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Pharmacokinetic Interaction Between the Hepatitis C Virus Protease Inhibitor Boceprevir and Cyclosporine and Tacrolimus in Healthy Volunteers

Ellen Hulskotte,[1] Samir Gupta,[2] Fengjuan Xuan,[2] Marga van Zutven,[1] Edward O'Mara,[2] Hwa-Ping Feng,[2] John Wagner,[2] and Joan Butterton[2]

The hepatitis C virus protease inhibitor boceprevir is a strong inhibitor of cytochrome P450 3A4 and 3A5 (CYP3A4/5). Cyclosporine and tacrolimus are calcineurin inhibitor immunosuppressants used to prevent organ rejection after liver transplantation; both are substrates of CYP3A4. This two-part pharmacokinetic interaction study evaluated boceprevir with cyclosporine (part 1) and tacrolimus (part 2). In part 1, 10 subjects received single-dose cyclosporine (100 mg) on day 1, single-dose boceprevir (800 mg) on day 3, and concomitant cyclosporine/boceprevir on day 4. After washout, subjects received boceprevir (800 mg three times a day) for 7 days plus single-dose cyclosporine (100 mg) on day 6. In part 2A, 12 subjects received single-dose tacrolimus (0.5 mg). After washout, they received boceprevir (800 mg three times a day) for 11 days plus single-dose tacrolimus (0.5 mg) on day 6. In part 2B, 10 subjects received single-dose boceprevir (800 mg) and 24 hours later received boceprevir (800 mg) plus tacrolimus (0.5 mg). Coadministration of boceprevir with cyclosporine/tacrolimus was well tolerated. Concomitant boceprevir increased the area under the concentration-time curve from time 0 to infinity after single dosing (AUC_{inf}) and maximum observed plasma (or blood) concentration (C_{max}) of cyclosporine with geometric mean ratios (GMRs) (90% confidence interval [CI]) of 2.7 (2.4-3.1) and 2.0 (1.7-2.4), respectively. Concomitant boceprevir increased the AUC_{inf} and C_{max} of tacrolimus with GMRs (90% CI) of 17 (14-21) and 9.9 (8.0-12), respectively. Neither cyclosporine nor tacrolimus coadministration had a meaningful effect on boceprevir pharmacokinetics. *Conclusion:* Dose adjustments of cyclosporine should be anticipated when administered with boceprevir, guided by close monitoring of cyclosporine blood concentrations and frequent assessments of renal function and cyclosporine-related side effects. Administration of boceprevir plus tacrolimus requires significant dose reduction and prolongation of the dosing interval for tacrolimus, with close monitoring of tacrolimus blood concentrations and frequent assessments of renal function and tacrolimus-related side effects. (HEPATOLOGY 2012;56:1622-1630)

Boceprevir (800 mg three times a day), in combination with pegylated interferon-α (PEG-IFNα) and ribavirin, was approved in the United States and Europe for the treatment of genotype 1 chronic hepatitis C infection in adult patients with compensated liver disease. As a structurally novel ketoamide serine protease inhibitor of the hepatitis C virus (HCV) nonstructural 3 (NS3/4A) active site, boceprevir has been shown to significantly increase rates of sustained virologic response (SVR) when added to PEG-IFNα plus ribavirin as compared with treatment with PEG-IFNα plus ribavirin alone.[1,2] In treatment-

Abbreviations: AE, adverse event; AUC_{inf}, area under the concentration-time curve from time 0 to infinity after single dosing; AUC_{last}, area under the concentration-time curve from time 0 to time of last measurable sample; BMI, body mass index; CI, confidence interval; C_{max}, maximum observed plasma (or blood) concentration; CL/F, apparent total body clearance; CYP3A4/5, cytochrome P450 3A4 and 3A5; GMR, geometric mean ratio; HCV, hepatitis C virus; HIV, human immunodeficiency virus; HPLC, high-performance liquid chromatography; LLOQ, lower limit of quantification; NS3/4A, HCV nonstructural 3/4A protein; PEG-IFNα, pegylated interferon-α; P-gp, P-glycoprotein; PK, pharmacokinetics; SVR, sustained virologic response; $t_{1/2}$, terminal phase half-life; T_{max}, time to maximum observed plasma (or blood) concentration.

From the [1]Merck Sharp & Dohme Corp, Haarlem, Netherlands; and [2]Merck Sharp & Dohme Corp, Whitehouse Station, NJ.

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naive patients, SVR rates increased from 38% among patients treated with PEG-IFNα plus ribavirin to 63%-66% in those receiving boceprevir plus PEG-IFNα and ribavirin.[2] Similarly, in treatment-experienced patients, SVR rates were 21% with PEG-IFNα plus ribavirin and 59%-66% in those receiving boceprevir plus PEG-IFNα and ribavirin.[1] Boceprevir (800 mg three times a day) in combination with PEG-IFNα and ribavirin, was approved for the treatment of genotype 1 chronic hepatitis C infection in adult patients with compensated liver disease in the United States and Europe in 2011. Metabolism of boceprevir occurs by aldo-ketoreductase to form inactive keto-reduced metabolites and by cytochrome P450 3A4 and 3A5 (CYP3A4/5).[3] Boceprevir is also a substrate for the efflux pump P-glycoprotein (P-gp) and is an inhibitor of OATP1B1.[4]

