CHEST Online Supplement

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

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e-Appendix 1.

DESIGN

Part II subjects were to receive a dose of camostat or matched placebo in a blinded fashion on up to three occasions (Treatment Periods). Part II was initiated with a random assignment of 20 μg camostat and placebo in a 3:1 ratio. Allowable doses could subsequently be 20, 10, 5, 2, or 0.5 μg (placebo could also be assigned with a fixed probability of 25%). Doses were allocated using a Bayesian response-adaptive algorithm. An Emax model was assumed to characterize the camostat dose versus NPD response relationship. The dose-response analysis was updated five times during execution of Part II and the algorithm adapted the choice of doses in order to explore the dose-response relationship in an efficient manner (technically, this was achieved by choosing doses that seek to minimize variability on the potency (ED50) parameter of an Emax model). The actual doses administered in Part II are listed in Figure 1, Panel B.

METHODS

Pharmacokinetics (PK):

Blood, urine and nasal lavage samples were collected to assess the PK of camostat and its major metabolite QAY243. Blood and urine samples were collected in Part I of the study and the nasal lavage samples were collected in both Part I and II of the study. Where possible, the plasma PK parameters determined were AUC_{last}, C_{max} and T_{max} and the urine PK parameters determined were $Ae_{0\text{-}t}$, T_{max} and ER_{max}. Blood samplings were carried out pre-dose, and 5 min, 15 min, 30 min, 1 h, 2 h, 3 h and 6 h post-dose. Urine samples were collected at 0 to 3 and 3 to 6 h post-dose. Nasal lavage sampling times differed for Parts I and II of the study. In Part I nasal lavage fluid was

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collected at baseline and 3 h and 6 h post-dose in al treatment periods. In Part II nasal lavage fluid was collected at 3 h post dose in all treatment periods.

Analysis of camostat and QAY243 in plasma, urine and nasal lavage was performed using a validated LC/MSMS detection method using positive mode heated electrospray ionization (HESI). In all biological matrices, the lower limit of quantification (LLOQ) was 2 ng/mL expressed as the free base for camostat and 0.5 ng/mL expressed as the free base for QAY243.

The PK parameters of camostat and QAY243 were calculated using concentration-time data using non-compartmental methods with WinNonLin professional version 5.2. Rapid 100% conversion of camostat to QAY243 was assumed.

Safety Evaluation:

Safety and tolerability assessments included the monitoring and recording of all adverse Events (AEs) and serious adverse events (SAEs) and of concomitant medications/significant non-drug therapies, regular checks of routine blood chemistry, hematology and urine values, special laboratory tests, ECG recordings, measurements of vital signs and physical examinations. Safety assessments also included total nasal symptom score (TNSS) and Nasal examination rating scale (NERS).

Nasal Lavage

Nasal lavage was collected from the non-target nostril following installation and recovery of 5 ml of sterile PBS with a 10 ml syringe and nasal olive while the subject held their breath in a seated position. Leukocyte count and differential were measured by hemocytometer and Wright's stain, respectively. Neutrophil elastase activity was measured in batch by a fluorometric probe using a commercially available kit (EMD Millipore, Darmstadt, Germany).

Dose-response Analysis

Exploration of the dose-response relationship was conducted using the following Emax model:

$Yij=b0 + b0i + b1*(PREDij-mPRED) + Emax . DOSEij/(ED50 + DOSEij) + eij,$

where Yij denotes the response (change in maximal basal NPD from pre-dose to 2 hours post-dose) obtained at the jth occasion on the ith subject, PREDij denotes the corresponding maximal basal NPD pre-dose value and DOSEij

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the administered dose; mPRED is the mean of the PREDij's; b0, b1, Emax, and ED50 are model parameters; eij and b0i are random variables respectively representing within- and between-subject variability. Non informative or vague priors were assumed for all model parameters except ED50, which is a more sensitive parameter. The prior was chosen to somehow constrain ED50 in a reasonably small range (approximately 90% probability below 50 μg). Results shown in the paper correspond to the final analysis combining all data from Part I and Part II.

Statistical Analysis of Secondary Outcome Variables

The secondary NPD outcomes were the Ringer's PD, the change in PD with amiloride, change in PD with change zero [chloride] plus isoproterenol (Iso), and the change in PD with ATP at 2 h post-dose. Part I data were analyzed using an analysis of variance (ANOVA) model with treatment as factor and subject as random effect. The least squares mean (LSM) differences to placebo were reported with 80% CI.

