CHEST

Original Research

GENETIC AND DEVELOPMENTAL DISORDERS

Reduced Sodium Transport With Nasal Administration of the Prostasin Inhibitor Camostat in Subjects With Cystic Fibrosis

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Background: **Prostasin, a trypsin-like serine protease, is a channel-activating protease and major regulator of epithelial sodium channel-mediated sodium absorption. Its direct inhibition by camostat represents a potential approach to inhibiting sodium transport in cystic fibrosis (CF).**
Methods: **To determine whether a topical formulation of camostat represents an efficacious and tolerable approach to reducing Na+ transport in the CF airway, we conducted a two-part randomized, double-blind, placebo-controlled, crossover, ascending single-dose study to evaluate the pharmacodynamics, safety, and pharmacokinetics of camostat administered through a nasal spray pump in subjects with CF. Nasal potential difference (PD) was measured before and after treatment, and safety and pharmacokinetics were assessed by a standardized approach.**
Results: **In part 1, nine subjects were enrolled, and six completed crossover dosing at the maximally tolerated dose. The change in maximal (most polarizing) basal PD 2 h following administration of camostat was +13.1 mV (1.6-mg dose group) compared with −8.6 mV following placebo ($P < .005$). Intrasubject change in Ringer and amiloride-sensitive PDs exhibited similar and consistent responses. Bayesian analysis in an additional six subjects in part 2 estimated a dose of 18 μg/mL to provide 50% of the maximum effect. There was no significant change in chloride transport or total nasal symptom score, nasal examination rating, and laboratory parameters.**
Conclusions: **This study establishes the proof of concept that a reduction in sodium transport in the human CF airway can be achieved through inhibition of prostasin activity, identifying a potential therapeutic target in the disease.**
Trial registration: **ClinicalTrials.gov; No.: NCT00506792; URL: www.clinicaltrials.gov**

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Abbreviations: AE = adverse event; ASL = airway surface liquid; CAP = channel-activating protease; CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane regulator; ENaC = epithelial sodium channel; NPD = nasal potential difference; PD = potential difference; SAE = serious adverse event

Cystic fibrosis (CF) is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, which functions as a chloride and bicarbonate channel in epithelial plasma membranes.[1] Normal CFTR function promotes anion and fluid secretion into the airway lumen, a process that is balanced by the absorption of sodium and fluid by the epithelial sodium channel (ENaC). In this model, an adequate volume of airway surface liquid (ASL) is maintained in the lungs to ensure effective mucociliary clearance. CF is characterized by abnormal ion transport in the epithelia of various organs, including the sweat glands and lungs. In the airway, impaired CFTR-mediated anion secretion limits mucosal hydration, thereby reducing the volume of ASL available to support effective mucociliary clearance.[2] Consequently, chronic airway infections and inflammation develop in patients with CF, resulting in a progressive loss of lung function.[3]

A number of investigational therapies are aimed at improving ASL volume and, thus, mucociliary clearance in CF. Apart from restoring CFTR directly through gene therapy or small molecule CFTR modulators to enhance epithelial fluid secretion, other potential therapies directed at increasing ASL volume include potent and selective ENaC inhibitors[4] and inhaled

hypertonic saline[5,6] and mannitol,[7,8] which enhance ASL volume by osmotic loading. Inhaled hypertonic saline also reduces the frequency of CF pulmonary exacerbations, improves quality of life,[6] and is widely used by patients with CF.[9]

Prostasin, a trypsin-like serine protease, is a major channel-activating protease (CAP) of ENaC-mediated sodium currents in CF epithelia.[10-14] Attenuation of ENaC function by CAP inhibition is predicted to improve mucociliary clearance with potential downstream effects on pulmonary obstruction and clinical stability. Camostat is an inhibitor of prostasin that has been shown to inhibit ENaC function in vitro[15] and in vivo.[16] An oral formulation of camostat has been marketed in Japan since the early 1980s to treat acute pancreatitis and postoperative reflux esophagitis through its antiprotease activity and has a reasonable safety profile. In this study, we tested an investigational formulation of camostat (QAU145) to determine the tolerability of topical administration and whether ENaC activity in patients with CF could be inhibited through this mechanism.