Hepatitis C–related liver cirrhosis is a frequent cause of liver transplantation, and because recurrent viremia is common among patients who are viremic at the time of transplantation, treatment of HCV infection is frequently required after transplantation.[5] Cyclosporine and tacrolimus are calcineurin inhibitors widely used to prevent solid organ transplant rejection. Both agents are substrates for CYP3A[6,7] and P-gp.[8] Cyclosporine is also an inhibitor of several other transporter proteins, including OATP1B1 and OATP1B3.[9] Both agents have a narrow therapeutic index, with therapeutic monitoring being required to avoid either underexposure, which can result in organ rejection, or excess exposure, which may cause nephrotoxicity, neurotoxicity, hypertension, or gastrointestinal toxicity. Boceprevir is a strong inhibitor of CYP3A4/5 and would be anticipated to increase exposure to cyclosporine and tacrolimus upon coadministration, as was previously observed for another recently approved HCV NS3/4A protease inhibitor (telaprevir, Incivek, Vertex Pharmaceuticals, Inc.).[10] In this study, the pharmacokinetic (PK) interactions between boceprevir and tacrolimus/cyclosporine were separately evaluated.

Subjects and Methods

This was a single-center, two-part, open-label study. The study was conducted in accordance with the principles of Good Clinical Practice and was approved by the appropriate institutional review boards and regulatory agencies. All subjects provided written informed consent prior to participation in study-related procedures.

Subjects

Healthy adult male and female subjects aged 18-55 years with an inclusive body mass index (BMI) of 18-32 kg/m^2 were enrolled. All subjects were required to be free of any clinically significant disease and have clinical laboratory tests (including complete blood counts, blood chemistries, urinalysis, electrocardiogram, and vital signs) within normal limits or clinically acceptable to the investigator. Premenopausal women and men were required to use a medically accepted method of contraception. All subjects were required to provide written informed consent and to adhere to dose and visit schedules. No information on CYP3A4/5 polymorphisms in the study subjects was available prior to dosing.

Subjects who were pregnant, breastfeeding, or who (in the opinion of the investigator) were unable to participate optimally in the study were excluded. Additional exclusion criteria were: a surgical or medical condition that might significantly alter the absorption, distribution, metabolism, or excretion of any drug; a recent history of any infectious disease; and infection with hepatitis B, hepatitis C, or human immunodeficiency virus (HIV). Subjects with a history of alcohol or drug abuse in the past 2 years, who smoked >10 cigarettes or had equivalent tobacco use per day, or who had elevated liver function tests also were excluded.

Study Design

This study consisted of two parts, each with a fixed-sequence design. Part 1 was designed to assess the effect of cyclosporine on boceprevir PK and the effect of boceprevir on cyclosporine PK (Fig. 1A). In part 2, the effect of boceprevir on tacrolimus PK and the effect of tacrolimus on boceprevir PK were assessed (Fig. 1B). In both parts of the study, boceprevir was administered orally as 4 × 200-mg capsules swallowed (not crushed or chewed) with a glass of water. A meal or light snack preceded boceprevir. During hospitalization (part 1, days −1 to 5 and days 10 to 13; part 2a, days −1 to 5 and days 11 to 19; part 2b, days −1 to 3), the subjects were on standard meals, including a

Address reprint requests to: Ellen Hulskotte, Department of Clinical Pharmacology Merck Sharp & Dohme, P. O. Box 20, 53410 BH Oss, Netherlands. E-mail: ellen.hulskotte@merck.com; fax: (31)-412-66-2506.

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Potential conflict of interest: Drs. Xuan, Butterton, and Feng own stock in Merck.

