

Voclosporin Food Effect and Single Oral Ascending Dose Pharmacokinetic and Pharmacodynamic Studies in Healthy Human Subjects

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Abstract

Voclosporin (VCS) is a novel calcineurin (CN) inhibitor intended for prevention of organ graft rejection and treatment of lupus nephritis. These studies evaluated the single ascending dose pharmacokinetics (PK) and pharmacodynamics (PD, CN activity) of VCS and the effect of food. VCS was administered orally in single doses of 0.25 through 4.5 mg/kg in 62 subjects in the single ascending dose study and as a single oral 1.5 mg/kg dose to 18 subjects after fasting, consumption of a low-fat and high-fat meal. Non-compartmental PK, PD, and PKPD correlation were evaluated. Following single oral doses, systemic exposure increased in a linear manner and demonstrated 1:1 dose-proportional, first-order linear PK above 1.5 mg/kg. VCS inhibited CN activity in a dose-related fashion with maximal inhibition peaking at 3.0 mg/kg. PKPD correlation indicated an EC_{50} of 78.3 ± 6.8 ng/mL. Administration of VCS with a low-fat and high-fat meal decreased C_{max} by 29% and 53%, respectively, and AUC_{inf} by 15% and 25%, respectively. Following ascending single doses of VCS, exposure increased in a linear fashion. A food effect on exposure was demonstrated, with a more pronounced effect following a high-fat meal. VCS concentrations were also found to correlate with CN activity.

Keywords

pharmacodynamics, clinical pharmacology, food effect, voclosporin, calcineurin inhibitor, pharmacokinetics

Introduction

Voclosporin (VCS) is a novel calcineurin (CN) inhibitor intended for use in the prevention of organ graft rejection and the treatment of autoimmune diseases, such as lupus nephritis. VCS was created by adding a single carbon extension to the amino acid-1 region of cyclosporine A (CsA). X-ray crystallography studies have shown this increase in carbon length alters the way the cyclophilin–VCS complex binds to a composite surface of catalytic and regulatory subunits in CN (latch region). This change in binding has increased the potency of VCS compared to CsA¹ which has been confirmed in vivo using a ³²P-labeled CN activity assay.² CN activity has been studied as a potential biomarker throughout the development of VCS. As amino acid-1 is the primary site of CsA's metabolism, modification of this site has shifted the primary site for VCS metabolism to the amino acid-9 position. In vitro studies suggest that the primary metabolites for CsA and VCS are AM1 and IM9, respectively, which are equipotent in their ability to prevent T-cell activation (internal data) and approximately 10% of the parent compound activity. Critically, IM9 is produced in significantly smaller amounts than AM1 resulting in less competitive antagonism of their respective parent molecules. The combination of increased potency and a change in metabolite profile for VCS allows for

administration of lower doses, less pharmacokinetic–pharmacodynamic variability, and a potentially improved safety profile compared to CsA.

Efficacy and safety of VCS has been demonstrated against tacrolimus (TAC) in renal transplant recipients³ and against placebo and CsA in patients with plaque psoriasis.^{4,5} At efficacious doses, VCS exhibits CN inhibitor-class adverse events (AEs), but with lesser magnitude. VCS's effect on serum lipids is distinctly different from CsA lacking a rise in serum triglycerides and presenting a lipid profile similar to TAC in renal transplant patients. VCS also demonstrated less New Onset of Diabetes Mellitus After Transplant (NODAT) than TAC in renal transplantation.

VCS is a large molecular weight, highly lipophilic molecule which suggests it would favor biliary excretion and hepatic metabolism as the primary elimination routes. It is estimated that over 99% of the drug is eliminated as

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metabolite and that the parent molecule is 97% protein bound.

This report describes the single ascending dose pharmacokinetics (PK) and pharmacodynamics (PD) of VCS and the effect of food on PK of VCS in healthy adult volunteers.

Methods

Single Ascending Dose Study

The single ascending dose study was a single-center, double-blind, randomized, placebo-controlled, single oral ascending dose design. Sixty-two subjects were randomly assigned to one of five treatment groups: In each cohort, six subjects were randomly assigned to receive VCS and two subjects to receive placebo. Due to incomplete urine collections, cohorts 1 and 3 were repeated, therefore, 56 healthy adult male and female subjects completed five treatment groups. Single doses of 0.25 mg/kg, 0.5 mg/kg, 1.5 mg/kg, 3 mg/kg, and 4.5 mg/kg VCS were studied. VCS 50 mg/mL liquid and placebo were diluted in 240 mL of apple juice and were administered to subjects in a fasted state.