Materials and Methods

Study Population

Patients aged 18 to 50 years with CF (confirmed by clinical manifestations and evidence of CFTR dysfunction by sweat chloride and nasal potential difference [NPD] testing or by two known genetic mutations) were eligible for this study. Patients with a history of clinically significant ECG abnormalities, autonomic dysfunction, acute or chronic bronchospastic disease, allergies affecting the nasal or sinus passages, upper respiratory tract infection, any structural nasal abnormalities, history of immunodeficiency diseases, evidence of liver disease or injury, renal impairment, positive hepatitis B test results, or history of drug or alcohol abuse were excluded.

Study Design

This randomized, double-blind, placebo-controlled, alternating panel, ascending single-dose study was conducted in two parts as shown in Figure 1. Part 1 of the study comprised two panels (A and B) of three subjects each. In treatment periods 1 and 2, ascending single doses of camostat 0.2, 0.8, and 1.6 mg were administered. In treatment period 3, subjects received camostat 1.6 mg in a balanced crossover fashion with respect to treatment period 2. Different subjects within each panel were randomized to receive placebo in each treatment period. Each treatment period consisted of dose administration on day 1 and postdose evaluations up to 6 h postdose.

Part 2 of the study estimated the lowest efficacious dose of camostat. Six subjects completing period 3 in part 1 comprised part 2. Further details are provided in e-Appendix 1.

This study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonisation Good Clinical Practice guidelines. Approval was obtained from an independent institutional review board (Western Institutional Review Board, Olympia, WA; W20070738), and all subjects provided written informed consent before participating in the study.

Study Drug

Investigational camostat for nasal spray solution and placebo (lactose) were prepared by Novartis Pharma US and provided as lyophilized powder in vials. Both were reconstituted in 0.9% saline prior to administration.

Assessments

Nasal Potential Difference: The primary outcome was the change in maximal basal NPD from predose to 2 h postdose. The maximal basal NPD at predose and 2 h postdose was obtained as the maximum absolute value among measurements at 0.5, 1, 1.5, 2, and 3 cm in the target nostril. NPD is a validated model for proof of concept studies for ENaC inhibition in CF.[17-20]

In part 1, NPD was measured at baseline (day −1 of treatment period 1, ~24 h prior to dosing on day 1) in each nostril and at the end of the study as previously described.[18] In part 2, basal NPD was measured at predose in both nostrils for all subjects. At 2 h postdose, basal NPD was measured in the nontarget nostril, and a traditional NPD was performed in the target nostril.

A

Panel	Period 1		Period 2		Period 3	
			CROSSOVER			
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
A N=3 (A:P)	0.2 mg (2:1)		1.6 mg/MTD (2:1)		1.6 mg/MTD (1:2)	
B N=3 (A:P)		0.8 mg (2:1)		1.6 mg/MTD (2:1)		1.6 mg/MTD (1:2)

B

Subject number	Treatment Period 1	Treatment Period 2	Treatment Period 3
1	20 mcg	5 mcg	10 mcg
2	20 mcg	5 mcg	10 mcg
3	20 mcg	10 mcg	Placebo
4	Placebo	5 mcg	5 mcg

Figure 1. Study design, with randomization schemes for part 1 and part 2. A, Part 1 included two panels of subjects (three subjects per group) with repeat dosing and crossover design. B, Part 2 doses were determined using a Bayesian dose-response analysis and administered in the crossover design. A:P = active:placebo dosing ratio; MTD = maximally tolerated dose up to 1.6 mg.

