval between acebilustat treatments and throughout the treatment period when compared with predose levels for a dose group ($P < 0.01$) or to levels for the placebo group ($P < 0.01$). Even at the highest dose (200 mg); however, acebilustat treatment did not completely eliminate LTB4 production in blood. Instead, increasing dosages appeared to reach a point of maximal LTB4 inhibition, leaving at least 10% residual LTB4 near the peak acebilustat concentration in the plasma (the 3-h collection point). In healthy volunteers, there was a general trend toward lower residual LTB4 production at the end of treatment (after steady state had been reached). For example, the level of residual LTB4 (mean \pm SD) at the trough level of acebilustat (24 h after the prior

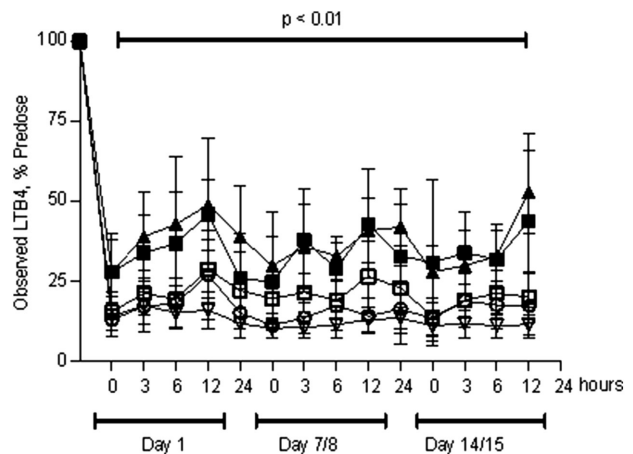


Figure 5 Pharmacodynamic effect of once-daily oral acebilustat on LTB4 production in *ex vivo* stimulated whole blood of healthy volunteers (open symbols) and CF patients (closed symbols) during the course of treatment. For healthy volunteers, doses of 100 mg (open squares), 150 mg (open circles), and 200 mg (open inverted triangles) are shown. For CF patients, doses of 50 mg (closed triangles) and 100 mg (closed squares) are shown. The data are presented as percent predose (mean \pm SD) for 24 h at the first dose, mid-course dose, and last dose of the study.

dose) for the 100 mg dose group was $29 \pm 9.0\%$ ($n = 9$) after the first treatment, $27 \pm 11\%$ ($n = 9$) after the mid-course treatment, and $20 \pm 8.0\%$ ($n = 9$) after the final treatment. Over the 24 h after the final acebilustat treatment, there was little to no variation in the levels of residual LTB4 in the 100 mg (peak $14 \pm 5.1\%$, $n = 9$; trough $19 \pm 8.4\%$, $n = 9$), 150 mg (peak $14 \pm 5.1\%$, $n = 9$; trough $19 \pm 8.4\%$, $n = 9$) or 200 mg (peak $11 \pm 3.9\%$, $n = 9$; trough $12 \pm 3.7\%$, $n = 9$) dose group of the healthy volunteer study. In contrast, there was

