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ARTICLE

A phase I study of high dose camostat mesylate in healthy adults provides a rationale to repurpose the TMPRSS2 inhibitor for the treatment of COVID-19

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Abstract

Camostat mesylate, an oral serine protease inhibitor, is used to treat chronic pancreatitis and reflux esophagitis. Recently, camostat mesylate and its active metabolite 4-(4-guanidinobenzoyloxy)phenylacetic acid (GBPA) were reported to inhibit the infection of cells by severe acute respiratory syndrome coronavirus 2 by inhibiting type II transmembrane serine protease. We conducted a phase I study to investigate high-dose camostat mesylate as a treatment for coronavirus disease 2019. Camostat mesylate was orally administered to healthy adults at 600 mg 4 times daily under either of the following conditions: fasted state, after a meal, 30 min before a meal, or 1 h before a meal, and the pharmacokinetics and safety profiles were evaluated. In addition, the time of plasma GBPA concentration exceeding the effective concentration was estimated as the time above half-maximal effective concentration (EC_{50}) by using pharmacokinetic/pharmacodynamic modeling and simulation. Camostat mesylate was safe and tolerated at all dosages. Compared with the fasted state, the exposure of GBPA after a meal and 30 min before a meal was significantly lower; however, no significant difference was observed at 1 h before a meal. The time above EC_{50} was 11.5 h when camostat mesylate 600 mg was administered 4 times daily in the fasted state or 1 h before a meal. Based on the results of this phase I study, we are currently conducting a phase III study.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Camostat, an oral serine protease inhibitor, is used to treat chronic pancreatitis and reflux esophagitis. Recently, camostat and its active metabolite were reported to inhibit the infection of severe acute respiratory syndrome coronavirus 2 to cells by inhibiting type II transmembrane serine protease.

WHAT QUESTION DID THIS STUDY ADDRESS?

The recommended dosage and regimen for a phase III study of coronavirus disease 2019 (COVID-19).

TRIAL REGISTRATION: NCT04451083.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

For the treatment of COVID-19, higher and more frequent doses than those currently approved should be considered. Camostat mesylate 600 mg 4 times daily (q.i.d.) was safe and well-tolerated under all dosing conditions tested. Food intake reduced the plasma exposure, but there was no significant difference in plasma exposure between administration under fasted conditions and 1 h before a meal. When 600 mg q.i.d. was administered, plasma concentrations are expected to exceed the half-maximal effective concentration of 11.5 h.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Camostat mesylate 600 mg q.i.d. in the fasted state/1 h before a meal was selected as the dosage and regimen of our phase III study of COVID-19.

INTRODUCTION

Infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the novel coronavirus infection that was first identified in December 2019 in Wuhan, Hubei Province of the People's Republic of China. As of December 7, 2020, this virus had infected more than 65.8 million cases worldwide and caused more than 1.5 million deaths.[1] SARS-CoV-2 is a type of coronavirus classified as a positive-sense single-stranded RNA virus, whose spike protein (S protein) binds to angiotensin-converting enzyme II (ACE2), a functional receptor on the host cell membrane. Subsequently, the S protein is cleaved to S1 and S2 by host-derived protease activity. The S1 fragment binds to ACE2 and the S2 fragment is cleaved by type II transmembrane serine protease (TMPRSS2) on the host cell membrane to promote the fusion of the viral envelope (outer membrane) with the cell membrane.[2] It has been reported that ACE2 and TMPRSS2 are essential in airway epithelial cells for infection of coronavirus including coronavirus disease 2019 (COVID-19).[3–9]

In March 2020, Hoffmann et al. reported that the serine protease inhibitor camostat mesylate, dimethylcarbamoylmethyl 4-(4-guanidinobenzoyloxy)phenylacetate monomethanesulfonate, inhibited TMPRSS2 and prevented SARS-CoV-2 infection of a cell line derived from human airway epithelial cells (Calu-3).[2] Camostat mesylate is an oral serine protease inhibitor used in Japan and South Korea for the treatment of acute symptoms related to chronic pancreatitis and the treatment of postoperative reflux esophagitis.[10] Camostat mesylate was launched in 1985 and has been in clinical use for over 35 years. As pharmacokinetic and pharmacodynamic (PK/PD) characteristics of camostat mesylate, rapid metabolism is noted. Camostat mesylate is rapidly metabolized to the active metabolite 4-(4-guanidinobenzoyloxy)phenylacetic acid (GBPA) by the esterase after oral administration and is not observed in the plasma. Camostat mesylate and GBPA have similar protease inhibitory activities.[11–14] Although camostat mesylate has long been suggested to be effective against Middle East respiratory syndrome and severe acute respiratory syndrome coronaviruses based on in vitro studies,[3,4,8,15] no clinical trials have been conducted to investigate its effect in these infectious diseases. However, about 1 month after the report by Hoffman et al., Aarhus University in Denmark announced that it would start clinical trials to evaluate the efficacy of camostat mesylate for COVID-19 (NCT04321096). Subsequently, several medical institutions and universities announced the start of clinical trials of camostat mesylate for patients with COVID-19. As of December 9, 2020, there have been 17 trials including our phase III study (NCT04657497) that have been registered on Clinical trial.gov in 10 countries including, the United States and European countries. In addition, the results of in vitro studies have also been updated. Hoffman et al. reported the half-maximal effective concentration (EC_{50}) of camostat mesylate,[16] followed by the EC_{50} of GBPA.[17]