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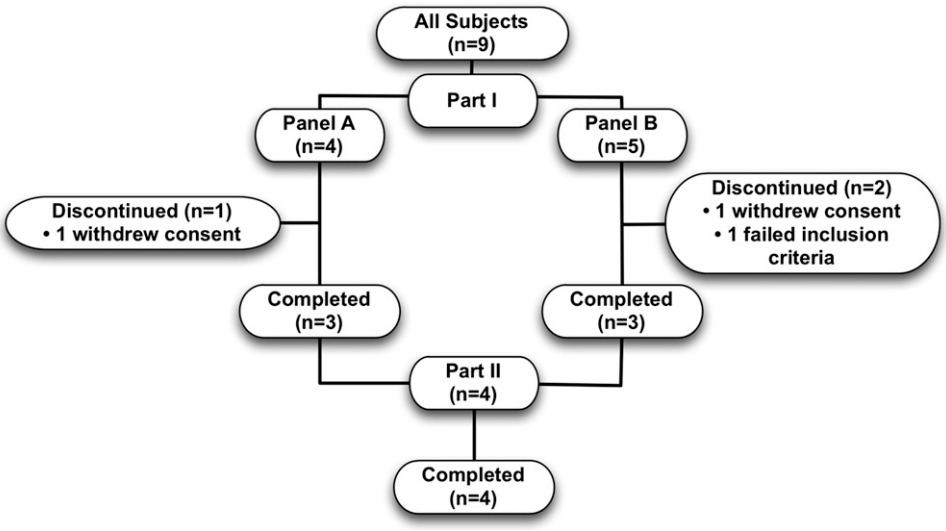

FIGURE 2. Flow of subjects through the study.

Pharmacokinetics and Safety Evaluation

Blood, urine, and nasal lavage samples were collected to assess the pharmacokinetics of camostat and its major metabolite QAY243. Blood and urine samples were collected in part 1 of the study, and the nasal lavage samples were collected in both parts 1 and 2 from the nontarget nostril. Safety and tolerability assessments included the monitoring and recording of all adverse events (AEs) and serious adverse events (SAEs). Further details are provided in e-Appendix 1.

Statistical Analysis

For part 1, an analysis of covariance model was used, including a fixed factor for treatment (placebo or dose of camostat received), a random factor for subject, and the predose NPD value as a covariate. The primary contrast of interest was the difference between camostat at the maximum tolerated dose (1.6 mg) and placebo. After completion of part 1, an interim analysis was performed at which a Bayesian posterior distribution for this contrast was obtained with a noninformative prior. Dose response analyses, analysis of secondary NPD variables, and sample size calculations are described in e-Appendix 1.

RESULTS

Subject Disposition

Nine subjects (four in panel A and five in panel B) were enrolled and randomized in part 1 of the study. Six subjects completed the study and received study drug in all three treatment periods. One subject was discontinued from panel A (withdrew consent), two were discontinued from panel B (one withdrew consent, and one did not meet inclusion criteria because of a CFTR-related disorder and was withdrawn prior to dosing) (Fig 2). Four subjects who completed part 1 of the study enrolled in part 2, two each from panels A and B. All four subjects completed part 2. The mean age of the study subjects was 30.3 years. The demographic characteristics of the study subjects are shown in Table 1.

Effect of Camostat on NPD

Six subjects completed randomized crossover testing at the maximum dose tested (1.6 mg). Representative predose and postdose basal potential difference (PD) tracings for camostat and placebo are shown in Figures 3A and 3B, respectively, and a pronounced reduction in basal PD with camostat administration was demonstrated. In part 1, the change in maximal (most polarizing) basal PD 2 h following administration of 1.6 mg camostat was +13.1 mV compared

Table 1—*Demographic Characteristics of Study Participants*

Characteristic	All Patients (N = 9)
Age, y	
Mean ± SD	30.3 (9.43)
Median (range)	31.0 (20-49)
Female sex	3 (33.3)
Race	
White	8 (88.9)
Black	1 (11.1)
CF genotype	
F508del/F508del	3 (33.3)
F508del/G551D	1 (11.1)
F508del/unknown	1 (11.1)
F508del/2184delA	1 (11.1)
F508del/S549N	1 (11.1)
E60×/621+1 G→T	1 (11.1)
Unknown	1 (11.1)
Body weight, mean (range), kg	67.9 (49.8-91.4)
BMI, mean (range), kg/m²	25.1 (18.0-35.8)

Data are presented as No. (%), unless otherwise indicated. CF = cystic fibrosis.