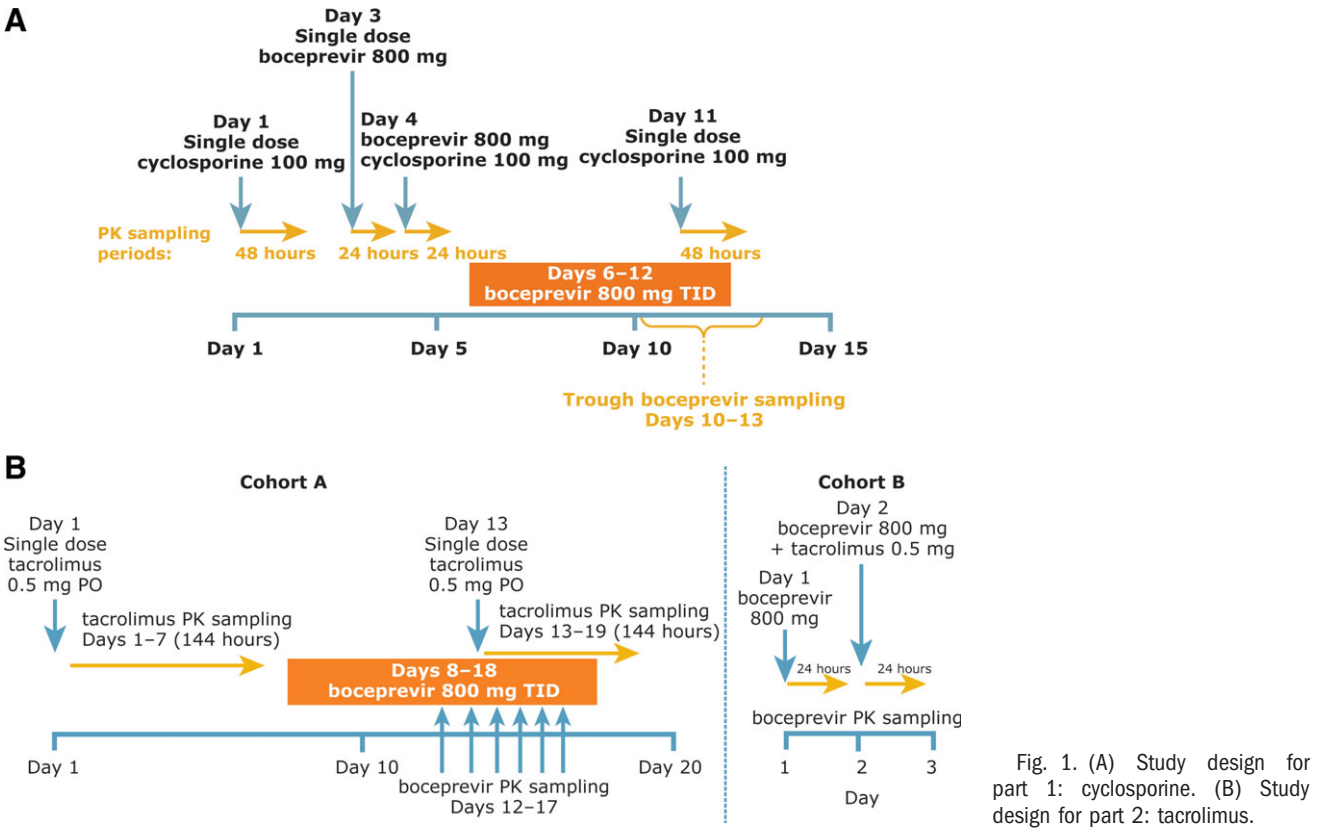

Fig. 1. (A) Study design for part 1: cyclosporine. (B) Study design for part 2: tacrolimus.

standard breakfast comprising 828 kcal (20.2% fat, 14.5% protein, 65.2% carbohydrates). Neoral (soft gelatin capsule, 100 mg) was used for cyclosporine treatment and Prograf (capsule, 0.5 mg) was used for tacrolimus treatment. In cases of boceprevir and cyclosporine or tacrolimus coadministration, drugs were taken concomitantly with 240 mL of water.

Part 1: Cyclosporine. On day 1, after a standard breakfast, all subjects received a single dose of oral cyclosporine (100 mg). PK samples for cyclosporine determination were obtained predose on day 1 and then at selected time points until 48 hours postdose on day 3. After the 48-hour sample on day 3, all subjects received a single oral dose of boceprevir (800 mg) with PK samples obtained predose and then at selected intervals until 24 hours postdose (on day 4). After the final boceprevir PK sample had been obtained on the morning of day 4, all subjects received single doses of boceprevir (800 mg) and cyclosporine (100 mg) and PK samples for boceprevir were again obtained at intervals up to 24 hours postdose.

From the morning of day 6 through the evening of day 12, all subjects received boceprevir 800 mg three times a day. Plasma samples for trough boceprevir levels were obtained before morning dose on days 10, 11, 12, and 13. In addition, on day 11, all subjects received a single 100-mg oral dose of cyclosporine together with their scheduled dose of boceprevir. PK samples for cyclosporine concentrations (at steady state boceprevir) were then collected before cyclosporine dosing on day 11 until 48 hours postdose on the morning of day 13. All subjects then returned for final clinic safety assessments on day 20.

Part 2: Tacrolimus. Because of the anticipated long half-life of tacrolimus, 2 separate enrollment cohorts were employed to study the PK interactions between tacrolimus and boceprevir. Cohort A was designed to evaluate the effect of boceprevir on tacrolimus, and cohort B was designed to evaluate the effect of tacrolimus on boceprevir.

In cohort A, following a standard breakfast on day 1, all subjects received a single dose of oral tacrolimus (0.5 mg). PK samples were obtained predose and then at selected intervals until the morning of day 7 (equivalent to a postdose period of 144 hours). From the morning of day 8 through the evening of day 16, subjects then received boceprevir 800 mg three times a day. Plasma samples for trough levels of boceprevir were obtained before the morning dose on days 12, 13, 14, 15, 16, and 17. In addition, on day 13, subjects received a single oral dose of tacrolimus (0.5 mg) and PK samples for evaluation of tacrolimus levels (at

steady state boceprevir) were collected from day 13 predose until the morning of day 19 (equivalent to 144 hours postdose). All subjects returned to the clinic for a final safety assessment on day 24.

In cohort B, on the morning of day 1 all subjects received a standard breakfast and were then administered a single oral dose of boceprevir (800 mg). PK samples for boceprevir determination were obtained predose and at selected intervals until 24 hours postdose. After the final PK sample was obtained on day 2, subjects received another single dose of boceprevir (800 mg) together with a single dose of tacrolimus (0.5 mg). PK samples for boceprevir (in the presence of tacrolimus) were collected predose and then at selected intervals until the morning of day 3 (equivalent to 24 hours postdose). On day 3, after the last PK sample had been obtained, safety assessments were performed, and subjects were then discharged. All subjects returned to the clinic for final safety assessments on day 10.