In June 2020, with growing expectations, Ono Pharmaceutical decided to develop camostat mesylate as a therapeutic candidate for COVID-19. At the start of development, PK/PD preliminarily modeling and simulation analyses were performed using past phase I data (in-house data[14]) to estimate the dosage to be used in a phase III study. For the PK/PD analysis to estimate efficacy, the time when the plasma GBPA concentration is above the EC_{50} value (time above EC_{50}) was used. On the basis of the simulation results, safety, and adherence, we hypothesized that camostat mesylate 600 mg 4 times daily was an appropriate regimen candidate for the phase III study. However, the currently approved maximum dose of camostat mesylate is 200 mg 3 times daily; a camostat mesylate dose of 600 mg 4 times daily has not been used in previous clinical trials. Furthermore, no study has reported whether food intake affects the PK of camostat mesylate. Therefore, prior to the phase III study, we conducted a phase I study to: (1) clarify the safety, tolerability, and PK of camostat mesylate 600 mg administered to healthy adults 4 times daily; (2) explore the impact of food on the PK of camostat mesylate; and (3) update PK/PD modeling and simulation to estimate the time above EC_{50} in various regimens.

METHODS

Ethics

This phase I study was conducted in accordance with the study protocols, Good Clinical Practice, and the Declaration of Helsinki, and is registered on ClinicalTrials.gov (identifier: NCT04451083). All subjects provided written informed consent. The study protocols were approved by independent ethics committees.

Study design

This was an open-label phase I study to assess the safety, tolerability, and PK of multiple doses of 600 mg camostat mesylate 4 times daily (q.i.d.) in healthy Japanese male subjects. Eligible subjects were healthy Japanese males, aged 18–45 years, with normal body weight (body mass index ≥18.0 and ≤25.0 kg/m^2). The schedules for dosing and PK sampling are shown in Figure 1. Camostat mesylate 600 mg (100 mg tablet ×6) was taken with 200 ml of water in the morning (9 a.m.), at midday (1 p.m.), in the evening (5 p.m.), and at night (9 p.m.) on day 1 and day 3 through to day 9 (no administration on day 2). This study consisted of two cohorts. In cohorts 1 and 2, the night dose was administered under fasted conditions. In cohort 1, the morning dose of day 1 was administered under fasted conditions, and the remaining daytime doses were administered after a meal. In cohort 2, the morning, midday, and evening doses of day 1 were administered 30 min before a meal, and the remaining daytime doses were administered 1 h before a meal.

PK assessment

The PK analysis set included all participants in the safety set with calculable PK data and without protocol deviations that might have affected PK data.

The plasma concentrations of camostat were not evaluated, but the plasma concentrations of GBPA were determined for PK assessment. The plasma concentrations of camostat were thought to be undetectable because camostat mesylate is metabolized rapidly with a half-life in human plasma within 1 min.[13] To determine GBPA plasma concentrations, blood was collected from each subject at day 1, before the first dose and then 0.33, 0.67, 1, 1.5, 3, 4, and 24 h after the first dose, and on days 3 and 9, before the morning dose, then 0.33, 0.67, 1, 1.5, 2, 3, 4, 8, 12, and 24 h after the morning doses on days 3 and 9, respectively.

Plasma PK parameters of GBPA were calculated by noncompartmental analyses using Phoenix WinNonlin (version 7.0; Certara USA Inc.). The maximum observed concentration (C_{max}) and time to reach C_{max} (T_{max}) were generated by Phoenix WinNonlin from the observed concentration–time data. The area under the concentration–time curve (AUCs) values were calculated using the linear-trapezoidal method for ascending concentrations and the log-trapezoidal method for descending concentrations. At least three consecutive timepoints in the terminal phase (excluding the T_{max}) were used to determine the apparent terminal elimination rate constant (lambda z) so that the apparent terminal half-life ($t_{1/2}$) could be calculated with a meaningful value ($t_{1/2}$ = ln2/lambda z). Descriptive statistics were calculated for plasma concentrations of GBPA and derived PK parameters. The mean plasma concentration-time profile was plotted. The effects of food intake were evaluated with a mixed-effects model using natural log (ln)-transformed values for C_{max} and AUC_{24h}. The least squares mean for differences among dosing conditions and its 95% confidence intervals were constructed for the ln-scale values of each parameter and back-transformed and expressed as the geometric mean ratio.