Sample size calculation

The literature data indicated that the standard deviation (SD) of the change in maximal basal PD was approximately 12 mV and that the correlation of repeated maximal basal PD measurements on the same subject was approximately 0.3 (so that the SD of intra-individual differences was 12*√SQRT(2*(1-0.3)) = 14.2).[1,2] With six evaluable subjects at the maximum tolerated dose (MTD) of camostat, a one-sided t-test at α=0.1 had ca 85% power to detect a 15 mV difference between the mean maximal basal PD at 2h post-dose.

RESULTS

Pharmacokinetics:

Following intranasal administration, camostat was rapidly metabolized to QAY243. At all doses in Part I plasma and urine levels of camostat were below LLOQ following intranasal dosing of camostat. Plasma levels of QAY243 were below LLOQ following intranasal dosing of 0.2 mg camostat, but could be detected up to the sampling point of 0.5 h post dose in one out of two subjects following intranasal dosing of 0.8 mg camostat and up to the sampling point of 3 hr post dose in all seven subjects of the 1.6 mg dose group. Following intranasal administration of 1.6 mg camostat mean dose normalized AUClast for QAY243 was 2.873 (h*ng/mL)/mg. The mean dose normalized plasma Cmax for QAY243 was 1.85 (ng/mL)/mg, with the median Tmax occurring at 0.25h post dose. Urine levels of QAY243 were low in all dose groups in Part I following intranasal administration of camostat. The amount of QAY243 recovered over 6 hours (ng) increased with increasing dose. The average urinary excretion of QAY243 over 6 hours was low and less than 2% of the nominal camostat dose. Dose proportionality in nasal

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lavage concentrations for camostat and QAY643 was not observed and recovery of both in nasal lavage samples was low (< 1%). No clear relationship between dose and concentrations in nasal lavage could be made due to the low recovery of total administered dose and the small number of observations. Similarly, systemic levels were not correlated with PD findings.

Tolerability of intranasal camostat:

No deaths were reported and no subject was discontinued from the study due to adverse events (AEs). Three subjects experienced serious adverse events (SAEs) during the Part I of the study. Two subjects had CF pulmonary exacerbations after treatment with camostat at the 1.6 mg dose and one subject had appendicitis requiring appendectomy after placebo treatment. All the SAEs resolved without any clinical sequelae, and none of the SAEs were suspected to be related to study drug.

A slightly higher percentage of subjects treated with camostat 1.6 mg (85.7%) had AEs compared to the subjects treated with camostat 0.8 mg (50.0%), 5 μg (66.7%) and placebo groups (62.5%) (**Table 2**). None of the subjects who were treated with camostat 10 μg, 20 μg, and 0.2 mg reported AEs over the course of the study. A majority of the events were mild and judged by the investigator to be unrelated to the study. Six subjects had AEs which were considered to be related to the study drug, including epistaxis (mild), nasal mucosal disruption (1 out of 4+), and hematuria (all in the camostat 1.6 mg dose group); nasal sensitivity and rhinorrhea were also reported (both in camostat 0.8 mg dose group).

There were no meaningful changes in blood chemistry, hematology and urinalysis values, ECG recordings, measurements of vital signs and physical examination findings. There was no increase in sinus symptoms as assessed by the TNSS questionnaire at 1, 2, 3, and 6 h post-dose. The mean change in TNSS was 0.1 ± 0.7 for the camostat (1.6 mg dose group) whereas subjects treated with placebo had a -0.3 ± 0.5 mean change (P=NS). Similarly the change in NER 6 h post-dose was 1.1 ± 1.6 for the 1.6 mg dose group and 0.1 ± 0.8 for placebo (P=NS; positive changes associated with increased symptoms). The subject who experienced a transient change in NER was asymptomatic, and the observed change was not accompanied by an increase in TNSS.

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REFERENCES

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2 Clancy JP, Rowe SM, Bebok Z, et al. No detectable improvements in cystic fibrosis transmembrane conductance regulator by nasal aminoglycosides in patients with cystic fibrosis with stop mutations. Am J Respir Cell Mol Biol 2007; 37:57-66

e-Table 1. Number (%) of patients with adverse events by treatment group and preferred term for Parts I and II for all AEs noted in at least one subject assigned to active drug. Footnote: No AEs were reported for subjects treated with camostat 10 µg, 20 µg, or 0.2 mg. Only AEs that occurred at or after the first study drug intake were included. An AE starting in one Treatment Period and continuing into the next Treatment Period was counted only in the onset Treatment Period. N = number of subjects/periods studied and n = number of subjects/periods with at least one AE on the category.