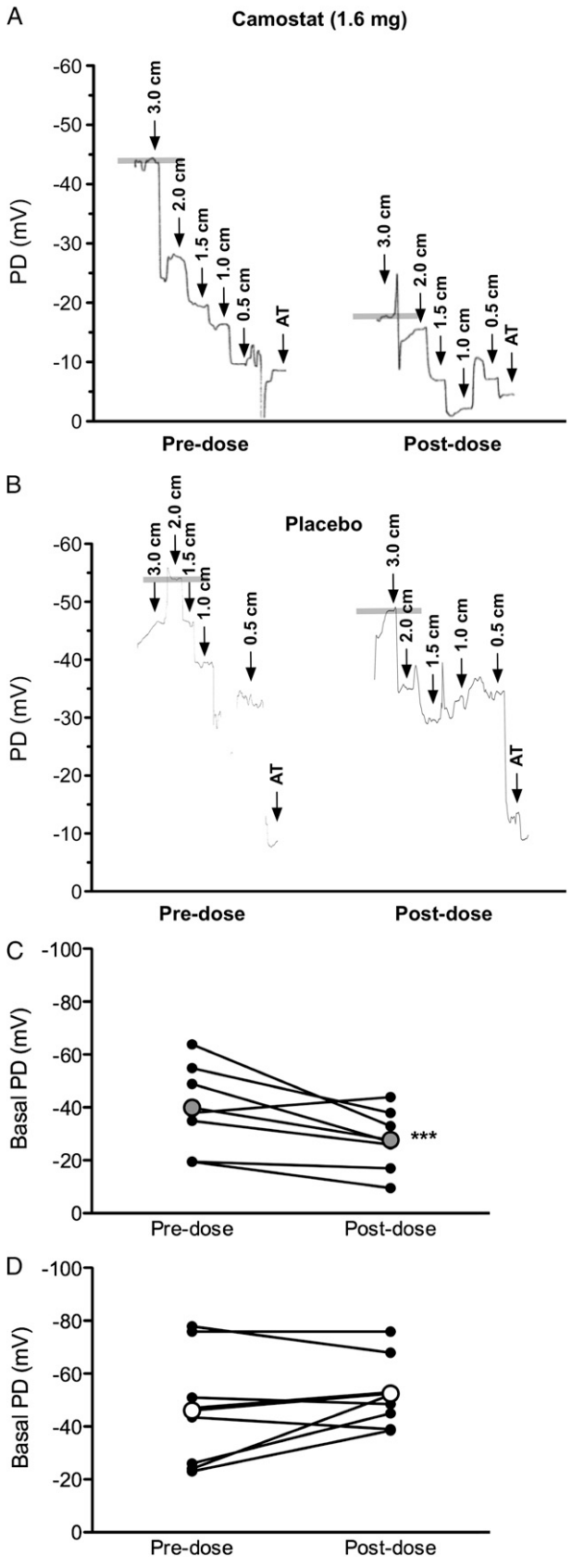

FIGURE 3. Effect of camostat on maximal basal PD. A, B, Representative basal PD tracing of subjects treated with camostat 1.6 mg (A) and placebo (B). Predose and 2-h postdose tracings are shown for the same nostril and the same subject on the left and right sides of A and B, respectively. Maximal basal PD is designated with the gray bar. C, D, Maximal basal PD for each individual subject (●) and the mean of all subjects (○) treated with camostat 1.6 mg (C) and placebo (D). ***$P < .005$. Data are presented as mean ± SEM. AT = anterior tip of the inferior turbinate; PD = potential difference.

with −8.6 mV following placebo ($P < .005$, analysis of covariance model) (Figs 3C, 3D). The effect of camostat 1.6 mg was also observed in the nontarget nostril 2 h postdose (+10.9 mV camostat vs −0.1 mV placebo, $P < .05$). Secondary NPD measurements were performed using a traditional NPD perfusion protocol immediately following the measurement of maximal basal PD in the target nostril only so that the contralateral nostril could be used to estimate intranasal pharmacokinetics. NPD tracings following camostat 1.6 mg are shown in Figure 4A. Consistent with maximal basal PD results, NPD tracings revealed a reduced Ringer PD in subjects treated with camostat compared with placebo ($P = .05$) (Figs 4B, 5B). The mean Ringer PD in the camostat-treated group (−20.4 ± 8.4 mV) was intermediate compared with a population of normal subjects (−16.1 ± 9.3 mV) and subjects with CF (−35.2 ± 12.5 mV) available in the University of Alabama at Birmingham database (n = 144 and 135, respectively). Although the change in amiloride-sensitive PD is generally less sensitive than the change in basal and Ringer PDs,[21] camostat also demonstrated a trend toward reduced amiloride-sensitive PD (Figs 4A, 4B, 5C), approaching statistical significance for the 1.6-mg dose group compared with placebo ($P = .088$). The mean amiloride-sensitive PD with camostat 1.6 mg (13.3 ± 6.6 mV) was also intermediate compared with normal subjects (8.8 ± 5.2 mV) and subjects with CF (18.7 ± 9.0 mV) in the database. In total, these results indicate that the Ringer PD and the effect of amiloride, an ENaC blocker, were reduced following camostat administration and further indicated inhibition of prostasin-mediated ENaC activation by camostat. Evaluation of lower dose levels of camostat tested within the dose-ascending component of part 1 (eg, 0.2 and 0.8 mg) suggested that the maximal effect had been achieved at these lower doses ($P < .001$ and $P < .005$ for the 0.2 mg and 0.8 mg doses, respectively) (Fig 5A), although the relative dose response was more apparent from the NPD parameters (Ringer PD, Δamiloride) derived from the perfusion tracings (Figs 5B, 5C).