Modeling and simulation

GBPA concentrations obtained from the current and previous phase I studies were used for population PK (PopPK) modeling.

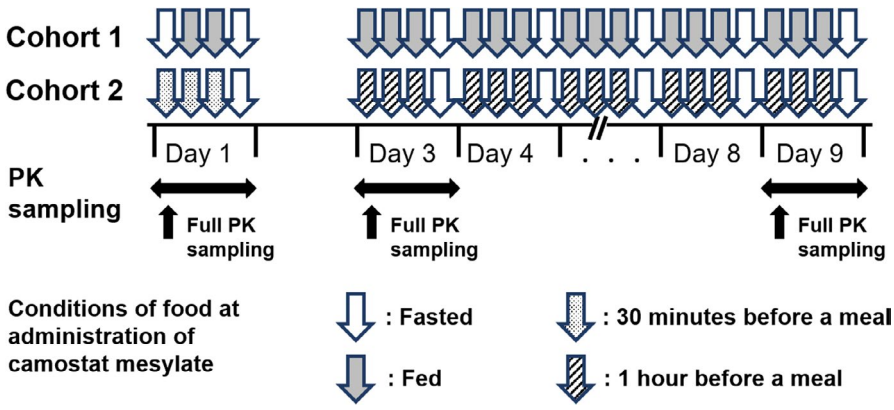

FIGURE 1 Study design of a phase I study in healthy subjects. Camostat mesylate 600 mg was administrated four times per day under the described conditions. PK, pharmacokinetic

In the previous phase I study, a single dose of camostat mesylate 200 mg or 600 mg ($n = 5$ per dose) was administered to healthy adult men under fasted conditions, and GBPA plasma concentrations were determined at 0.33 (200 mg only), 0.67, 1.33, 2, 3, 5, and 7 h after administration.[14] PopPK analysis was performed using plasma GBPA concentrations at 372 points obtained from 14 subjects in the current phase I study and 47 points obtained from 10 subjects in the previous phase I study.

PopPK models were developed using NONMEM (version 7.4.1; ICON Development Solutions). The PPK of GBPA was investigated in a stepwise manner. All models were fitted using the first-order conditional estimation method with the interaction option. The conversion process from camostat mesylate to GBPA was not investigated because camostat mesylate is thought to be rapidly converted to GBPA and thus to become undetectable. To estimate the PopPK parameters for GBPA, the doses were converted into the GBPA base equivalent by molecular weight ratio (GBPA/camostat mesylate). One- and two-compartment models with linear elimination were evaluated to describe the disposition of GBPA with first-order rate constant absorption. In terms of the residual variability, the additive error model, proportional error model, or a combination of these were tested. Between-subject variability and interoccasion variability (IOV) in the model parameters were evaluated assuming that they were log-normally distributed. Because this was a healthy subject study, no covariates were evaluated except for dosing conditions in the current and previous studies. Model determination was guided by the minimum objective function value, Akaike Information Criterion, and visual inspection of diagnostic plots. The adequacy of the PK model to describe both populations was assessed by a visual predictive check.

After the final PopPK model for GBPA was established, the plasma GBPA concentrations at steady-state were predicted at various dosages and regimens of camostat mesylate. The time above EC_{50} was calculated as the time where the predicted concentration exceeded the EC_{50} value of GBPA that inhibited SARS-Cov-2 infection, which was 178 nM.[17] The time above EC_{50} was plotted against daily doses and the relationship between the dose level and time above EC_{50} was evaluated using a power model.

Bioanalytical methods

Plasma samples were analyzed by reversed-phase liquid chromatography-tandem mass spectrometry (LC-MS/MS). To 100 µl of each plasma sample, 500 µl of ethanol was added for protein precipitation. After shaking with MixMate (Eppendorf) for 30 s and centrifugation at 2000 × *g* for 3 min at 4°C, 60 µl of supernatant was mixed with 20 µl of the internal standard working solution. Following the filtration of the whole mixture through a MultiScreen Solvinert filter plate (0.45 µm, Merck Millipore) at 720 × *g* for 2 min, the filtrates were diluted with 420 µl of water and then subjected to LC-MS/MS analysis.

LC-MS/MS analysis was performed using the Nexera HPLC system (Shimadzu Corporation) coupled to QTRAP 5500 (AB SCIEX) in the positive ion mode. Chromatographic separation was achieved using a two-solvent gradient consisting of 0.1% formic acid in water (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B) with ACQUITY UPLC BEH C18 (2.1 mm i.d. ×50 mm, particle size 1.7 µm; Waters) at a column temperature of 40°C. The flow rate was set at 0.40 ml/min. Initially, the ratio of mobile phase B was set at 15% for 2 min. Thereafter, the ratio of mobile phase B was rapidly increased to 90% in 0.1 min, and held for 3 min for column washing. The ratio of mobile phase B was then decreased to 15% in 0.1 min, and held for 5 min. Quantification was carried out in the multiple reaction monitoring mode. GBPA was monitored at a transition