Food Effect Study

The food effect study was a single center, randomized, single-dose, open-label, 3-way crossover food interaction study to assess the effects of fasting (Treatment A), consumption of a low-fat meal (Treatment B), and high-fat meal (Treatment C) on the PK of VCS. Subjects were randomized to receive VCS as a single oral dose of 1.5 mg/kg body weight administered in 50 mL of apple juice with an additional 10 mL of apple juice rinse separated by a washout period of 12 days. The randomization schedule included three sequences: ABC, BCA, and CAB.

Subjects were dosed after a supervised overnight fast of at least 10 hours. For Treatment B and C drug was administered within 5 minutes after completion of a low-fat and high-fat meal, respectively. The low-fat meal was comprised of $\frac{3}{4}$ cups of corn flakes with 240 mL skim milk, one slice of whole wheat bread with butter, and four tsp strawberry jelly, one banana, and 120 mL of orange juice. The high-fat meal was comprised of 64 g hash brown potatoes, 16 g slices of bacon, two eggs cooked with butter, two slices of toast with butter, and 200 mL of whole milk.

Subjects

Subjects included male and females aged 18–45 years, body mass index (BMI) between 18 kg/m² and 30 kg/m² inclusive and weight between 55 kg and 100 kg. Subjects were required to adhere to the following restrictions: limited caffeine (of no more than eight cups per day), and/or methylxanthine containing beverages (of more than 1 L per day), and limited alcohol consumption of no more than two drinks per day in a week.

Main exclusion criteria included: any clinically significant abnormality (including clinically significant ECG or vital sign abnormalities) or abnormal laboratory test results, presence of malignancy, and/or undiagnosed skin lesions, or reception of a flu vaccine within 14 days. Medication exclusion included: history of significant alcohol or drug abuse within 2 years prior to the screening visit, use of any drugs known to induce or inhibit hepatic drug metabolism within 30 days (or five half-lives). Main laboratory exclusions included: positive test for hepatitis B, hepatitis C, or HIV, hemoglobin <120 g/L, positive test for drugs of abuse.

Blood Sampling

For the single ascending dose study, blood samples were taken at predose and at 0.5 hours, 1 hours, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 16 hours, and 24 hours postdose for the measurement of VCS. Additional samples were taken at 48 hours postdose in Cohorts 3, 4, and 5 and at 72 hours postdose in Cohorts 4 and 5. Blood samples were taken at predose and at 1 hours, 2 hours, 4 hours, 8 hours, and 24 hours postdose for the measurement of CN inhibition. For the food effect study, blood samples were taken at predose and at 0.5 hours, 1 hours, 1.5 hours, 2 hours, 2.5 hours, 3 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 16 hours, 24 hours, 36 hours, 48 hours, and 72 hours postdose in each period.

The single ascending dose study was conducted at one clinical site in Phoenix, Arizona, and one site in Montreal, Quebec. The protocol and all modifications and appropriate consent procedures were reviewed and approved by a Research Ethics Board or Institutional Review Board (REB/IRB) at each site in accordance with the current regulations. All patients signed written informed consents at the screening visit. The principal investigator at each institution ensured that this study fully adhered to the principles outlined in guideline for good clinical practice (GCP) International Conference on Harmonization (ICH) Tripartite Guideline (January 1997) which is based on the principles of the Declaration of Helsinki (1996).

Sample Analysis

Whole blood VCS concentrations were measured using a validated LC/MS/MS method.⁶ The limit of quantitation (LOQ) for VCS was 1 ng/mL. Calcineurin activity (CNa) in whole blood was determined indirectly with a validated method that measured ³²Phosphate release from a protein substrate of CN (Isotechnika Pharma, Inc., 5120-75th Street, Edmonton, Alberta, Canada T6E 6W2). Blood contains the protein phosphatases PP1, PP2a, PP2b (CN), and PP2c. In order to measure CNa activity, two separate assays are required. Okadaic acid is utilized in the first assay to inhibit PP1 and PP2a allowing for the measurement of PP2c and PP2b (CN) together. In a

second assay, saturating doses of CsA are added to inhibit PP1, PP2a, and PP2b (CN) allowing for the measurement of PP2c. By subtracting the two results, it is possible to indirectly measure CN activity. ^{32}P release from the peptide was determined by measuring counts per minute on a Beckman Liquid Scintillation counter. CNa was expressed as a rate in pmol/min/mg.