The robust effects of camostat at the lowest dose tested within part 1 led us to next test lower doses of camostat (5, 10, and 20 μg) in part 2 to establish a dose-response curve, the minimal efficacious dose, and a safety margin. Summary data indicating the change in maximal basal PD and the established dose-dependent reduction in PD following camostat

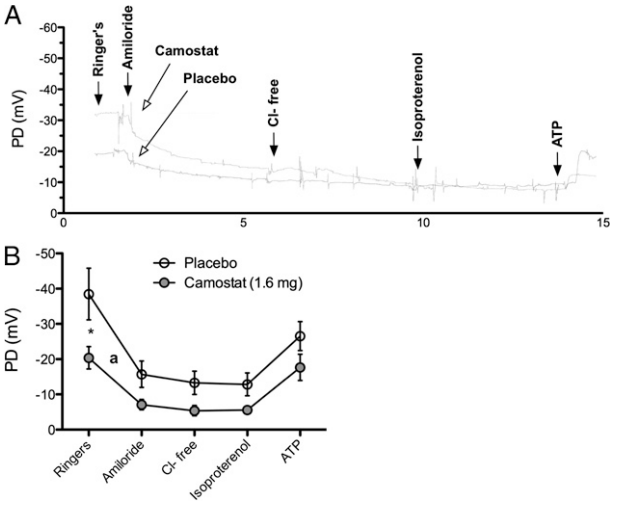

FIGURE 4. Effect of camostat on nasal PD perfusion tracings. Tracings were obtained in the target nostril about 2 h following intranasal administration of camostat or placebo. A, Representative nasal PD tracings for a subject treated with camostat 1.6 mg and placebo. The nostril was sequentially perfused with Ringer, amiloride (100 μmol/L), Cl-free gluconate, Cl-free gluconate plus isoproterenol 100 μmol/L, and ATP 10 μmol/L. B, Summary data indicating the mean PD of that shown in A for subjects treated with camostat 1.6 mg and placebo. The Ringer PD and change in PD following amiloride perfusion each reflect sodium transport, whereas the change with Cl-free (zero Cl^-), Cl-free isoproterenol and ATP reflect anion conductance. *$P = .05$; [a]$P = .09$. Data are mean ± SEM. ATP = adenosine triphosphate. See Figure 3 legend for expansion of other abbreviation.

administration compared with placebo are shown in Figure 5D. NPD perfusion tracings obtained 6 h postdose confirmed these observations (e-Fig 1) and indicated a reduction in Ringer PD (Fig 5E, e-Fig 1) and amiloride-sensitive PD (Fig 5F) compared with placebo.

The dose-response relationship (pooled data from parts 1 and 2) is shown in Figure 6. The results revealed that the maximal effect for the change in maximal basal PD from predose to 2 h postdose in the target nostril compared with placebo was 24.8 mV (95% CI, 17.0-32.8 mV). The dose providing 50% of the maximum effect was estimated to be 18 μg (95% CI, 5-50 μg). A priori, a change in maximal basal PD of 10 mV was believed to indicate a clinically meaningful change in PD on the basis of genotype-phenotype correlations in CF.[19] The dose providing an increase of 10 mV over placebo was estimated to be 14 μg (95% CI, 3-44 μg).