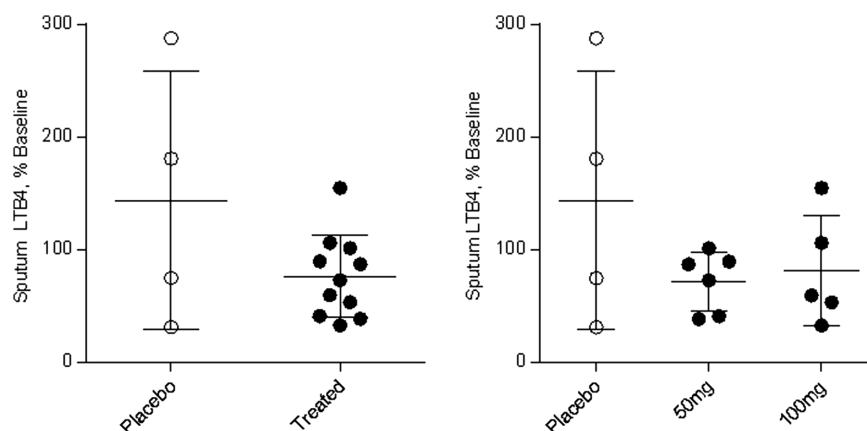


Figure 6 Pharmacodynamic effect of once-daily oral acebilustat (closed symbols) or placebo (open symbols) on LTB4 levels in sputum of CF patients at steady state (treatment day 9). Left panel shows total treated group vs. placebo, and right panel shows results by dose. The data are presented as percent baseline (mean \pm SD) for day 9 of the study.

somewhat greater residual LTB4 production in the blood of CF patients treated with 100 mg acebilustat than in healthy volunteers treated with the same dose level. There was also greater variation between residual LTB4 production at peak acebilustat concentrations vs. trough acebilustat concentrations on the same treatment day. After the final treatment, for example, the 100 mg dose group of CF patients showed $31 \pm 26\%$ ($n = 6$) residual LTB4 at peak and $44 \pm 27\%$ ($n = 6$) at trough, while the 50 mg dose group showed $28 \pm 8.0\%$ ($n = 6$) residual LTB4 production at peak and $53 \pm 13\%$ ($n = 6$) at trough.

Sputum LTB4 in CF patients

At steady state (treatment day 9), sputum LTB4 (mean \pm SD % of baseline) was reduced from baseline in CF patients treated with acebilustat ($76 \pm 37\%$, $n = 11$) and the treated group was significantly lower ($P = 0.046$) than placebo ($140 \pm 110\%$, $n = 4$) (Figure 6). No dose dependence was evident in the mean % baseline sputum LTB4 levels between the 50 mg ($72 \pm 26\%$, $n = 6$) and 100 mg ($81 \pm 49\%$, $n = 5$) dose groups. There was a trend toward a higher proportion of CF patients showing less than 60% of baseline LTB4 at the higher dose of acebilustat; one of four in the placebo group, two of six in the 50 mg acebilustat group, and three of five in the 100 mg acebilustat group.

Safety

Acebilustat was generally well tolerated in healthy adult males treated for up to 14 days with oral doses ranging from 50–200 mg once daily in a multiple ascending dose study; in healthy adult males and females treated for 10 days with an oral dose of 100 mg once daily in a CYP3A4 induction study; and in adult CF patients treated with oral doses of 50 mg or 100 mg once daily for 15 days. There were no deaths in any of these studies. There were no serious adverse events (SAEs) or subject discontinuations due to adverse events in either healthy volunteer study. In the CF study, one SAE termed pulmonary exacerbation of CF occurred during the follow-up period 35 days after the final 50 mg dose of acebilustat, and this SAE was deemed by the clinical site investigator to be unlikely related to the study drug. Across the three studies, only two AEs were observed at rates of 10% or more

in the acebilustat treated patients ($n = 68$): headache 28% (19 patients) and oropharyngeal pain 13% (nine patients). By comparison, these two AEs were observed in the placebo-treated patients ($n = 17$) at rates of 18% (three patients) and 24% (four patients), respectively.

DISCUSSION

Three phase I studies were conducted assessing PK and PD in healthy volunteers and CF patients as well as food effect and CYP3A4 induction in healthy volunteers after multiple oral doses of acebilustat. In these studies, acebilustat was generally safe and well tolerated.