Pharmacokinetic Analysis

Pharmacokinetic parameters for VCS were calculated using non-compartmental analysis. Only whole blood concentrations greater than or equal to the LOQ for the assay were included in the pharmacokinetic analyses. Actual blood sampling times were used unless unavailable in which case nominal times were used. The maximum whole blood concentration ($C_{\max}$) and time to $C_{\max}$ ($T_{\max}$) were taken directly from the data without interpolation. The area under the curve 0 to infinity (AUC_{inf}) was calculated using the linear trapezoidal method. Secondary pharmacokinetic parameters determined were apparent oral clearance (CL/F), and volume of distribution (V_z/F). Apparent terminal elimination half-life ($T_{1/2}$) was determined using concentrations above LOQ at three time points in the log-linear portion of the elimination phase.

All pharmacokinetic calculations were performed in WinNonlin Professional Version 5.2.1 (Pharsight®, Cary, NC).

Pharmacodynamic Analysis

Pharmacodynamic parameters were evaluated using non-compartmental analysis. CN data was analyzed as percent inhibition from time 0 (CN inhibition [%CNI]). Maximum %CNI ($E_{\max}$) and time to $E_{\max}$ ($TE_{\max}$) were determined from the percent CN inhibition time curve without interpolation. An effect half-life was determined using least squares linear regression of the 4-hour, 8-hour, and 24-hour time points.

Pharmacokinetic–Pharmacodynamic Correlation

Drug concentration was plotted against CN activity (pmol/min/mg of protein). CN activity was fit for pooled subjects using an inhibitory $E_{\max}$ model. Percent CN inhibition data was fit using a simple $E_{\max}$ model. $E_{\max}$ and EC_{50} was summarized using descriptive statistics.

Statistical Analysis

Demographic parameters were summarized using the range and mean and standard deviation. Baseline subject characteristics were presented to evaluate whether there was adequate balance after the randomization, but no statistical tests were planned.

PK and PD were summarized using descriptive statistics. Dose proportionality was tested in an exploratory manner by plotting $C_{\max}$ or AUC_{inf} versus dose

utilizing linear regression on the linear scale and the power model on the log–log scale. Dose proportionality was further evaluated by plotting dose-normalized $C_{\max}$ and AUC_{inf} versus dose and calculating the ratio of change in $C_{\max}$ and AUC_{inf} relative to the change in dose at each dose level.

PK PD correlation was performed using an inhibitory $E_{\max}$ model for CN activity and a simple $E_{\max}$ model for percent CN inhibition. Goodness of fit was assessed using correlation, standard error of the visual inspection of the residuals, and the Akaike information criterion.

For the food effect study, ANOVA was performed on ln-transformed AUC_{inf} and $C_{\max}$. Ratios of least-squares means and 90% geometric confidence intervals were calculated for ln-transformed AUC_{inf} and $C_{\max}$. As per protocol, absence of a food effect was concluded if the 90% geometric confidence intervals of the ratios of means (B/A and C/A) were fully included within 80% to 125% for ln-transformed AUC_{inf} and within 70–143% for ln-transformed $C_{\max}$.

Statistical analyses were performed using SAS® for Windows® Version 6.12, Bioequiv Version 3.40, and GraphPad Prism 4 for Windows Version 4.00 (La Jolla, CA). Graphs were produced using ggplot2 in R version 2.15.0 (Vienna, Austria).

Safety Evaluation

Safety assessments included physical examinations, AEs, vital signs (heart rate and blood pressure), ECG, physical examination, and clinical laboratory tests. AEs were graded as mild, moderate, or severe and monitored continuously throughout the study. Vital signs were assessed at the time of blood work on screening, Day-1 to end of study and follow-up using clinically acceptable methods and devices. The measurements were taken only after the subject had remained in a supine position for at least 5 min and prior to any blood sampling. The body weight was measured and this value was used to calculate the exact dose of medication required on a mg/kg basis rounded to 10 mg. A 12-lead safety ECG was taken before dosing at screening and Day-1 to ensure the subject's eligibility, and throughout the study for subject safety.

Results

Analysis Population

A total of 62 subjects entered the single ascending dose study and were randomized to study treatment; 56 subjects completed the study. Eighteen (18) subjects were entered into the food effect study, and 15 subjects completed all treatment periods and were included in the pharmacokinetic analyses. The demographics of the study subjects are summarized in Table 1.