Pharmacokinetics

The pharmacokinetics of camostat and QAY243 are reported in e-Appendix 1. No clear relationship among NPD, dose, and concentrations in nasal lavage, plasma, or urine could be made because of the low recovery of the total administered dose and the small number of observations.

Tolerability

No deaths were reported, and no subject was discontinued from the study because of AEs. Three subjects experienced SAEs; each resolved without clinical sequelae, and none were suspected to be related to the study drug. A slightly higher percentage of subjects treated with camostat 1.6 mg (85.7%) had AEs compared with the subjects treated with camostat 0.8 mg (50.0%), 5 μg (66.7%), and placebo (62.5%) (e-Table 1). None of the subjects treated with camostat 10 μg, 20 μg, and 0.2 mg reported AEs. The majority of the events were mild and unrelated to the study drug. Six subjects had AEs that were considered to be related to the study drug, including epistaxis (mild), nasal mucosal disruption (1 out of a range of 1-4), and hematuria (all in the camostat 1.6 mg group); nasal sensitivity and rhinorrhea were also reported (both in the camostat 0.8 mg group). There was no effect of camostat on leukocyte count, differential, or neutrophil elastase activity recovered in nasal lavage. Additional details are provided in e-Appendix 1.

DISCUSSION

To our knowledge, this study is the first to assess whether administration of a prostasin inhibitor can attenuate airway ENaC activity in human subjects with CF. The results establish the proof of concept that inhibition of prostasin can block ENaC-mediated sodium transport in CF. Results from part 1 indicated that camostat 1.6 mg produces a robust inhibition of baseline maximal (basal PD and Ringer perfusion PD) voltage in addition to exhibiting a trend toward decreased amiloride-sensitive voltage, providing strong evidence that inhibition of ENaC was efficacious following intranasal administration. Lower doses tested within part 1 (eg, 0.2 and 0.8 mg) also demonstrated similar efficacy. Hence, the threshold for efficacy was reached at the lowest dose tested in part 1, enabling the subsequent studies in part 2. Part 2 confirmed the initial observations, and by nature of the Bayesian design, they provided an estimate of the minimal efficacious dose and the dose-response curve. We estimated the 50% maximum effect to be 18 μg, which also induces an effect magnitude predicted to cause clinically meaningful changes in ENaC activity (eg, >10 mV).[19] The consistency of findings across study parts, dose groups, and NPD parameters of sodium transport offers further confidence that the findings are internally valid and reproducible.

The results were predicted by positive findings in vitro and in animal studies. Camostat has been shown to inhibit ENaC activity in vitro[16] whereupon it partially normalized ENaC activity as indicated by a reduction in an amiloride-sensitive short-circuit current (on the