Bioanalysis

Concentrations of cyclosporine and tacrolimus in collected human blood samples were determined using high-performance liquid chromatography (HPLC) and HPLC–tandem mass spectrometry, respectively, at PharmaNet Canada (Quebec, Quebec, Canada). The lower limit of quantification (LLOQ) for the cyclosporine assay was 2 ng/mL; the linear calibration range was 2-1,002 ng/mL. The LLOQ for the tacrolimus assay was 50.52 pg/mL; the linear calibration range was 50.52 to 50,520 pg/mL. Concentrations of boceprevir and its metabolites in collected human plasma samples were determined using HPLC–tandem mass spectrometry at PPD (Middleton, WI). Concentrations of boceprevir were determined as the sum of concentrations of two enantiomers of boceprevir: SCH 534128 and SCH 534129. Concentrations of SCH 629144, an inactive metabolite of boceprevir, were obtained as the sum of concentrations of four analytes: SCH 783004, SCH 783005, SCH 783006, and SCH 783007. The overall LLOQ for boceprevir was 4.80 ng/mL, and the overall LLOQ for SCH 629144 was 2.50 ng/mL.

Endpoints

Standard PK variables were assessed, including area under the concentration-time curve from time 0 to the time of the last measurable sample (AUC_{last}); area under the concentration-time curve from time 0 to infinity after single dosing (AUC_{inf}); maximum observed plasma (or blood) concentration (C_{max}); time to maximum observed plasma (or blood) concentration (T_{max}); terminal phase half-life ($t_{1/2}$); and apparent total body clearance (CL/F). Safety variables including vital signs, electrocardiograms, adverse events (AEs), hematology, and blood chemistries also were monitored regularly.

Statistical Analysis

Assessment of safety and tolerability included all subjects who received at least one dose of boceprevir, and PK analyses were based on the per-protocol population, which included all protocol-compliant subjects. PK parameters were summarized by treatment using descriptive statistics and graphics. The log-transformed AUC and C_{max} values were analyzed using mixed effect modeling extracting the effect due to treatment as fixed effect, and subject as random effect.

Geometric mean ratios (GMRs) and associated 90% confidence intervals (CIs) were calculated using the following predefined limits to define clinically meaningful drug-drug interactions. In view of the narrow therapeutic window and high degree of intersubject variability of cyclosporine, confidence bounds for the 90% CI for AUC or C_{max} of 0.80-1.25 were chosen to assess the effect of boceprevir on cyclosporine levels. Tacrolimus monitoring using trough concentrations is generally easier and more reliable than cyclosporine monitoring using the modified AUC format, which is prone to greater individual point variability. The effect of boceprevir on tacrolimus was considered not clinically meaningful if the 90% CI for AUC and C_{max} of tacrolimus with boceprevir versus tacrolimus alone would be between 0.7 and 1.43. Analysis of the available clinical data for 800 mg three times a day boceprevir in healthy volunteers and patients indicated that confidence bounds for the 90% CI for AUC or C_{max} of (0.50-2.00) would be appropriate to control resistance generation and/or treatment failure as well as prevent clinically significant safety concerns (data on file).

Results

Interaction of Boceprevir and Cyclosporine

Ten subjects were enrolled and completed the cyclosporine study. There were seven females and three males, all of Hispanic or Latino ethnicity. The overall mean age was 36 years (SD 7.1 years), and the mean BMI was 26.8 kg/m^2 (SD 2.8 kg/m^2).

Effect of Boceprevir on PK Parameters of Cyclosporine. Coadministration of boceprevir with cyclosporine resulted in increased cyclosporine exposure, with the mean AUC_{inf} increasing from 1,800 ng/hour/mL to 4,870 ng/hour/mL and mean C_{max} levels

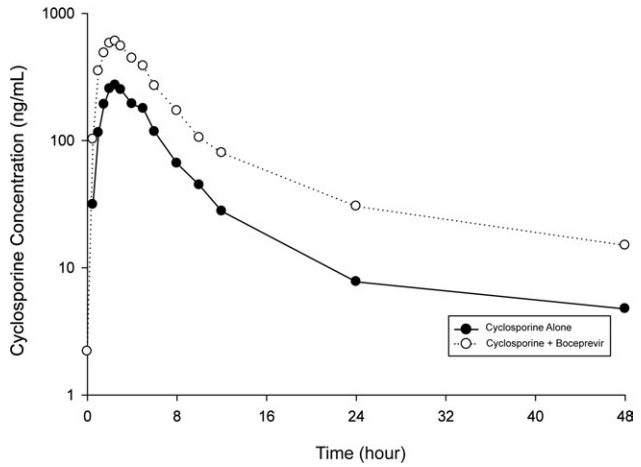

Fig. 2. Mean plasma concentration-time profiles of cyclosporine alone or concomitantly with multiple doses of boceprevir.

increasing from 388 ng/mL to 737 ng/mL (Fig. 2, Table 1). The GMRs for AUC_{inf} and C_{max} parameters for the comparison of cyclosporine plus boceprevir versus cyclosporine alone were 2.7 and 2.0, with 90% CIs for the GMRs falling outside the predefined range for defining clinically meaningful drug-drug interactions of 0.80-1.25 (Table 2). Consistent with the increase in exposure, there was an approximately 2-fold reduction in apparent cyclosporine clearance in the presence of boceprevir (mean CL/F of 21.0 L/hour versus 58.8 L/hour when administered alone; Table 1). The mean cyclosporine half-life increased by approximately 25%, from 11.3 hours to 15.7 hours, in the presence of boceprevir versus cyclosporine alone.