Table 1. Summary of Demographic Data

Demographics	SAD study	Food effect study
N	62	15
M/F	32/30	12/6
Age	30 (18–45)	32 (19–45)
Race		
Black	5	4
Caucasian	18	7
Hispanic	39	6
Other	0	1
Height (cm)	166.9 (146.3–189.2)	170.1 (152.0–179.0)
Weight (kg)	72.3 (56.3–98.4)	71.7 (56.5–86.4)
Size	Small frame: 14 Medium frame: 45 Large frame: 3	BMI 24.8 (20.3–28.9)

Pharmacokinetics

Single Dose Pharmacokinetics. In each cohort five to seven subjects received single ascending doses of 0.25 mg/kg, 0.5 mg/kg, 1.5 mg/kg, 3.0 mg/kg, and 4.5 mg/kg of VCS. The 0.25 mg/kg and 1.5 mg/kg cohorts were repeated due to incomplete collection of urine. The repeated cohorts demonstrated similar concentrations and profiles and were thus pooled and presented here as a single group for clarity. The whole blood VCS concentrations increased in a dose-related manner (Figure 1).

Whole blood PK parameters for VCS are summarized in Table 2.

Whole blood concentrations peaked within 2 hours of drug administration at all dose levels. VCS demonstrated multi-exponential decline following $C_{\max}$ with increasing apparent half-life of elimination with doses up to 1.5 mg/kg after which the mean apparent terminal half-life was 17–18 h. Consistent with this observation, the oral clearance of VCS decreased with increasing doses up to 1.5 mg/kg and then appeared unchanged at the 1.5 mg/kg, 3.0 mg/kg, and 4.5 mg/kg doses. Although whole blood concentrations could not be followed for the full 72 hours after administration of the lower doses due to assay sensitivity, the decay profiles are reasonably parallel for all doses through 24 hours, and at the higher doses through 72 hours suggesting no dose-dependency in distribution or elimination. Exposure to VCS as determined by $C_{\max}$ and $AUC_{\inf}$ increased in a dose-related manner. Linear regression of $C_{\max}$ versus dose and $AUC_{\inf}$ versus dose yielded statistically significant relationships ($P < .05$) and demonstrated strong linear relationships with correlation coefficients 0.9655 and 0.9994 for $C_{\max}$ and $AUC_{\inf}$ respectively (Figure 2).

Dose proportionality was assessed using the power model. As shown in Figure 2, log–log plots of the mean $C_{\max}$ and $AUC_{\inf}$ versus dose are linear, but the slopes suggest greater than 1:1 dose-proportionality.

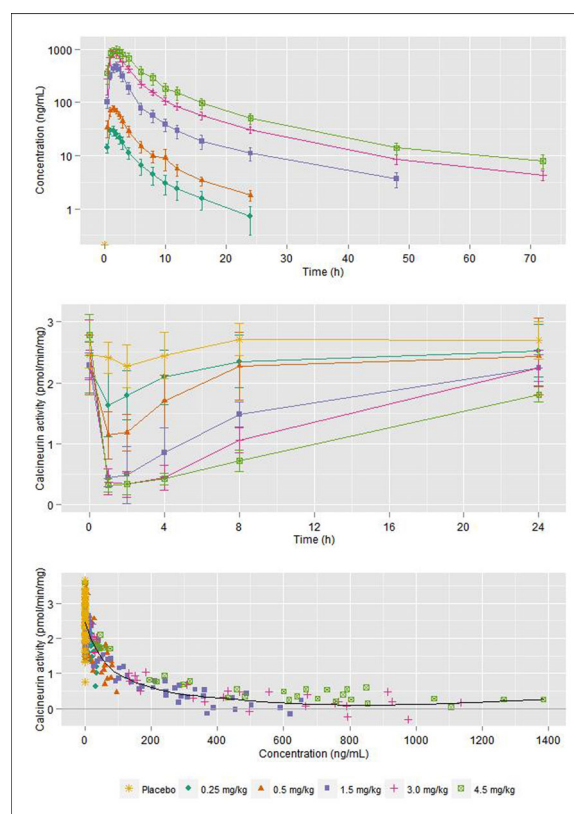


Figure 1. Mean ($\pm 95\%$ confidence interval) whole blood VCS concentration versus time (top), calcineurin activity versus time (middle), and calcineurin activity versus concentration (bottom) profiles following single oral administration at 0.25–4.5 mg/kg doses.

The estimates for $AUC_{\inf}$ were underestimated at the lower doses due to the shorter period of time over which concentrations were quantifiable, resulting in an overestimate of the slope. The estimation of $C_{\max}$ should not be affected by assay sensitivity and thus it may be a better estimate of the linearity. For both $C_{\max}$ and $AUC_{\inf}$ dose-proportionality approaches unity at doses above 1.5 mg/kg. Dose-adjusted $C_{\max}$ and $AUC_{\inf}$ increased from 0.25 to 1.5 mg/kg doses and were approximately proportional to dose at the 1.5 mg/kg, 3.0 mg/kg, and 4.5 mg/kg doses.