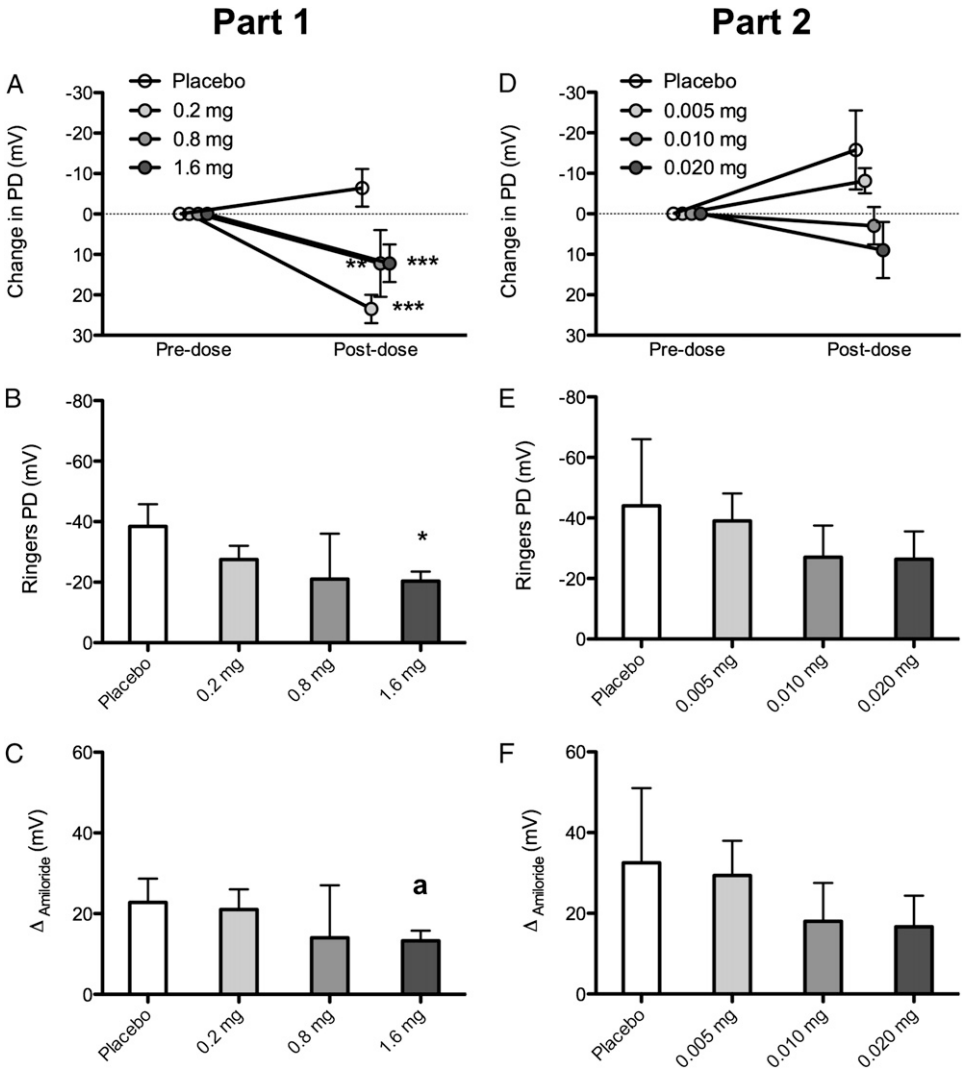

FIGURE 5. Effect of various doses of camostat on the maximal basal PD. Maximal basal PD was measured predose and 2 h postdose. A, B, and C, Part 1 included camostat 0.200, 0.800, and 1.600 mg and placebo. D, E, and F, Part 2 included camostat 0.005, 0.010, and 0.020 mg and placebo. The Ringers PD by dose (B and E) and change in PD following amiloride perfusion by dose (C and F) for each part of the study are shown. $^{*}P < .05$; $^{**}P < .005$; $^{***}P < .001$; $^{a}P = .09$; mean ± SEM. See Figure 3 legend for expansion of abbreviation.

range of 30 nmol/L-3 μmol/L) in F508del homozygous primary human bronchial epithelial cells over a brief exposure window. In vitro results were similar to aprotinin, an inhibitor of CAPs. Similar to the present findings in human subjects, the baseline short-circuit current was also reduced in F508del homozygous cells pretreated with camostat for 90 min.[16] These results also translated to non-CF guinea pig in vivo, exhibiting an approximate 7.5 mV decrease in trachea PD following 100 μg/kg intratracheal administration of camostat, an effect comparable with that observed with amiloride.[16] The mucociliary clearance of normal sheep was also augmented with camostat inhalation 2 and 5 h postdose,[16] suggesting that effective inhibition of prostasin in patients with CF, as observed by the present studies, should also confer increased mucociliary clearance and, thus, clinical benefit.

The results provide an initial step toward the development of a pharmaceutical strategy directed toward improving sodium transport. Further studies will be needed to demonstrate that camostat exhibits a sustained duration of action and is safe and effective in the lower airway when delivered by nebulizer. As opposed to protease inhibitors such as aprotinin and α_1-antitrypsin, the spectrum of camostat is relatively narrow and has no detectable effect on human neutrophil elastase or matriptase in vitro.[16] Whether this relative specificity is a strength or liability in the inflamed CF airway remains to be determined, but it did not prevent the detection of an effect with camostat administration despite elevated levels of human