Effect of Cyclosporine on PK Parameters of Boceprevir. Boceprevir AUC_{inf} and C_{max} increased 16% and 8%, respectively (Table 2). The 90% CIs were within the predefined limits of 0.5 and 2.00, so that the observed increase in boceprevir concentrations is not considered clinically meaningful (Table 2). An approximate 2-fold increase in mean C_{max} and AUC_{inf} of the inactive metabolite SCH 629144 was observed following coadministration of boceprevir and cyclosporine (data not shown).

Safety. No subjects discontinued treatment because of an AE, and there were no serious AEs or deaths. Furthermore, no clinically meaningful changes in blood chemistry, hematology, blood pressure, pulse rate, oral body temperature, or electrocardiogram parameters were observed. A total of 21 AEs were reported by eight subjects in the cyclosporine study, all of which were of mild intensity, with 17 considered possibly drug-related. Dysgeusia was the most frequently reported possibly drug-related AE and occurred after cyclosporine-only treatment (n = 1), cyclosporine-boceprevir coadministration (n = 2), and boceprevir-only treatment (n = 7). Flatulence and headache were both reported twice, headache after boceprevir-only treatment and cyclosporine-boceprevir coadministration, flatulence after cyclosporine-only treatment and cyclosporine-boceprevir coadministration. Abdominal distension, abdominal discomfort, and flushing were each reported once.

Interaction of Boceprevir and Tacrolimus

Effect of Boceprevir on PK Parameters of Tacrolimus (Cohort A). Twelve subjects were enrolled and completed cohort A of the tacrolimus study (female, n = 5; male, n = 7; all Hispanic or Latino) with a mean age of 32.9 years (SD 10.8 years) and a mean BMI of 27.0 kg/m^2 (SD 3.03 kg/m^2).

Tacrolimus exposure was markedly increased in the presence of boceprevir (Fig. 3, Table 1); the mean AUC_{inf} increased from 21.8 ng/hour/mL to 345 ng/hour/mL upon concomitant administration of tacrolimus and boceprevir, while the mean C_{max} levels

Table 1. Mean (Coefficient of Variation %) Pharmacokinetic Parameters for Cyclosporine, Tacrolimus, and Boceprevir

Treatment	C_{max} (ng/mL)	AUC_{last} (ng/hour/mL)	AUC_{inf} (ng/hour/mL)	$t_{1/2}$ (h)	CL/F (L/h)	T_{max}, Hours, Median (Range)
Cyclosporine ± boceprevir						
Cyclosporine alone (n = 10)	388 (48)	1,770 (23)	1,800* (25)	11.2* (36)	58.8* (26)	2.50 (1.00-5.00)
Cyclosporine + boceprevir (n = 10)	737 (27)	4,520 (13)	4,840* (16)	15.5* (23)	21.2* (17)	2.50 (1.00-5.00)
Boceprevir ± cyclosporine						
Boceprevir alone (n = 10)	2,130 (31)	8,100 (15)	8,660* (22)	2.71* (76)	95.9* (20)	3.00 (2.00-4.00)
Boceprevir + cyclosporine (n = 10)	2,240 (17)	9,810 (17)	9,900 (17)	1.96 (28)	82.9 (17)	3.00 (2.00-4.00)
Tacrolimus ± boceprevir						
Tacrolimus alone (n = 12)	0.808 (36)	18.3 (59)	21.8 (53)	36.7 (22)	29.6 (57)	5.00 (2.00-12.0)
Tacrolimus + boceprevir (n = 12)	7.80 (25)	275 (27)	345 (32)	61.3 (18)	1.60 (32)	6.00 (4.00-24.0)
Boceprevir ± tacrolimus						
Boceprevir alone (n = 10)	1,945 (27)	7,227 (26)	7,292 (26)	1.54 (22)	115 (20)	4.00 (2.00-6.00)
Boceprevir + tacrolimus (n = 10)	1,920 (30)	7,186 (26)	7,318 (26)	1.79 (33)	116 (24)	3.00 (2.00-6.00)

*n = 9.