The dose-proportionality as expressed as ratio of change in $C_{\max}$ and $AUC_{\inf}$ to the change in dose demonstrates an almost twofold increase in exposure from 0.5 to 1.5 mg/kg but near 1:1 proportionality from 1.5 to 4.5 mg/kg doses (Table 2).

Overall, these analyses indicate VCS exposure increases linearly with dose. Some dose-dependency is apparent at lower doses that disappear above 1.5 mg/kg indicating first-order, linear PK.

Single Dose Pharmacodynamics. CN activity as assessed by turnover rate was suppressed by VCS in a dose-related manner up to the 3.0 mg/kg dose (Figure 1). The 4.5 mg/kg dose yielded no further maximal inhibition. CN activity

Table 2. Pharmacokinetic Parameters for VCS After Single Oral Administration of 0.25–4.5 mg/kg Doses in Healthy Human Subjects

Dose (mg/kg)	T _{1/2} (h)	T _{max} (h)	C _{max} (ng/mL)	AUC _{inf} (ng h/mL)	CL/F (L/h)	Vz/F (L/kg)	Dose proportionality	
							C _{max}	AUC _{inf}
0.25	5.7 ± 2	1 (1–2)	32 ± 9.9	142.9 ± 66.9	2.1 ± 0.9	15.1 ± 3.4	—	—
0.5	7.9 ± 1.4	1.5 (1–2)	77 ± 12.9	354.4 ± 69.9	1.5 ± 0.3	16.7 ± 4.9	1.20	1.24
1.5	14.3 ± 2.9	1.5 (1–2.5)	459 ± 150.8	2166.5 ± 769.8	0.8 ± 0.3	16.1 ± 6.9	1.99	2.04
3	17.4 ± 2.8	2 (1–2)	874.9 ± 167.4	5079.6 ± 698.7	0.6 ± 0.1	15 ± 2.7	0.95	1.17
4.5	18.1 ± 2.1	1.8 (1–3)	955.5 ± 251.7	7693.6 ± 2253.8	0.6 ± 0.2	16 ± 3.4	0.73	1.01

Data presented as mean ± SD except T_{max} presented as mean (range).

Dose proportionality calculated relative to change from previous dose.

returned to baseline levels by 24 hours at doses up to 3.0 mg/kg but remained suppressed at 24 hours at the 4.5 mg/kg dose.

Peak CN inhibition was reached within 2 hours at all doses in correspondence with peak whole blood concentrations of VCS. The non-compartmental pharmacodynamic parameters for CN activity and percent inhibition are presented in Supplementary Online Material.

PKPD Relationship

Drug concentration was plotted against CN activity (Figure 1). CN activity was not fit for each individual subject due to the sparse sampling. Pooled concentration and CN data were utilized for all PK PD correlation. A simple E_{max} model was utilized because the sigmoidicity factor in a Sigmoid model was close to unity. E_{max} and EC₅₀ were summarized using descriptive statistics and r correlation.