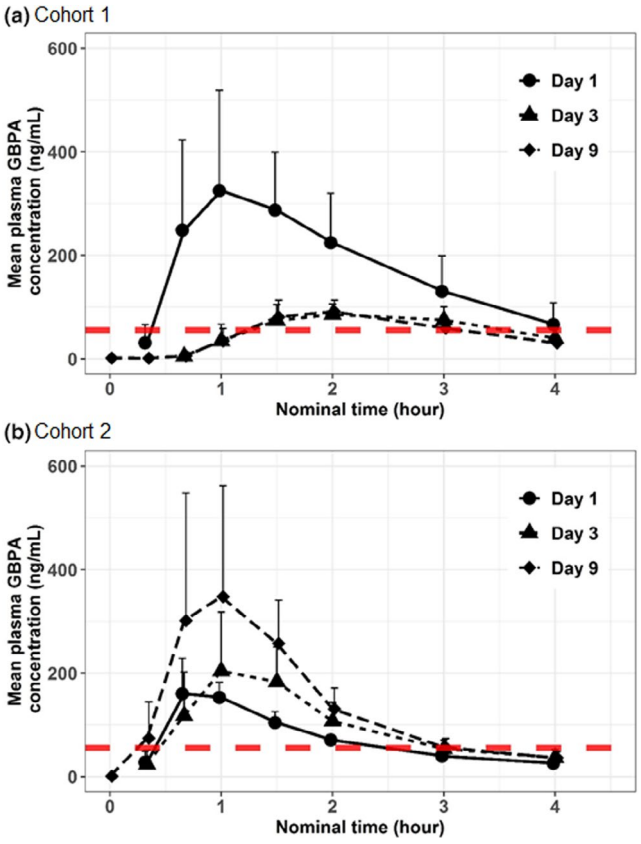

FIGURE 2 GBPA concentration–time profiles in (a) cohort 1 and (b) cohort 2. Data are the arithmetic mean ± SD from the pharmacokinetic analysis set. In some samples, GBPA concentrations were below the lower limit of quantification (1.00 nmol/L). Broken lines represent EC_{50} of half-maximal effective concentration.[17] EC_{50}, half-maximal effective concentration; GBPA, 4-(4-guanidinobenzoyloxy)phenylacetic acid

TABLE 1 Summary statistics for the plasma PKs of GBPA in plasma following a single oral dose of 600 mg camostat mesylate

	Cohort 1			Cohort 2		
	Day 1 Fasted	Day 3 Fed	Day 9 Fed	Day 1 30 min before a meal	Day 3 1 h before a meal	Day 9 1 h before a meal
C_{max} (ng/ml)	371 (165)	95.8 (25.2)	93.5 (22.1)	177 (54.5)	218 (99.9)	367 (209)
T_{max} (h)	1.00 (0.667–2.00)	2.00 (1.50–3.00)	2.00 (1.50– 2.00)	1.00 (0.667–1.00)	1.00 (1.00–3.92)	1.00 (0.667–1.50)
AUC4h (ng*h/ml)	683 (241)	206 (56.0)	194 (48.2)	227 (45.1)	366 (139)	556 (268)
$t_{1/2}$ (h)	1.58 (1.70)[a]	1.33 (NC)[b]	1.16 (0.150)[c]	1.35 (0.346)	1.05 (0.245)[a]	0.962 (0.157)
Comparison	Geometric mean ratio (95% confidence interval)					
C_{max} against fasted	——	0.28 (0.18, 0.42)	0.27 (0.17, 0.42)	0.50 (0.31, 0.86)	0.57 (0.30, 1.08)	0.95 (0.51, 1.75)
AUC4h against fasted	——	0.31 (0.23, 0.43)	0.30 (0.21, 0.43)	0.43 (0.29, 0.64)	0.53 (0.31, 0.91)	0.79 (0.46, 1.37)

Note: Data are expressed as the arithmetic mean (SD) from the PK analysis set, except that T_{max} is expressed as the median (range).

Abbreviations: AUC4h, area under the plasma concentration–time curve in the dosing interval; C_{max}, maximum observed plasma concentration; GBPA, 4-(4-guanidinobenzoyloxy)phenylacetic acid; NC, not calculated; PK, pharmacokinetic; $t_{1/2}$, terminal half-life; T_{max}, time to reach C_{max}.

$n = 7$, [a]$n = 6$, [b]$n = 2$, [c]$n = 3$.

of m/z 314 to 145 and the internal standard was monitored at m/z 255 to 120. This method was validated for selectivity, accuracy, precision, dilution integrity, and stability of GBPA in plasma.

Safety assessments

Safety was assessed throughout the study by monitoring subjective symptoms, physical examination, vital signs (blood pressure/pulse rate [supine position], body temperature, percutaneous oxygen saturation, and body weight), laboratory tests (hematology, blood biochemistry, blood coagulation test, and urinalysis [spot urine]), electrocardiograms (ECGs: 12-lead ECG and monitor ECG), and adverse events. The safety analysis included all participants who received at least one dose of camostat mesylate.