Table 2. Summary Statistics for Cyclosporine, Tacrolimus, and Boceprevir

	Parameter	Treatment	No. of Patients	Geometric Mean*	Comparison	GMR	90% CI for GMR
Cyclosporine study							
Cyclosporine†	C_{max} (ng/mL)	Cyclosporine + boceprevir	10	712	Cyclosporine + boceprevir versus	2.01	1.69-2.40
		Cyclosporine alone	10	354	cyclosporine alone		
	AUC_{last} (ng/hour/mL)	Cyclosporine + boceprevir	10	4,481	Cyclosporine + boceprevir versus	2.59	2.34-2.86
		Cyclosporine alone	10	1,731	cyclosporine alone		
	AUC_{inf} (ng/hour/mL)	Cyclosporine + boceprevir	9	4,762	Cyclosporine + boceprevir versus	2.68	2.38-3.03
		Cyclosporine alone	9	1,774	cyclosporine alone		
Boceprevir‡	C_{max} (ng/mL)	Boceprevir + cyclosporine	10	2,209	Boceprevir + cyclosporine versus	1.08	0.967-1.20
		Boceprevir alone	10	2,052	boceprevir alone		
	AUC_{last} (ng/hour/mL)	Boceprevir + cyclosporine	10	9,678	Boceprevir + cyclosporine versus	1.21	1.13-1.29
		Boceprevir alone	10	8,015	boceprevir alone		
	AUC_{inf} (ng/hour/mL)	Boceprevir + cyclosporine	10	9,777	Boceprevir + cyclosporine versus	1.16	1.06-1.26
		Boceprevir alone	9	8,459	boceprevir alone		
Tacrolimus study							
Tacrolimus§	C_{max} (ng/mL)	Tacrolimus + boceprevir	12	7.58	Tacrolimus + boceprevir versus	9.90	7.96-12.3
		Tacrolimus alone	12	0.77	tacrolimus alone		
	AUC_{last} (ng/hour/mL)	Tacrolimus + boceprevir	12	265	Tacrolimus + boceprevir versus	17.0	13.3-21.7
		Tacrolimus alone	12	15.6	tacrolimus alone		
	AUC_{inf} (ng/hour/mL)	Tacrolimus + boceprevir	12	328	Tacrolimus + boceprevir versus	17.1	14.0-20.8
		Tacrolimus alone	12	19.2	tacrolimus alone		
Boceprevir‖	C_{max} (ng/mL)	Boceprevir + tacrolimus	10	1,839	Boceprevir + tacrolimus versus	0.972	0.837-1.13
		Boceprevir alone	10	1,892	boceprevir alone		
	AUC_{last} (ng/hour/mL)	Boceprevir + tacrolimus	10	6,985	Boceprevir + tacrolimus versus	0.991	0.936-1.05
		Boceprevir alone	10	7,048	boceprevir alone		
	AUC_{inf} (ng/hour/mL)	Boceprevir + tacrolimus	10	7,104	Boceprevir + tacrolimus versus	0.999	0.946-1.06
		Boceprevir alone	10	7,111	boceprevir alone		

*Model-based (least squares) geometric mean based on a mixed effect model extracting the effect due to treatment as fixed effect and subject as the random effect.

†single dose cyclosporine with and without multiple dose boceprevir.

‡single dose boceprevir with and without single dose cyclosporine.

§single dose tacrolimus with and without multiple dose boceprevir.

‖single dose boceprevir with and without single dose tacrolimus.

increased from 0.8 ng/mL to 7.8 ng/mL (Fig. 3, Table 1). The AUC_{inf} and C_{max} GMRs for the comparison of tacrolimus plus boceprevir versus tacrolimus alone indicated a 17- and 9.9-fold rise, respectively, with 90% CIs falling outside the predefined range for defining clinically meaningful drug-drug interactions of 0.7 to 1.43 (Table 2). The mean apparent clearance of tacrolimus was approximately 18 times lower after coadministration of tacrolimus and boceprevir (Table 1). There was also an approximate doubling of the mean $t_{1/2}$ of tacrolimus in the presence of boceprevir.

Effect of Tacrolimus on PK of Boceprevir (Cohort B). Ten subjects were enrolled and completed cohort B (Hispanic/Latino, n = 9; African American, n = 1). The mean age was 45.4 years (SD 7.9 years) and the mean BMI was 27.27 kg/m^2 (SD 3.60 kg/m^2). Eight female subjects and two male subjects were included.

The AUCs and C_{max} values of boceprevir were essentially unchanged in the presence of tacrolimus compared with boceprevir administration alone (Table 1). The CL/F and the $t_{1/2}$ of boceprevir were also similar following concomitant administration of boceprevir and tacrolimus. GMRs were close to unity for C_{max}, AUC_{last}, and AUC_{inf}, and 90% CIs were all within the predefined range (0.50-2.00), indicating no clinically meaningful effect of tacrolimus on boceprevir PK. The PK parameters of the major metabolite SCH

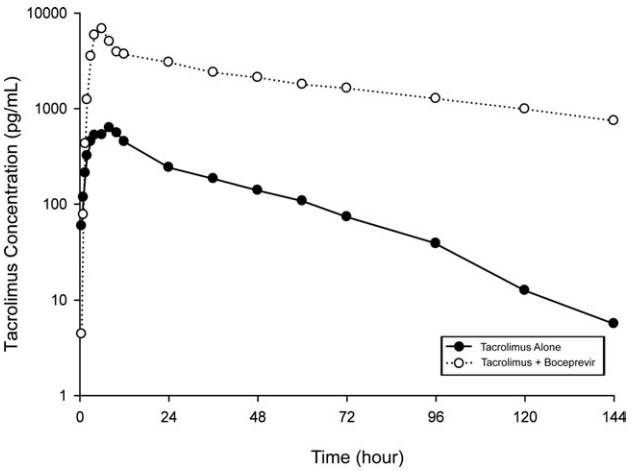

Fig. 3. Mean plasma concentration-time profiles of tacrolimus alone or in combination with multiple doses of boceprevir.