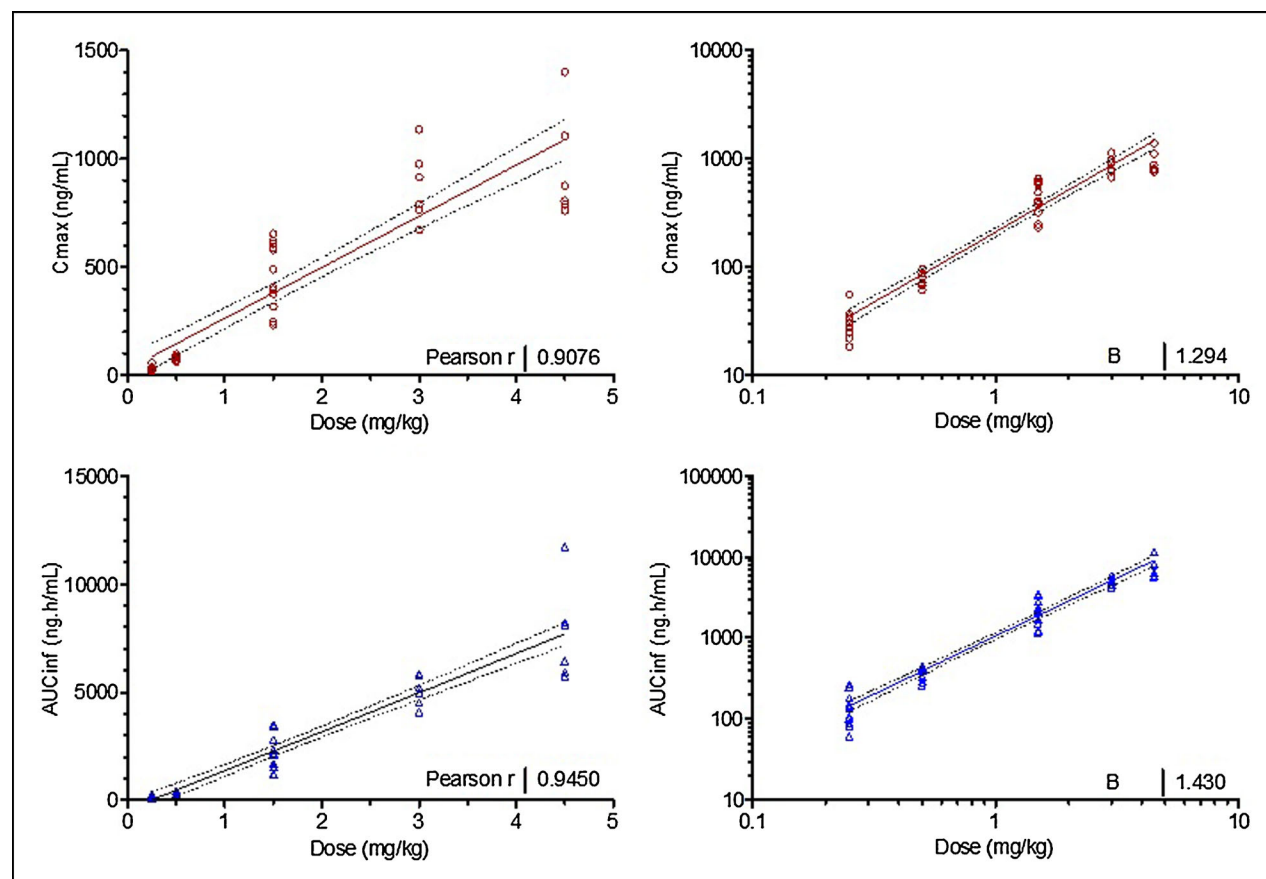


Figure 2. Scatter plots of peak concentration (C_{max}, top panels) and area under the concentration–time curve (AUC_{inf}, bottom panels) versus dose. Regression lines (solid) and 90% confidence intervals (dashed lines) are shown for linear regression (left panels) and power model regression (right panels).

Food Effect Study

Mean whole blood concentrations of VCS were decreased after administration with a low-fat and high-fat meal (Figure 3, top panel; the first 12 hours expanded in bottom panel).

Mean $C_{\max}$ and AUC_{inf} values were lower following administration after a low-fat and high-fat meal (Table 3).

According to ANOVA and Duncan's multiple range test, AUC_{inf} and $C_{\max}$ was greater under fasting conditions than after low-fat meal and high-fat meal. The least-squares means ratios (low-fat/fasted and high-fat/fasted), the 90% geometric confidence intervals and intra-subject CVs were determined and summarized in Table 3.

Safety and Tolerability

Single Ascending Dose Study. A total of 61 treatment-emergent AEs were reported by 28 (45%) of the 62 subjects dosed. No serious adverse events (SAEs) occurred and no subjects were discontinued from the study due to AEs. All of the AEs were mild in severity and resolved by the end of the study. "Feeling hot" was the most common drug-related AE, reported by 16% of the subjects (all VCS subjects). No clinically relevant trends were observed regarding vital sign or physical examination findings with respect to the differing treatments. A transient slight decrease (-0.2 mg/dL) in mean serum magnesium levels 5 hours postdose was noted in the 0.5 mg/kg VCS and higher dose levels.

Creatinine clearance measurements based on 24-hour urine collections were taken during the study to detect any subtle changes in renal function. Creatinine clearance values varied greatly during the study; however the variance was felt to be due to non-compliance with urine collections, or incomplete collections. Serum creatinine changes were modest during the study.

Food Effect Study. A total of 99 postdose AEs occurred during the study, of which 95 can be analyzed per treatment group. The number of AEs experienced per treatment group is as follows: 26 AEs (26.3%) following fasting, 44 AEs (44.4%) following administration of a low