RESULTS

Study population

All participants were included in the safety and PK analysis sets. Seven subjects were enrolled into each cohort, and all 14 subjects completed the study with a mean (SD) age of 24.9 (4.9) years in cohort 1, 29.6 (4.7) years in cohort 2, and 27.2 (5.3) years for the whole study group. The mean (SD) body weight and body mass index in cohort 1 were 62.31 (6.37) kg and 21.36 (1.27) kg/m^2, 64.86 (6.23) kg and 22.46 (1.90) kg/m^2 in cohort 2, and 63.59 (6.20) kg and 21.90 (1.66) kg/m^2 in the whole study group.

Pharmacokinetics

The plasma concentration-time curves of GBPA are shown in Figure 2 and the summary of PK parameters is described in Table 1. The C_{max} of GBPA occurred with the median T_{max} of 1 h when camostat mesylate was administered under fasted conditions, at 30 min before a meal, and at 1 h before a meal. The T_{max} was delayed under fed conditions with a median T_{max} of 2 h. Plasma concentrations rapidly declined following the C_{max}, on each day, and the mean $t_{1/2}$ was in the range 0.962 to 1.58 h in the plasma concentration-time profile after the morning dose. The values of C_{max} and AUC obtained when camostat mesylate was administered after a meal and at 30 min before a meal were not more than 50% of the values obtained after the administration under fasted conditions. The geometric mean ratios of the C_{max} and AUC at 1 h before a meal on days 3 to those under fasted conditions were 0.57 (0.30 to 1.08) and 0.53 (0.31 to 0.91), and the geometric mean ratios of the C_{max} and AUC at 1 h before a meal on day 9 to those under fasted conditions were 0.95 (0.41 to 1.75) and 0.79 (0.46 to 1.37). The C_{max} and AUC values obtained after the repeated administration of camostat mesylate at 1 h before a meal were comparable with those under fasted conditions.

Modeling and simulation

The PK profile of GBPA was best described by a two-compartment disposition model with a first-order absorption model with a lag time and first-order elimination. The IOV was estimated on absorption rate constant and relative bioavailability, and the residual variability was modeled with a proportional error model. Between-subject variability was not significantly incorporated in any population parameters, suggesting that the difference of dosages or previous and present studies would not affect the pharmacokinetics of GBPA in the range of 200 to 600 mg. We evaluated the effects of different dosing conditions on PK parameters considering the IOV. The administration of camostat mesylate under fed conditions was incorporated in the relative bioavailability, and the administration under fed conditions and at 30 min before a meal was incorporated in the absorption rate constant with the same magnitude of effect. The administration at 1 h before a meal did not affect any of the PopPK parameters. All population parameters were well estimated with relative standard errors below 50% for the fixed effect parameters. Parameter estimates with relative standard errors for the final PopPK model are shown in Table 2. Overall, the goodness-of-fit plots presented in Figure S1 show good agreement between the observed and predicted data. The plots of conditional weighted residuals versus time or versus population prediction showed a random distribution of data points around the zero line. The distribution of the conditional weighted residuals indicated no major deviation from normality. The results of the visual predictive check presented in Figure 3 confirmed that the model captured the central tendency and the IOV of the PK of GBPA.

The predicted times above EC_{50} for various dosing regimens are shown in Table 3, and the relationship between the dosage and the predicted time above EC_{50} at steady-state when camostat mesylate was administered 4 times per day at 4-h intervals under fasted conditions is shown in Figure 4. The time above EC_{50} was estimated to be shorter when camostat mesylate was administered 30 min before a meal than those for other dosing conditions. Along with the prolongation of dosing intervals, the time above EC_{50} under fed conditions tended to be shortened whereas that under fasted conditions (or administration at 1 h before a meal) was over 11 h regardless of the dosing intervals.

TABLE 2 Population PK parameter estimates for GBPA in plasma

Parameter	Estimate	RSE (%)
Fixed effect parameters		
Absorption rate constant (KA) [/h]	5.91	49.2
Clearance (CL) [L/h]	680	9.78
Volume of distribution of the central (V2/F) [L]	904	8.83
Intercompartmental clearance (Q) [L/h]	25.6	15
Volume of distribution of the peripheral (V3/F) [L]	151	19.9
Lag time [h]	0.319	2.39
Covariate effect parameters		
Food effect against fasted on KA; $KA \times \theta^1_{FOOD}$		
Fed	0.0807	42.9
30 min before a meal	1.0 FIXED	–
1 h before a meal	1.0 FIXED	–
Food effect against on FA; $FA \times \theta^2_{FOOD}$		
Fed	0.527	9.17
30 min before a meal	0.527	9.17
1 h before a meal	1.0 FIXED	–
Random effect parameters		
Interoccasion variability (IOV)		
IOV on KA [% CV]	105	22.7
IOV on FA [% CV]	35.8	10.7
Intra-subject variability		
Proportional residual error [% CV]	0.39	4.46
Proportional additional error [SD]	–	–

Abbreviations: CV, coefficient of variation; GBPA, 4-(4-guanidinobenzoyloxy) phenylacetic acid; PK, pharmacokinetic; RSE, relative standard error.

