APPENDIX I

STUDY 21-96-201: MASS BALANCE STUDY

AN OPEN-LABEL STUDY OF THE ABSORPTION, DISTRIBUTION, METABOLISM AND EXCRETION OF CILOSTAZOL FOLLOWING ORAL ADMINISTRATION OF ^{14}C-CILOSTAZOL IN HEALTHY HUMAN SUBJECTS

Reference: Volumes 59 to 63
Investigator:
Study Location:
Objective:

To determine the absorption, distribution, metabolism and excretion of a single oral dose of 50 mg [^{14}C]-cilostazol (200 μCi/50 mg) in healthy caucasian male subjects.

Radiolabeled Forms:

Details of which carbon was labeled has not been provided (may be same as used in study 21-94-303 which has radiolabel on the quinolinone ring on the carbon containing the ketone).

Study Design:

This is a single-center, open-label, single dose study of the metabolism of radiolabeled cilostazol. 10 healthy, non-smoking, caucasian male volunteers of age 18 - 55 years participated in the study. These subjects received a single oral dose of an ethanol solution of ^{14}C-cilostazol (200 μCi/50 mg/17 ml). Blood was drawn at 0, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72, 96, 120, 144, 192, 240 and 288 hours after dosing for determination of plasma concentration of the drug and radioactivity in plasma. Urine samples were collected at baseline, 0 - 12, 12 - 24, 24 - 48, 48 - 72, 72 - 96, 96 - 120, 120 - 144, 144 - 168, 168 - 192, 192 - 216, 216 - 240, 240 - 264 and 264 - 288 hours after dosing. Feces were collected at 24 hour intervals up to 288 hours after dosing and radioactivity determined.

DETERMINATION OF TOTAL RADIOACTIVITY:
METABOLITE PROFILING, ISOLATION AND IDENTIFICATION: Carried out at Radioactivity was determined analysis. Metabolite profiling was done in plasma and urine by method with Urine and fecal samples were analyzed for identification and quantitation of cilostazol and its metabolites using a system For quantitation of the metabolites, the radioactivity of each analyte was converted to percent of dose.

Data Analysis:

Statistical analysis was limited to summary statistics for PK parameters, recoveries, adverse events and clinical lab data.

Results:

Recovery of radioactivity in urine and feces between 0 and 144 hours following administration of the radiolabeled doses is shown in the table below. Approximately 74% of dose was eliminated in urine and about 21% in feces. No unchanged drug was found in urine.

MASS BALANCE:

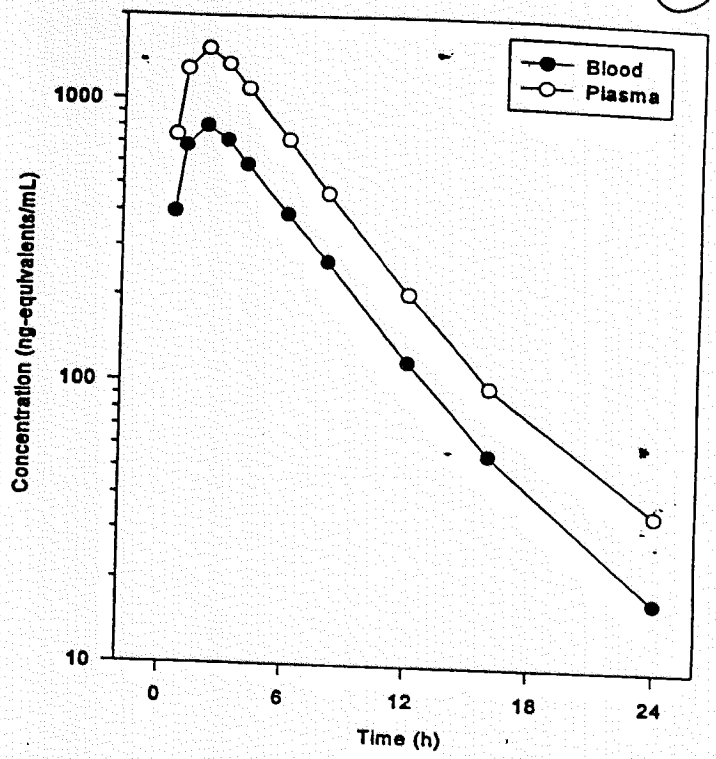
MOIETY	% OF DOSE IN URINE	% OF DOSE IN FECES
Total radioactivity	73.8 ± 8.12% (69.77% in first 24 hours)	21.11 ± 4.47%
Radioactivity as known metabolites in 144 hours	64.14 ± 8.94%	11.36 ± 4.93%
Cilostazol (OPC-13013)	None	1.25 ± 2.64%
OPC-13015	0.13 ± 0.38%	0.89 ± 0.44%
OPC-13213	29.57 ± 6.76%	2.58 ± 0.6%
OPC-1533	7.99 ± 2.07%	1.59 ± 0.87%
OPC-13217	7.67 ± 2.56%	0.93 ± 0.49%
OPC-13269	7.27 ± 0.98%	1.66 ± 0.78%
OPC-13326	3.99 ± 1.22%	1.28 ± 0.97%
OPC-13366	3.38 ± 0.54%	0.59 ± 0.45%
OPC-13371	2.40 ± 0.76%	0.28 ± 0.19%
OPC-13211	1.87 ± 0.67%	0.31 ± 0.22%
Radioactivity as known metabolites, in first 24 hours	59.86 ± 8.59%	