	QAU145 5 µg N = 3 n (%)	QAU145 0.8 mg N = 2 n (%)	QAU145 1.6 mg N = 7 n (%)	Placebo N = 8 n (%)
Subjects with AE(s)	2 (66.7)	1 (50.0)	6 (85.7)	5 (62.5)
Preferred term				
Condition aggravated	0 (0.0)	0 (0.0)	2 (28.6)	0 (0.0)
Epistaxis	0 (0.0)	0 (0.0)	1 (14.3)	1 (12.5)
Hemoptysis	0 (0.0)	0 (0.0)	1 (14.3)	1 (12.5)
Hematuria	0 (0.0)	0 (0.0)	1 (14.3)	1 (12.5)
Infusion site pain	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)
Musculoskeletal chest pain	0(0.0)	0 (0.0)	1 (14.3)	0 (0.0)
Nasal mucosal disorder	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)
Oopharyngeal pain	1 (33.3)	0 (0.0)	0 (0.0)	1 (12.5)
Pain in extremity	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Paranasal sinus hypersecretions	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Rhinorrhea	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)
Sensory disturbance	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)

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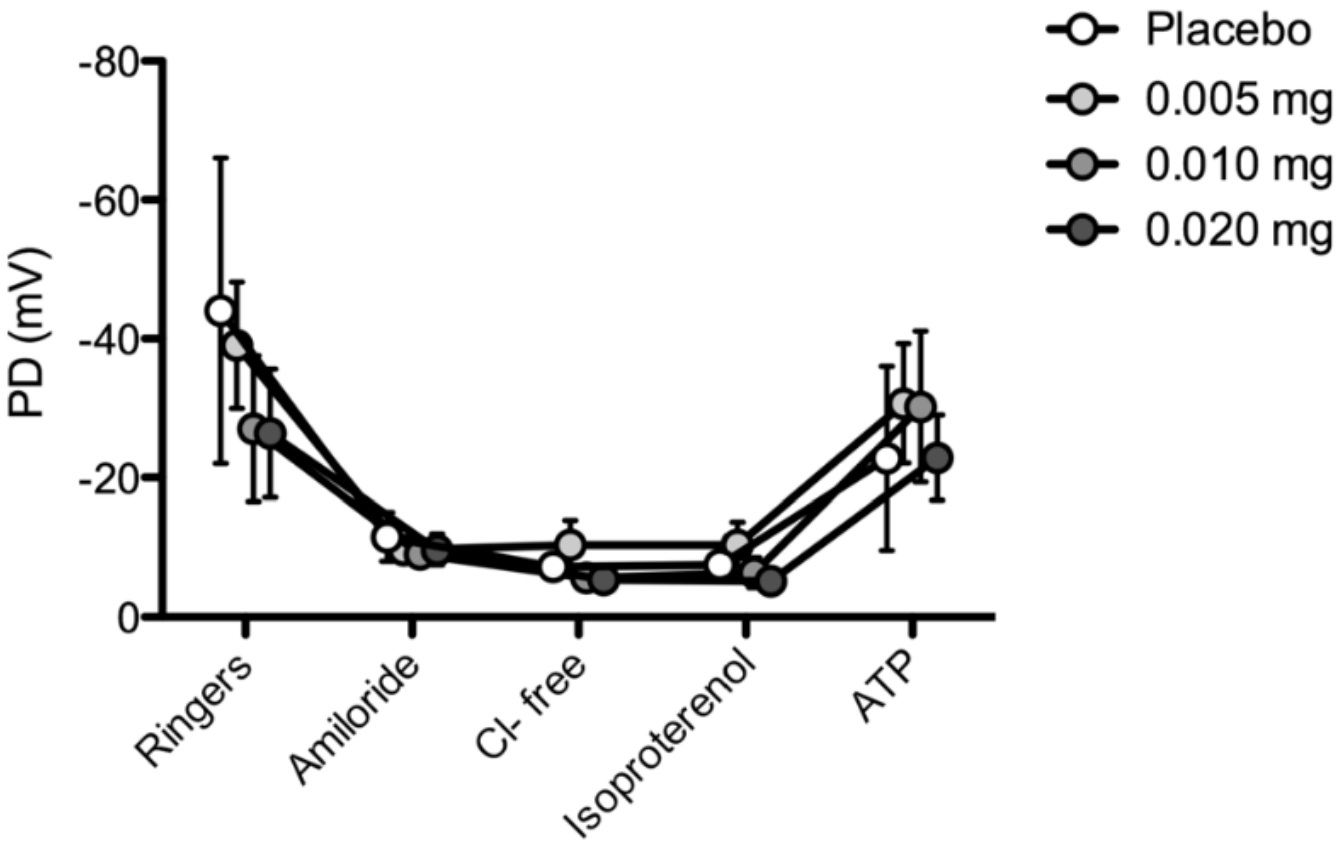
e-Figure 1. Effect of various doses of camostat on nasal potential difference tracings (Part 2). Tracings were obtained in the target nostril ~2 hours following intranasal administration of camostat or placebo, as also shown in Figure 4.



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