Safety

Multiple administration of camostat mesylate 600 mg q.i.d. was well-tolerated and no safety concerns were raised. There were no serious adverse events or deaths. Only one adverse event occurred in each cohort (1 of 7 participants [14.3%] in each cohort). Both adverse events (hyperuricemia [one event, one participant] in cohort 1, and aphthous ulcer [one event, one participant] in cohort 2) were mild and did not result in withdrawal from the study. No individual ECG finding was reported as an adverse event, and there were no clinically significant changes in the heart rate, or PR and QRS intervals, or in ECG diagnostic analyses. No subject had a corrected QT Fridericia's formula (QTcF) interval greater than 480 ms at any postbaseline visit, and there were no instances of a change from baseline of greater than 60 ms.

DISCUSSION

In 2020, camostat mesylate and its active metabolite, GBPA, were reported to inhibit TMPRSS2 indicating their potential as therapeutic drugs for COVID-19.[2,5,9,16–18] Ono

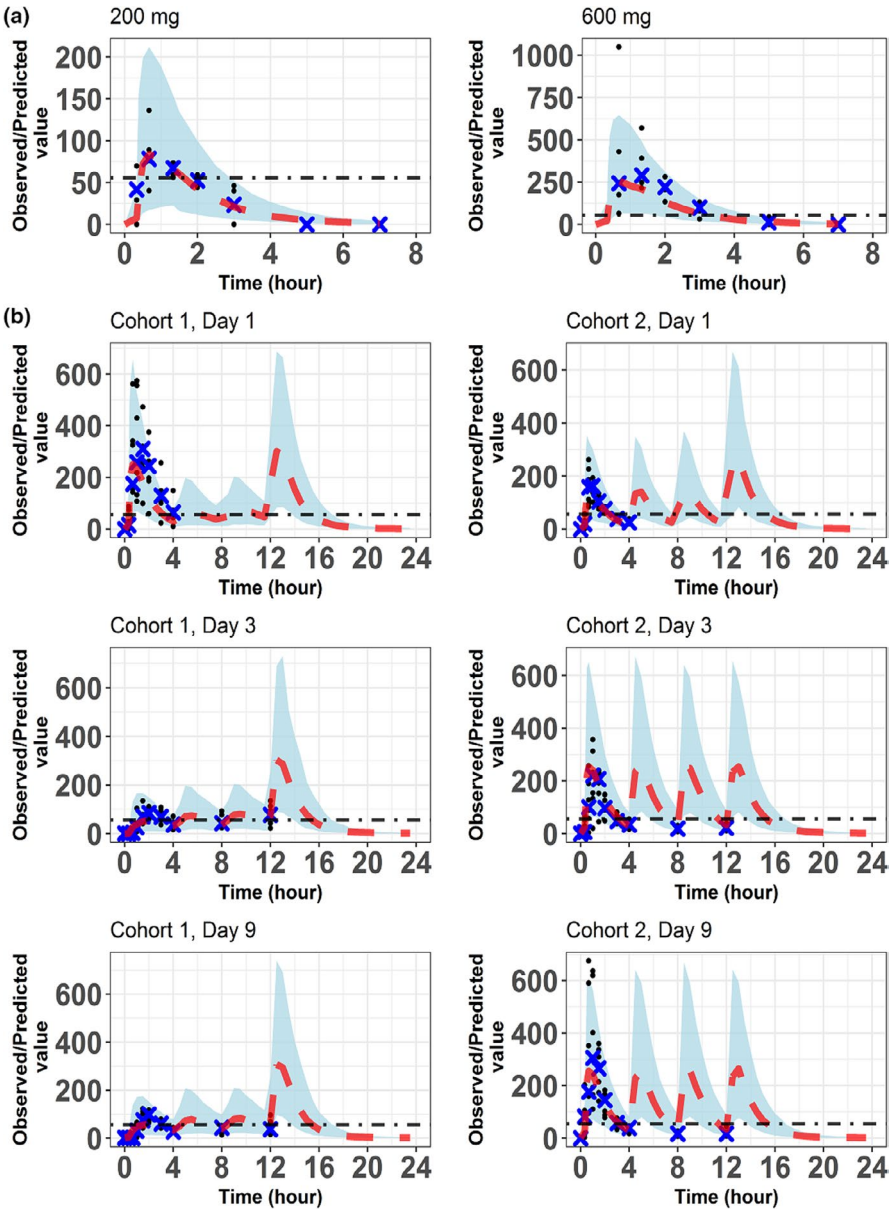

FIGURE 3 Visual predictive check for the final GBPA population pharmacokinetic model. Shaded areas represent the 5th, 50th, and 95th percentiles of the observed data. Dashed lines represent the median value of the simulated data. Dot dash lines represent EC_{50} of GBPA.[17] EC_{50}, half-maximal effective concentration; GBPA, 4-(4-guanidinobenzoyloxy)phenylacetic acid. (a) phase I single dose study,[14] (b) phase I multiple dose study

Pharmaceutical planned a phase III study of camostat mesylate targeting patients with COVID-19. Prior to the phase III study, we conducted a phase I study to set the dosage and treatment regimen in the phase III study, namely, the dose, dosing frequency, and food intake conditions.