In the first 24 hours, the known metabolites and their % found in urine were as follows: Cilostazol (OPC-13013): None; OPC-13015: Traces; OPC-13213: 27.39 ± 5.36%; OPC-1533: 7.86 ± 2.09%; OPC-13217: 7.36 ± 2.41%; OPC-13269: 6.90 ± 1.29%; OPC-13326: 3.79 ± 1.49%; OPC-13366: 3.39 ± 0.60%; OPC-13371: 2.08 ± 0.50% and OPC-13211: 1.73 ± 0.68%.

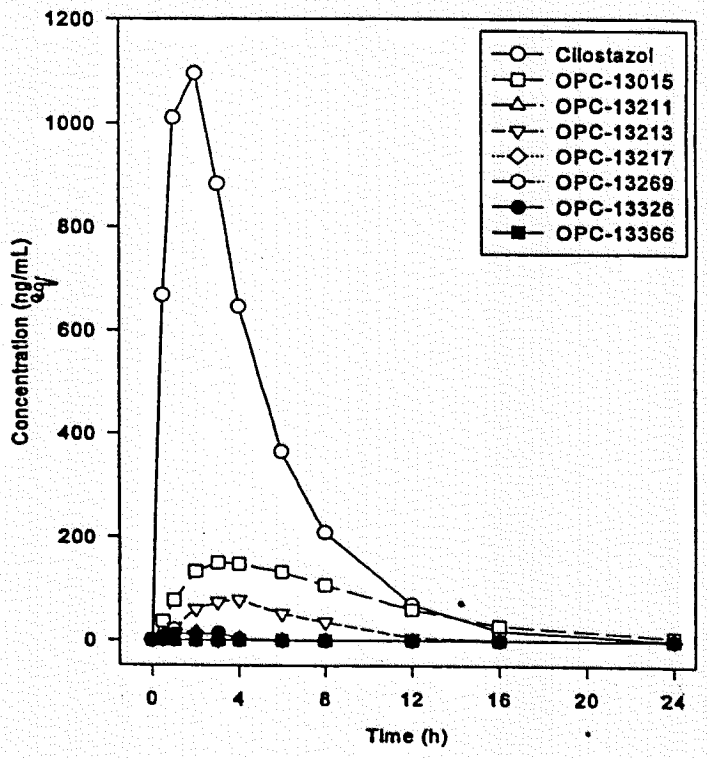
% unbound in plasma: cilostazol: 2.17 - 5.56%, OPC-13015: 1.94 - 4.63%, OPC-13213: 36.61 - 44.56%, OPC-13217: 20.50 - 36.84%.

BLOOD AND PLASMA TOTAL RADIOACTIVITY: The following figure shows the mean blood and plasma total radioactivity-time profiles following administration of 50 mg of radiolabeled cilostazol orally. The mean ± SD of radioactivity in plasma reached a Cmax of 1585 ± 301 ng-equiv/ml at 1.78 ± 0.67 hrs (Tmax). Radioactivity in blood reached a mean ± SD Cmax of 841 ± 144 ng-equiv/ml at a Tmax of 1.8 ± 0.7 hours and subsequently declined with a terminal half-life of 3.57 ± 0.72 hours. The mean blood to plasma ratio ranged

Blood and Plasma Activity-Time Profiles (ng-equiv/mL) of Total tivity Following Oral Administration of 50 mg of ^{14}C-Cilostazol (Semi- ale)



PHARMACOKINETICS OF PLASMA CILOSTAZOL, METABOLITES AND UNIDENTIFIED RADIOACTIVITY: Of the total radioactivity in plasma: 56% was due to cilostazol, 15% due to OPC-13015 and 4% due to OPC-13213 based on mean AUC0-t. The mean plasma concentration-time profiles for cilostazol and its metabolites are shown in the following figure:.