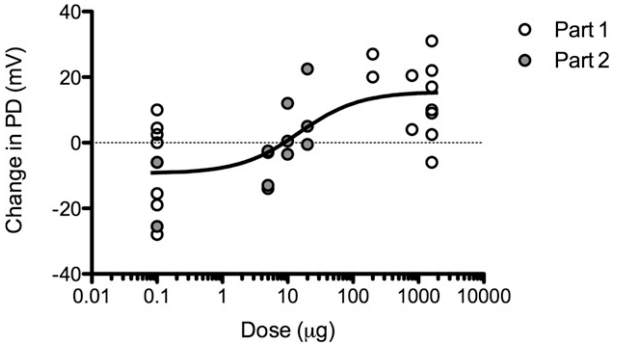

FIGURE 6. Dose-response relationship for camostat. The change in maximal basal PD is plotted for each individual subject for parts 1 and 2. The dose-response curve was obtained as a result of the Bayesian analysis. Placebo values were assigned a small (0.1 μg) value for graphical presentation in this figure (log scale). See Figure 3 legend for expansion of abbreviation.

neutrophil elastase activity in the nasal lavage of the present subjects with CF (mean 3.9 [95% CI 0.2-16.3] MeoSuc-Ala-Ala-Pro-Val AMC substrate/mg/4 h for CF placebo group predose vs undetectable in non-CF control group). Conversely, because the change with amiloride and Ringer PD did not completely normalize to within the range expected for patients without CF, it is possible that the results reflect a liability of a specific approach to block ENaC activation through prostasin.

Inhibition of CAPs represents just one of a number of approaches being pursued to target ENaC activity in CF. Other pharmaceutical approaches include long-acting amiloride derivatives that are intended to directly inhibit ENaC.[4] SPLUNC1 (short palate, lung, and nasal epithelial clone 1) is a recently identified protein that acts as a volume sensor of the airway surface and could be targeted to improve ASL depth in CF.[22,23] Broad-spectrum antiprotease therapy could also confer benefit on ENaC activation, aside from providing other desirable effects on the CF proinflammatory environment. Inhibition of CAPs along with other approaches that target ENaC relies on the principle that inhibition of ENaC will augment delayed mucociliary transport in CF. Given renewed questions regarding the role of sodium hyperabsorbtion in CF pathogenesis[24,25] and the failure of inhaled amiloride to confer benefit in prior human studies,[26,27] this hypothesis remains unresolved at present.

Camostat was generally well tolerated. Of the three SAEs reported, none were suspected to be related to the study medication. One subject experienced an asymptomatic episode of nasal mucosal disruption at the highest dose (1.6 mg), and an increase in the nasal examination rating score was observed. It is possible that 1.6 mg represents the upper limit of local tolerability. Whether this translates to potential toxicity when administered to the lung will require careful assessment, but according to the relatively broad dose response, targeting prostasin through this mechanism appears feasible. On the basis of the efficacy and tolerability demonstrated here and the beneficial effects of camostat on mucociliary clearance in vivo, inhibition of ENaC by blocking CAPs deserves further exploration as a therapeutic approach for CF.

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Author contributions: Dr Rowe had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Dr Rowe: contributed to the conception of the experiments, conduct of the research, data analysis, project supervision, and writing of the manuscript.
Ms Reeves: contributed to the conduct of the research, approved the submitted manuscript, and had an opportunity to provide edits as required.
Ms Hathorne: contributed to the conduct of the research, approved the submitted manuscript, and had an opportunity to provide edits as required.
Dr Solomon: contributed to the conduct of the research, approved the submitted manuscript, and had an opportunity to provide edits as required.
Dr Abbi: contributed to the data analysis, approved the submitted manuscript, and had an opportunity to provide edits as required.
Dr Renard: contributed to the data analysis, approved the submitted manuscript, and had an opportunity to provide edits as required.
Dr Lock: contributed to the data analysis, approved the submitted manuscript, and had an opportunity to provide edits as required.
Dr Zhou: contributed to the data analysis, approved the submitted manuscript, and had an opportunity to provide edits as required.
Dr Danahay: contributed to the conception of the experiments, data analysis, approved the submitted manuscript, and had an opportunity to provide edits as required.
Dr Clancy: contributed to the conception of the experiments, approved the submitted manuscript, and had an opportunity to provide edits as required.
Dr Waltz: contributed to the conception of the experiments, data analysis, project supervision, approved the submitted manuscript, and had an opportunity to provide edits as required.
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Additional information: The e-Appendix, e-Figure, and e-Table can be found in the "Supplemental Materials" area of the online article.

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