An important PK feature of camostat mesylate is that when orally administered, it is rapidly metabolized by esterases.[11,13,14] Camostat mesylate is not detectable in the plasma but rather is present as the active metabolite GBPA. In addition, GBPA is rapidly eliminated with a half-life of less than 2 h. Therefore, frequent dosing is required to maintain effective plasma concentrations. Based on its mechanism of action, the target patient population of camostat mesylate is assumed to include patients with asymptomatic, mild, and moderate COVID-19 who is requiring no supplemental oxygen. Considering the adherence of the target patient population, we assumed the q.i.d. administration of camostat mesylate comprising of dosing in the morning, at midday, in the evening, and before bedtime would be acceptable. Specifically, the time above EC_{50} for the same daily dose 3 times daily and 4 times daily are compared in Table 3. The time above EC_{50} of camostat mesylate 800 mg 3 times daily and 600 mg 4 times daily was 9.8 h and 11.5 h, respectively. The results of PK/PD simulations also suggested the advantage of increasing the dosing frequency rather than increasing the dose per administration.

Another important PK feature of camostat mesylate is that its bioavailability is markedly affected by food intake. Although camostat mesylate is soluble,[10] its chemical structure suggests poor permeability through the gastrointestinal mucosa. Compounds with such physicochemical properties belong to class 3 of the Biopharmaceutics Classification System-based Biowaivers.[19] There is a report that exposure is reduced by administration after a meal in 61% of class

TABLE 3 Predicted value of time above EC_{50} for various dosages and regimens

Regimen and dosing interval		Dose (mg)	Dose/day (mg/day)	Time above EC_{50} (h)		
				Fasted and 1 h before a meal[d]	30 min before a meal	Fed
t.i.d.[a]	4[c]	200	600	4.0	0.5	0.0
		300	900	5.8	2.9	0.0
		400	1200	6.8	4.3	1.6
		600	1800	8.6	5.9	8.1
		800	2400	9.8	7.1	11.4
q.i.d.[b]	4[c]	200	800	5.4	2.0	1.5
		300	1200	7.8	4.8	2.1
		400	1600	9.3	6.6	4.3
		600	2400	11.5	8.8	11.5
		800	3200	13.1	10.3	14.6
	5[c]	600	2400	11.5	8.8	10.2
	6[c]	600	2400	11.2	8.8	9.8

[a]Three administrations per day.

[b]Four administrations per day.

[c]Dosing interval (h).

[d]No difference was detected between dosing under fasted conditions and at 1 h before a meal. Half-maximal effective concentration is the estimated concentration to inhibit the cell entry of the S protein of severe acute respiratory syndrome coronavirus 2.

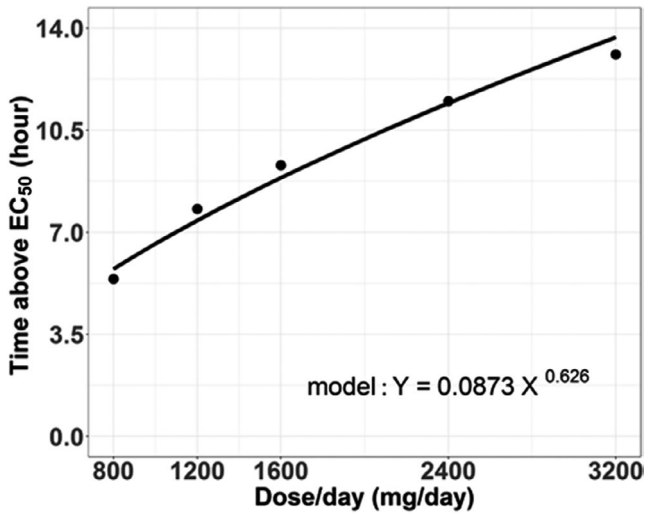

FIGURE 4 The relationship between the daily dose and time above EC_{50} after the administration of camostat mesylate four times daily at 4-h intervals under fasted conditions or at 1 h before a meal. The y-axis represents the time above EC_{50}, and the x-axis represents the daily dose. The estimated value of the time above EC_{50} is plotted for each daily dose. The bold line represents the result of power model fitting. EC_{50}, half-maximal effective concentration