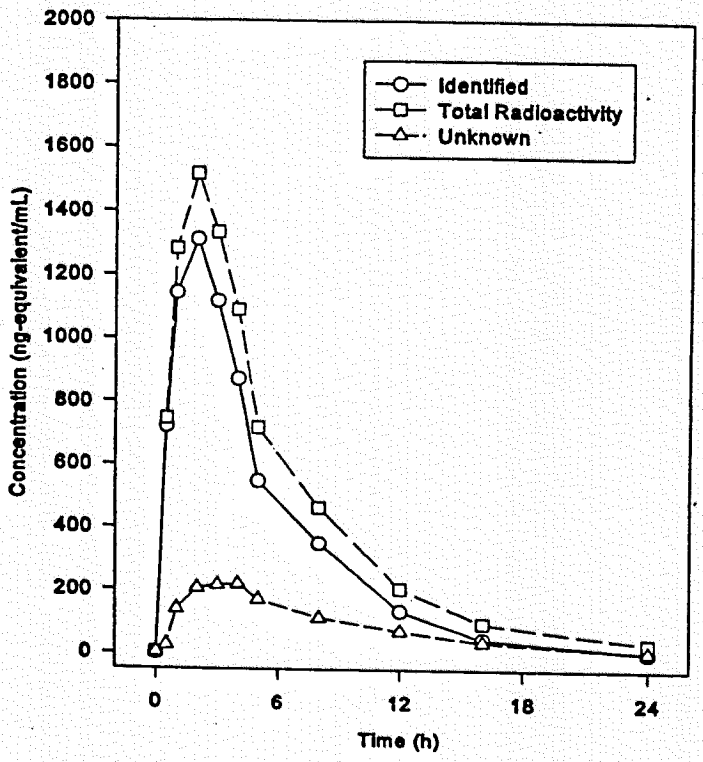


Summary of the pharmacokinetic parameters is shown in the table below:

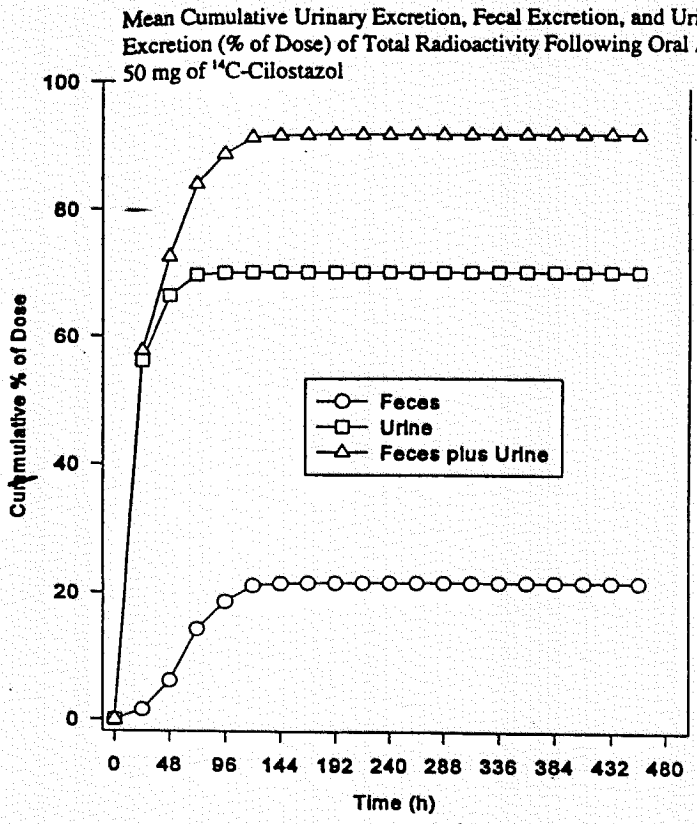
Moiety	Tmax (hrs)	Cmax (ng-eq/ml)	AUC0-t (ng-eq.hr/ml)	t1/2 (hrs)
Cilostazol	1.8 ± 0.7	1183 ± 253	5604 ± 1288	2.45 ± 0.35
OPC-13015	4.0 ± 1.2	156 ± 30	1512 ± 571	4.69 ± 1.27
OPC-13213	3.6 ± 0.5	79.3 ± 20.7	435.1 ± 129.9	Not calculated
Total ^{14}C	1.78 ± 0.67	1585 ± 301	10021 ± 2158	3.97 ± 0.49

The mean identified and unidentified radioactivity in plasma are shown in the following figure:

Mean Plasma Activity-Time Profiles (ng-equiv/mL) of Total Radioactivity, Identified Radioactivity, and Unidentified Radioactivity Following Oral Administration of 50 mg of ^{14}C-Cilostazol (Cartesian Scale)



Cumulative urinary and fecal excretion of total radioactivity is shown in the following figure:



Conclusions:

Approximately 95% of the administered radioactivity is recovered with 74% in urine and 21% in feces. This indicates that at least 74% of cilostazol is absorbed either as the parent itself or its metabolites. Of the total radioactivity in plasma, 56% was due to cilostazol, 15% was due to OPC-13015 and 4% due to OPC-13213.