Overall, PK was very similar between CF patients and healthy volunteers at the 50 mg and 100 mg doses that both populations received (Table 1). This is an important finding, since CF patients may exhibit altered absorption of nutrients and drugs in the gut due to the CFTR mutation, and this effect has hampered development of other small-molecule drugs.³³ A notable difference was seen in the latter half of the elimination phase, starting 12 h after treatment, where healthy volunteers appeared to exhibit slower elimination. This is evident in the PK half-lives observed for the two populations, with the healthy volunteers exhibiting a longer elimination half-life (15–17 h) than CF patients (9 h). However, it is the CF patients who exhibit a near first-order elimination during this period, whereas the healthy volunteers exhibit a potential second-order effect. Whether this is an actual effect or an artifact specific to this study remains to be determined. In both cases, the plasma concentrations of acebilustat remain substantially higher over the entire 24-h period between treatments than required for pharmacologic activity; 64 nM (~ 31 ng/ml), the IC_{50} for inhibition of LTB4 production by acebilustat in human whole blood.

Production of LTB4 in blood was substantially reduced 3 h after acebilustat administration, indicating rapid target engagement. The overall PK-PD profile of acebilustat suggests a largely direct correlation between plasma concentrations and pharmacodynamic effect. Therefore, the net pharmacologic effect of acebilustat may be related to time-averaged plasma concentration or driven by minimum

Table 1 Summary of acebilustat blood PK and PD properties in healthy volunteers and CF patients

Study Population	HV			CF			HV			CF			HV			HV		
	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Dosing Day	14			15			14			15			14			14		
Dose (mg/day)	50			50			100			100			150			200		
C_{max} (ng/mL)	1,100	280	9	1,100	190	6	2,300	520	8	2,400	1,300	6	4,900	1,700	9	8,100	1,100	9
C_{3hr} (ng/mL)	850	240	9	1,100	180	6	2,000	650	8	2,200	1,200	6	4,100	1,400	9	7,000	1,500	9
C_{min} (ng/mL)	190	110	9	160	69	6	580	320	8	340	220	6	1,200	720	9	2,800	1,200	9
AUC_{0-24} (ng•h/mL)	11,000	3,700	9	12,000	1,700	6	32,000	9,300	8	26,000	16,000	6	66,000	30,000	9	120,000	30,000	9
T_{max} (h)	1.4	0.58	9	2.0	0.90	6	1.7	1.1	8	2.0	0.58	6	2.1	1.8	9	1.6	1.7	9
$T_{1/2}$ (h)	16 ^a	4.2	6	9.3	4.2	6	16 ^a	5.7	6	8.6	2.3	6	18 ^a	4.4	6	16 ^a	4.7	4
Ratio of C_{max} to blood LTB4 IC_{50}	34			37			75			79			158			262		
Ratio of C_{3hr} to blood LTB4 IC_{50}	28			34			66			70			132			228		
Ratio of C_{min} to blood LTB4 IC_{50}	6			5			19			11			39			91		
Peak LTB4 inhibition (%)	ND			73			86			69			86			89		
Trough LTB4 inhibition (%)	ND			58			80			67			82			88		

HV, healthy volunteers; CF, Cystic fibrosis patients; LTB4, leukotriene B4; AUC_{0-24} , area under the time–concentration curve; C_{max} , maximum observed plasma concentration; C_{3hr} , plasma concentration observed at 3hr (PD) timepoint; C_{min} , minimum observed plasma concentration; IC_{50} , concentration causing 50% inhibition (0.064 μ M, 30.8 ng/mL); $T_{1/2}$, elimination half-life; ND, not determined.

^a $T_{1/2}$ determined after a single dose.