3 compounds, although the reduction is about 20% to 30% in most cases.[20] Therefore, we did not expect that camostat mesylate would be affected by food intake to such a great extent. The study design for cohort 1 was only designed to assess the effect of food as a cautionary measure. Cohort 2 was added as an emergency in response to the results of cohort 1. The cause of this large food effect is unknown, but the following factors might be involved: (1) camostat mesylate forms micelles with bile acids, which reduces the partitioning of camostat mesylate into the gastrointestinal mucosa,[21] (2) camostat mesylate is adsorbed by the dietary components,[21] (3) metabolism in the gastrointestinal tract is enhanced with the prolongation of residence time in the gastrointestinal tract,[21] and (4) camostat mesylate reacts with digestive enzymes.[22]

Regarding the timing of dosing in the phase III study, it is not realistic to set all dosing under fasted conditions because q.i.d. dosing is assumed. Although the impact was not as great as that when administered after a meal, the exposure of GBPA when administered 30 min before a meal was still greatly affected by food. The interpretation of the results of the administration of camostat mesylate 1 h before a meal is complex. The exposure of cohort 2 on day 9 (seventh day of administration 1 h before a meal) was similar to that after administration under fasted conditions, and the effect of food was negligible. However, the exposure of cohort 2 on day 3 (first day of administration 1 h before a meal) was ~ 53% of that under fasted conditions. At 1 h after the first dosing, the conditions of administration 1 h before a meal were the same as that when administered under fasted conditions. Thus, the exposure of administration 1 h before a meal and under fasted conditions should theoretically be the same up to 1 h after administration. It is unknown why the exposure on day 3 up to 1 h after administration was approximately one-half of that after administration under fasted conditions. In the PopPK analysis using all the data including

day 3 and day 9, administration of camostat mesylate 1 h before a meal did not significantly affect the absorption rate based on administration under fasted conditions. Therefore, the effect of food can be concluded as follows. When administered after a meal and at 30 min before a meal, the exposure of GBPA was significantly decreased by food. When administered at 1 h before a meal, the exposure tended to be decreased to a lesser extent, but the effect of food was not significant. Based on this result, we judged it appropriate to set the administration timing for the phase III study as fasted administration or administration at least 1 h before a meal. In the current study, the time above EC_{50} was 11.5 h for administration after a meal and 1 h before a meal. However, the exposure was remarkably decreased after a meal. Although we used the time above EC_{50} as the efficacy index, whether this index alone correlates with efficacy is unknown. Therefore, we concluded that postprandial administration, which greatly reduces exposure, should be excluded from the dosage regimen.

Multiple administrations of camostat mesylate 600 mg q.i.d. were well-tolerated. There were no clinically significant findings in safety laboratory values, vital signs, or ECG parameters. However, in a repeated-dose toxicity study in dogs, camostat 300 mg/kg decreased body weight and food intake, induced vomiting and effects on the gastrointestinal tract, including gastrointestinal injury, and caused death, with a no-observed adverse effect level (NOAEL) of 100 mg/kg.[23] The human equivalent dose converted from the dog NOAEL is 3333 mg.[24] Based on the results of this animal study, 800 mg q.i.d. might also be a candidate dosing regimen for the phase III study. Even if a drug is tolerated in a phase I study, the higher the dose, the greater the risk of unexpected side effects when administered to a broader population. This is especially important when considering a therapeutic drug for COVID-19 because the target population is global. The selection of a dose with a narrow margin to NOAEL may increase the risk. Furthermore, the relationship between the daily dose and time above EC_{50} was not linear in the PK/PD simulation, and the time above EC_{50} was not prolonged concurrent with the increase of the dose (Figure 4). Based on the overall balance between these efficacy and safety risks, 600 mg q.i.d. was determined to be an appropriate dose for the phase III study.

We determined the dosage and regimen for a camostat mesylate phase III study based on the phase I study result and PK/PD modeling and simulation data. However, it should be noted that the time above EC_{50}, which was used as an index to determine the dosage and regimen, has not been confirmed the correlation with efficacy in animal studies. The relationship between the time above EC_{50} and clinical efficacy will be evaluated after obtaining clinical data. The efficacy and safety of camostat mesylate 600 mg q.i.d. are currently being evaluated in an ongoing Japanese phase III study in patients with COVID-19.

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CONFLICTS OF INTEREST

J.K., H.I., H.A., J.M., K.F., S.O., and S.N. are employees of Ono Pharmaceutical Co., Ltd. T.H. is a consultant and N.U. is a paid consultant of Ono Pharmaceutical Co., Ltd. All other authors declare no competing interests for this work.

AUTHOR CONTRIBUTIONS

J.K. and S.N. wrote the manuscript. J.M., K.F., S.N., and N.U. designed the research. J.M., K.F., and M.H. performed the research. J.K., H.I., H.A., S.O., and T.H. analyzed the data.

DISCLAIMER

As an Associate Editor of *Clinical and Translational Science*, Naoto Uemura as not involved in the review or decision process for this paper.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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