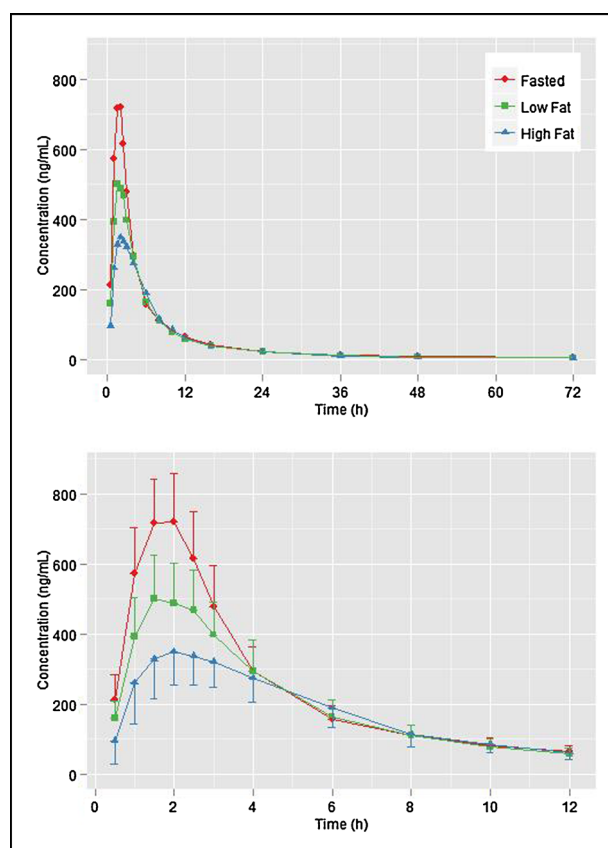


Figure 3. Mean whole blood concentration–time profiles of VCS following oral administration of 1.5 mg/kg in healthy subjects after an overnight fast and after a low-fat and high-fat meal (top panel, full profiles; lower panel, expanded view from 0 to 12 hours [$\pm 95\%$ CI]).

fat meal, and 25 AEs (25.3%) following administration of a high fat meal. The remaining AEs (4; 4.0%) were associated with abnormal vital signs results or laboratory results and could not be assigned to a treatment group (date and/or time of onset is not known). Seventy-seven AEs were graded as mild and six were graded as moderate. Ten percent of the AEs were judged as unrelated. Seven subjects reported a burning sensation, a sensation of heat,

Table 3. Pharmacokinetic Parameters and Statistical Comparisons for VCS Following a 1.5 mg/kg Oral Dose After Fasting, a Low-Fat and High-Fat Meal

Treatment	$T_{1/2}$ (h)	$T_{\max}$ (h)	$C_{\max}$ (ng/mL)	AUC_{inf} (ng h/mL)	GMR (90% confidence interval)	
					$C_{\max}$	AUC_{inf}
Fasted	22.72 $\pm$ 6.33 (27.85%)	1.77 $\pm$ 0.46 (25.78%)	789.43 $\pm$ 269.25 (34.11%)	4054.15 $\pm$ 1774.03 (43.76%)		
Low-fat	20.81 $\pm$ 3.57 (17.15%)	1.90 $\pm$ 0.6 (31.71%)	562.52 $\pm$ 207.95 (36.97%)	3458.47 $\pm$ 1786.51 (51.66%)	71.44 (64.25–79.44)	84.75 (78.39–91.62)
High-fat	20.01 $\pm$ 3.39 (16.96%)	2.32 $\pm$ 0.86 (37.28%)	385.03 $\pm$ 202.16 (52.5%)	3103.43 $\pm$ 1745.5 (56.24%)	47.47 (42.69–52.78)	74.74 (69.13–80.81)

Data presented as mean $\pm$ SD (CV%).

GMR, geometric least squares mean ratio (%) compared to fasted.

or an increased sensitivity in the mouth or throat; four subjects reported a burning sensation or a sensation of heat in the stomach; five subjects reported heartburn; and one subject reported a burning sensation in the mid-thoracic region. No difference among treatments was observed with respect to either those above-listed AEs, in general gastro-intestinal AEs, with the possible exception of heartburn, which was observed more frequently in fasting conditions. Four subjects reported a burning sensation or a sensation of heat in the hands, toes, forearm, head, or whole body. Those AEs were only reported with Treatments B and C. There were no SAEs or significant AEs in this study with the exception of one subject who had a significant AE "High CK." A repeat of this test performed 1 day later yielded a not clinically significant result. The AE was judged to be remotely related to study medication.