plasma concentration (C_{min}) between treatments. Comparing the peak reduction in whole-blood LTB4 production in healthy volunteers after the 100 mg dose (86% reduction) to that for the 150 mg dose (86% reduction) and the 200 mg dose (89% reduction), the maximal PD effect appears to be expressed at plasma concentration to whole-blood IC_{50} ratios of 75 and above, which occurs at doses of 100 mg and higher. Even when acebilustat plasma concentrations are well in excess of this level (e.g., ratio of 262 at the 200 mg dose), the maximal observed PD effect only approaches 90% inhibition of LTB4 production. The reason for less than 100% inhibition and the origin of the 10% residual LTB4 production are not presently understood, but could be a feature of the mechanism or an artifact of the analysis. Together, these findings suggest that the PD response level observed at the 200 mg dose is a maximal effect for acebilustat on LTB4 production. The PD response appeared to be somewhat lower at equivalent plasma levels in CF patients compared with healthy volunteers, and there was only a small increase in PD response at the 100 mg dose in CF patients compared with the 50 mg dose. While the basis for these findings is not currently understood, they could indicate a true difference in PD response between CF patients and healthy volunteers or a methodological inconsistency between the two studies. The finding of little difference between the 50 mg and 100 mg doses in CF patients in the presence of doubled plasma concentrations suggests that even the 50 mg dose may achieve near maximal inhibition of the pathway. Unfortunately, PD response in blood was not tested at the 50 mg dose in healthy volunteers. Therefore, future studies of acebilustat PD will include lower doses and a control that enables calibration of maximal pathway inhibition between groups.

Sputum LTB4 levels were reduced at steady state in acebilustat treated CF patients when compared with placebo ($P < 0.05$) or to individual baseline values. The mean reduction from baseline in sputum LTB4 in patients treated with 100 mg acebilustat did not significantly differ from the mean reduc-

tion in patients treated with 50 mg acebilustat, although a greater proportion of patients treated with the 100 mg dose did show a large reduction from baseline. While the absence of a quantitative difference in sputum LTB4 between the two dose levels mirrors the lack of difference in residual LTB4 production in blood, the magnitude of effect was markedly greater in blood (50–70% reduction from baseline) than in sputum (20–30% reduction from baseline). The impact of this difference remains unknown, but could reflect reduced distribution of acebilustat to the lung or could simply reflect the high variability and the small number of patients in the study. It remains to be determined whether distribution to the lung is required for therapeutic effect in CF, as recent advances in the understanding of the function of LTB4 as a neutrophil-to-neutrophil signal relay system suggests that the focal point of the LTB4 effect in CF may not be within the lumen of the lung, but rather at the interface between blood and lung epithelium.^{7,9} A deeper understanding of the relationship of sputum LTB4 to blood LTB4 and to neutrophil influx into the lung requires further investigation.

While consumption of a high-fat diet immediately prior to administration of a single dose of acebilustat in healthy volunteers resulted in an increase in T_{max} and a modest decrease in C_{max} , the lack of a change in AUC_{0-24} suggests that there may be little difference in exposure at steady state in the fed state vs. fasting. This is important, as CF patients are frequently prescribed a high-fat diet in order to help maintain body weight. Induction of CYP3A4 is another important consideration for treatment of CF patients, since some current CF therapies, notably ivacaftor, are subject to metabolism by CYP3A4.^{34,35} After reaching steady state over 7 days of once-daily treatment with 100 mg oral acebilustat, there was no induction of CYP3A4, indicating that acebilustat is suitable for administration in combination with current care for CF patients, including CFTR modulators.

In summary, these results indicate that there is minimal, if any, PK difference in CF patients compared with healthy

volunteers. Consumption of a high-fat diet is also not expected to significantly alter PK in CF patients, and acebilustat does not induce CYP3A4. A once-daily dose of 100 mg acebilustat produces a near-maximal pharmacodynamic response on the targeted pathway, LTB₄ production, in blood. Acebilustat treatment resulted in a reduction from baseline LTB₄ in the sputum of CF patients. Taken together, these results support further development of acebilustat for treatment of CF using once-daily oral doses of 50 mg and 100 mg. A phase II study assessing clinical outcomes in CF patients after 48 weeks of acebilustat treatment is currently in progress.³⁶

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Conflict of interest. L.B., R.G., S.A., and E.B.S. are employees of Celtaxsys, Inc. J.S.E. was a consultant for Vertex, Novartis, Raptor, and Celtaxsys during the conduct of this study.

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