629144 were essentially the same following coadministration of boceprevir and tacrolimus compared with boceprevir alone administration (data not shown).

Safety. No subjects discontinued treatment because of an AE, and there were no serious AEs or deaths. Furthermore, no clinically meaningful changes in blood chemistry, hematology, blood pressure, pulse rate, oral body temperature, or electrocardiogram parameters were observed. In cohort A, 21 AEs were reported by seven subjects receiving tacrolimus either alone or in combination with boceprevir. All AEs were of mild intensity, and 18 were considered to be possibly drug-related. Dysgeusia was the most frequently reported drug-related AE (n = 7; only reported in subjects receiving boceprevir with or without tacrolimus, not in subjects only receiving tacrolimus), followed by headache (n = 2; occurring once each after tacrolimus-only and boceprevir-only treatment), gastroesophageal reflux (n = 2; occurring once each after tacrolimus-only and boceprevir-only treatment), abdominal discomfort (n = 2; after boceprevir-only treatment), and chills (n = 2; once after boceprevir-only treatment and once after tacrolimus-boceprevir coadministration). All other possibly drug-related AEs (ie, asthenia, fatigue, and palpitations) were reported once. In addition, five AEs were reported by five subjects in cohort B, all of which were of mild intensity. Two AEs were considered possibly drug-related (dysgeusia, n = 1; headache, n = 1; both after boceprevir-only treatment).

Discussion

There is a significant unmet clinical need for the treatment of recurrent hepatitis C after liver transplantation. SVR rates for patients receiving PEG-IFNα and ribavirin after liver transplantation are low, with less than one-third of patients achieving SVR.[11] Furthermore, treatment-related toxicity represents a significant barrier to completion of therapy.[12] Thus, the liver transplantation population represents a subgroup of patients with chronic hepatitis C who could potentially derive significant clinical benefit from the use of direct-acting antiviral agents. Calcineurin inhibitors, such as cyclosporine and tacrolimus, are routinely administered in these patients as immunosuppressants to prevent allograft rejection. Given the narrow therapeutic index within which these agents are effective, and the subsequent need for therapeutic monitoring, a clear and detailed understanding of their propensity for drug-drug interactions is required before their concomitant use with new pharmacologic agents.

Cyclosporine and tacrolimus are both substrates of CYP3A4/5. Because boceprevir is a strong inhibitor of CYP3A4, coadministration with boceprevir would be anticipated to increase exposure to these calcineurin inhibitors. The doses of cyclosporine (100 mg) and tacrolimus (0.5 mg) used in this study were optimized for investigation of the potential for drug-drug interactions between the individual drugs and boceprevir without jeopardizing subject safety. Consequently, doses were much lower (tacrolimus) than or at the lower end (cyclosporine) of standard therapeutic dosing in order to maintain a safety margin if significant elevations in immunosuppressant concentrations were observed upon boceprevir coadministration. In addition, cyclosporine and tacrolimus were each given as single doses to mitigate potential safety concerns (eg, those associated with accumulation). Boceprevir was dosed to steady state in order to ensure that the maximum inhibitory potential of the drug was assessed. Also, to avoid an extremely long study duration, the interaction of tacrolimus as substrate and as perpetrator was studied in different cohorts of subjects.

Concomitant boceprevir administration increased the AUC_{inf} and C_{max} of cyclosporine by 2.7- and 2.0-fold, respectively. Boceprevir coadministration had a substantial effect on the PK of tacrolimus, with coadministered geometric mean AUC_{inf} and C_{max} parameter values approximately 17-fold and 10-fold higher than when tacrolimus was administered alone. Drug interactions also have been identified between cyclosporine and tacrolimus and telaprevir, another recently approved HCV NS3/4A protease inhibitor.[10] Coadministration of telaprevir led to a 4.6- and 1.3-fold increase in the dose-normalized AUC_{inf} and C_{max} of cyclosporine and a 70- and 9.3-fold increase in the dose-normalized AUC_{inf} and C_{max} of tacrolimus, respectively.

Neither tacrolimus nor cyclosporine had any notable effect on the PK of boceprevir. Boceprevir is metabolized by two pathways: aldo ketoreductase, which leads to (among others) a reduced, inactive metabolite (SCH 629144), and CYP3A4/5.[3] Although the C_{max} and AUC of boceprevir were essentially unchanged in the presence of cyclosporine compared with boceprevir administration alone, a two-fold increase in the C_{max} and AUC of the metabolite SCH 629144 was observed after coadministration of boceprevir and cyclosporine. Because this metabolite is not active against HCV, this increase has no consequences with respect to clinical efficacy; however, it is not known whether the increase in metabolite exposure could potentially increase side effects. Because cyclosporine is an

inhibitor of several proteins in both the drug-metabolizing enzyme and the uptake/efflux transporter systems, data in the present study do not provide insight into whether the increase in SCH 629144 levels is due to its effect on the enzyme/transporter interplay, resulting in an increase in the formation of SCH 629144, a decrease in the elimination of the metabolite, or a combination of both. The contribution of cyclosporine-based P-gp inhibition on drug interactions could not be assessed in this study, given that the low cyclosporine dose used did not produce plasma concentrations at the levels predicted to incur clinically meaningful P-gp inhibition (1,000-5,000 ng/mL).[13] Furthermore, the potential for tacrolimus to inhibit the metabolism of boceprevir may not have been fully assessed in this study because of the low tacrolimus dose used to allow for a large enough safety margin to accommodate the increased concentrations that were expected upon boceprevir coadministration.