Discussion

Whole blood concentrations of VCS in healthy subjects demonstrate pronounced multi-exponential decay after single oral doses. This suggests extensive and possibly time-dependent distribution of VCS between blood and tissues. Following single oral doses of 0.25–4.5 mg/kg, the extent of systemic exposure increased in a linear but slightly greater than 1:1 dose-proportional manner. An evaluation of dose-normalized AUC data suggest that an apparent dose dependency was encountered between 0.25 mg/kg and 0.5 mg/kg which seems to disappear at the higher doses of 1.5 mg/kg, 3.0 mg/kg, and 4.5 mg/kg. The possibility of a parallel saturable process combined with a linear process could explain these results. As the dose is increased this effect disappears and the PK demonstrate 1:1 dose proportional, first-order, linear PK. Although 1:1 dose proportionality was not observed at the lowest doses, the doses did increase in a linear fashion. It has been shown that CsA also demonstrates subtle non-linearities in blood and tissue binding that appear to offset one another in such a fashion that conventional analysis demonstrates apparent dose-independent, linear PK. Interestingly, CL/F appeared constant at the higher dosing range while V/F remained constant throughout the entire dose range. Estimation of AUC_{inf} at low doses due to the limit imposed by assay sensitivity could be a possible confounding factor in this observation. The apparent elimination half-life of VCS ranged from 14.3 hours to 18.1 hours in the 1.5 mg/kg to 4.5 mg/kg dose range. Shorter half-lives calculated for the lower doses were a likely consequence of the assay sensitivity and the shorter sampling time.

VCS inhibited CN activity in a dose-related fashion with maximal inhibition appearing to peak at the 3.0 mg/kg dose. Time to peak inhibition appeared to correspond with T_{max} for whole blood concentration indicating a direct relationship between whole blood concentration of

VCS and pharmacologic activity in the blood. This was also demonstrated by correlation of whole blood concentration to CN activity and percent inhibition. Conversion of CN activity to percent inhibition demonstrates peak VCS inhibition of CN of up to 80% to 90% at the 1.5 mg/kg and 3.0 mg/kg doses, respectively. This finding may have implications for the potential efficacy and safety of VCS compared with other CN inhibitors, as VCS can reliably achieve a percent CN inhibition of >60% over a wide concentration range which may aide in titrating into a therapeutic window.

Administration of VCS with food decreased the absorption as measured by C_{max} and AUC_{inf}. This food effect was more pronounced following a high-fat meal than low-fat meal. Peak VCS concentrations were decreased by up to 52% and AUC_{inf} decreased by 25% after a high-fat meal. In addition, T_{max} was delayed following administration with a high-fat meal. VCS is a highly lipophilic drug which may be dependent on bile-salt emulsification for absorption.⁷ A drug–food interaction has been observed for other highly lipophilic drugs including CsA.^{8,9} Food also increased intersubject variability for C_{max} and AUC_{inf} with high-fat meals, again, having a greater effect. Based on these findings, and taking into account the potential consequences of decreased exposure, the recommendation for VCS administration should be to take on an empty stomach.

VCS appeared to be safe and generally well-tolerated up to the highest single oral dose (4.5 mg/kg) when administered to healthy volunteers. "Feeling hot" was the most common drug-related AE reported in the single ascending dose study. Approximately as many AEs were observed with the administration of the study drug after a fasting period and after the consumption of a high-fat meal. The number of AEs reported with the administration of the study drug after the consumption of a low-fat meal was higher. The majority of AEs reported were associated with the gastrointestinal system (including primarily throat irritation, gastric irritation, dyspepsia, loose stools, and nausea). The number of gastrointestinal-related AEs reported in this study was similar in the fasted and low-fat fed groups and slightly lower in the high-fat fed group. In the low-fat fed group, a notably higher number of nervous system-related AEs were reported. The majority of the AEs reported in this study were graded as mild and resolved spontaneously, indicating that the study formulations were well-tolerated, with no major side effects.

Conclusions

The Phase 1 pharmacokinetic studies presented in this paper were designed to support the development of VCS in autoimmune and organ transplant indications and to provide a rationale for selection of dose and dosing

regimen for later stage trials. VCS single dose PK demonstrated approximately linear, multiphasic exposure. Overall, dose-proportionality was slightly greater than 1, however as dose increased above 1.5 mg/kg, dose-proportionality approached 1:1 suggestive of parallel saturable-linear pharmacokinetic process at lower doses giving way to linear PK as dose increased. VCS appears to directly inhibit CN demonstrating inhibitory $E_{\max}$ relationship with CN activity and an $E_{\max}$ relationship with percent CN and a peak percent CN inhibition of $\sim 90\%$ at 3.0 mg/kg dose.

Administration of VCS with food resulted in diminished peak and extent of absorption and delayed peak that appeared related to fat content of the meal.

VCS was safe and well-tolerated in healthy volunteers after oral administration at doses ranging from 0.25 mg/kg to 4.5 mg/kg.

Declaration of Conflicting Interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare: P.R.M., S.Y.L., R.B.H., D.G.F., L.J.A., and R.T.F. had support from Isotechnika Pharma, Inc. for the submitted work; P.R.M., S.Y.L., R.B.H., D.G.F., L.J.A., and R.T.F. are employees of Isotechnika Pharma, Inc., and that there are no other relationships or activities that could appear to have influenced the submitted work.

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