Coadministration of boceprevir with cyclosporine or tacrolimus was safe and well tolerated in this group of healthy volunteers. Overall, tolerability was consistent with the known safety profile of boceprevir in healthy subjects[14-16] and patients with chronic hepatitis C.[1,2,16] All AEs were mild, there were no treatment discontinuations due to AEs, and dygeusia was the most frequently reported drug-related AE. There was one event of mild palpitations after multiple-dose boceprevir plus single-dose tacrolimus treatment. Palpitations have previously been identified as uncommon for tacrolimus and as common for boceprevir (when taken together with PEG-IFNα and ribavirin).[6,16]

The PK of coadministered boceprevir and the calcineurin inhibitors have not been studied in liver transplant patients, which is a limitation for interpretation of these data. The data in the present study were derived from healthy subjects, and the magnitude of the potential interaction between cyclosporine or tacrolimus and boceprevir in liver transplant patients is not known. Blood concentrations of the calcineurin inhibitors in liver transplant patients with recurrence of HCV are subject to a wider range of influences than those in healthy subjects, which in turn could result in greater interpatient variability. HCV infection itself appears to reduce the dose of cyclosporine or tacrolimus required to achieve a given blood level, probably because of down-regulation of hepatic CYP3A4, impaired function of hepatic P-gp, or both.[17] The effect is reversed when the HCV-associated inflammatory response is eliminated by antiviral therapy.[18] In addition, liver function can change with time after transplantation.[19]

Based on the results from the present study, dose reductions of cyclosporine should be anticipated when administered with boceprevir and should be guided by close monitoring of cyclosporine blood levels and frequent assessments of renal function and cyclosporine-related side effects. For tacrolimus, significant dose reduction and prolongation of the dosing interval will be required, along with close monitoring of tacrolimus concentrations and frequent assessments of renal function and tacrolimus-related side effects. Plasma concentrations of other commonly used immunosuppressants such as sirolimus and everolimus may also be increased during coadministration with boceprevir. Thus, close monitoring of immunosuppressant blood levels is recommended here as well. This situation is comparable to that of HIV-coinfected patients after liver transplantation who require treatment with ritonavir-boosted HIV protease inhibitors concomitantly with cyclosporine or tacrolimus. HIV protease inhibitors (eg, lopinavir, darunavir, atazanavir, and ritonavir) are all potent CYP3A4 inhibitors, and several reports describe dose reductions of up to 99% of the calcineurin inhibitors when coadministered with HIV protease inhibitors, with dosing schedules of less than once weekly to maintain adequate cyclosporine and tacrolimus concentrations, or both.[20-22] Similarly, a preliminary report of the use of telaprevir in a small number of recipients after liver transplantation suggests that tacrolimus dose reduction and prolongation of the dosing interval have been generally well tolerated.[23]

Another consideration for the concomitant use of tacrolimus or cyclosporine with boceprevir in liver transplant patients relates to the need to readjust the dose levels of cyclosporine or tacrolimus when boceprevir treatment is completed or discontinued. A previous study using midazolam as a sensitive CYP3A4 probe suggests that CYP3A4 activity returns to baseline levels 48 hours after discontinuation of boceprevir (data on file, Merck & Co., Inc.). Although it is anticipated that standard doses of either immunosuppressant could be resumed soon after boceprevir is discontinued, careful and potentially increased frequency of blood concentration monitoring of immunosuppressants will be required.

In the treatment of chronic HCV, boceprevir is used in combination with PEG-IFNα and ribavirin. These therapies are not expected to influence cyclosporine or tacrolimus levels. Neither inhibition nor induction of cytochrome P450 enzymes or exhibition of cytochrome P450 enzyme-mediated metabolism has been observed in *in vitro* studies of ribavirin.[24] PEG-IFNα has shown increases in activity of CYP2D6 and

CYP2C8/9, but not CYP3A4/5.[25] None of the PK parameters of boceprevir, PEG-IFNα, or ribavirin have been affected by coadministration.[16]

In conclusion, coadministration with boceprevir results in clinically meaningful increases in exposure to cyclosporine and tacrolimus in healthy subjects. The magnitude of the potential interaction between cyclosporine or tacrolimus and boceprevir in organ transplantation patients is not yet known but could potentially be higher and more variable than those seen in healthy subjects due to intersubject PK variability and variability associated with disease during the course of antiviral therapy. Therefore, dose adjustments of cyclosporine should be anticipated when administered with boceprevir and should be guided by close monitoring of cyclosporine blood concentrations and frequent assessments of renal function and cyclosporine-related side effects. Concomitant administration of boceprevir with tacrolimus requires significant dose reduction and prolongation of the dosing interval for tacrolimus, with close monitoring of tacrolimus blood concentrations and frequent assessments of renal function and tacrolimus-related side effects.

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