Comment: While this study indicates that at least 74% of cilostazol is absorbed either intact or as its metabolites, this study cannot really address the issue of how much of parent cilostazol is actually absorbed and how much is metabolized pre-systemically. No absolute bioavailability study has been conducted by the sponsor to obtain this information. Based on plasma AUC, it can be said that at least half the exposure to this drug (as parent and metabolites) is due to the parent itself.

Study summary:

Study 21-94-303: A study of the absorption, distribution, metabolism and excretion following oral administration of ^{14}C-cilostazol to healthy human volunteers: This mass balance study submitted in this NDA under volumes 1.65 to 1.67 was conducted using an oral capsule. Results indicated that the mean recovery of radioactivity was 85.59 ± 8.62%. Fecal elimination accounted for 59.69% and renal elimination accounted for 25.89% of the total radioactivity. No unchanged cilostazol was found in urine. % of parent and metabolites found in plasma and urine were similar to the study 21-96-201. However, the ratio of elimination in urine and feces is different with greater amounts eliminated in feces with the oral capsule compared to oral solution. This indicates that the results of this study could be influenced by the absorption problems with the oral capsule. Hence, the results of study 21-96-201, which utilized an oral solution will be used to calculate the mass balance and for further interpretation.

STUDY 21-93-204: DOSE-PROPORTIONALITY AND FOOD EFFECT STUDY

AN OPEN LABEL STUDY OF THE DOSE PROPORTIONALITY OF CILOSTAZOL IN HEALTHY SUBJECTS

Reference: Volumes 50 to 54
Investigator:
Study Location:

Objective: 1. To determine the pharmacokinetics, dose proportionality, and safety of three doses of cilostazol in healthy subjects.
2. To evaluate the effect of food on the pharmacokinetics of cilostazol.

Study design:

This study was a single-blind, single-dose, randomized, balanced, incomplete block design, three period, four treatment crossover trial conducted at a single center. Twenty healthy male volunteers of age 18 to 40 years participated in this study. The four treatment groups tested were: cilostazol 50 mg fasting, cilostazol 100 mg fasting, cilostazol 100 mg fed and cilostazol 200 mg fasting. Subjects were randomized to one of the four treatment sequences as shown below:

Sequence	Treatment period I	Treatment period 2	Treatment period 3
A	50 mg (1 x 50 mg tab)	100 mg (2 x 50 mg tab)	200 mg (4 x 50 mg tab)
B	100 mg (2 x 50 mg tab)	200 mg (4 x 50 mg tab)	100 mg fed (2 x 50 mg tab)
C	200 mg (4 x 50 mg tab)	100 mg fed (2 x 50 mg tab)	50 mg (1 x 50 mg tab)
D	100 mg fed (2 x 50 mg tab)	50 mg (1 x 50 mg tab)	100 mg (2 x 50 mg tab)

Randomization was balanced so that 5 subjects were assigned to each sequence. Hence, during each treatment period, 5 subjects received each treatment and a total of 15 subjects comprised each treatment group at the completion of 3 treatment periods. There was a one week washout period between each dosing period. Each dose was taken with 180 ml of water. Subjects in the fed group received a high fat breakfast within 10 minutes prior to dosing. After dosing, subjects refrained from eating lunch and dinner until 4 and 10 hours after dosing.

Cilostazol 50 mg tablets, lot # 2J76PB1

High fat meal composition: Two fried eggs, two pieces of bacon, two pieces of toast with butter, whole milk, and hash brown potatoes.

Blood samples for cilostazol analysis were drawn in each treatment period at 0 (pre-dose), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 12, 24, 36, 48, 60, 72, 96 and 120 hours after dosing. Urine samples were collected at baseline, 0 - 4, 4 - 8, 8 - 12, 12 - 24, 24 - 48 and 48 - 72 hours after drug administration (urine samples were not analyzed subsequently since previous study showed no quantifiable levels of cilostazol in urine).

PK parameters for cilostazol and its major metabolites were determined by methods. The kinetic parameters of AUC, Cmax, Cmax/dose, and AUC/dose were used to estimate the dose proportionality by SAS GLM (ANOVA) and REGRESS procedures. The log transformed AUC and Cmax were analyzed using ANOVA to evaluate the food effect. 90% confidence intervals were then calculated.

Results:

ASSAY PERFORMANCE:

CILOSTAZOL (OPC-13013):
Method used:
Range:
Linearity: Linear within the range,
QC samples:
Precision:
Accuracy:

Specificity:

OPC-13015:
Method used:
Range:
Linearity: Linear within the range,
QC samples:
Precision:
Accuracy:

Specificity:

OPC-13213:
Method used:
Range:
Linearity: Linear within the range.
QC samples:
Precision:
Accuracy:

Specificity:

Assays were found to be acceptable.

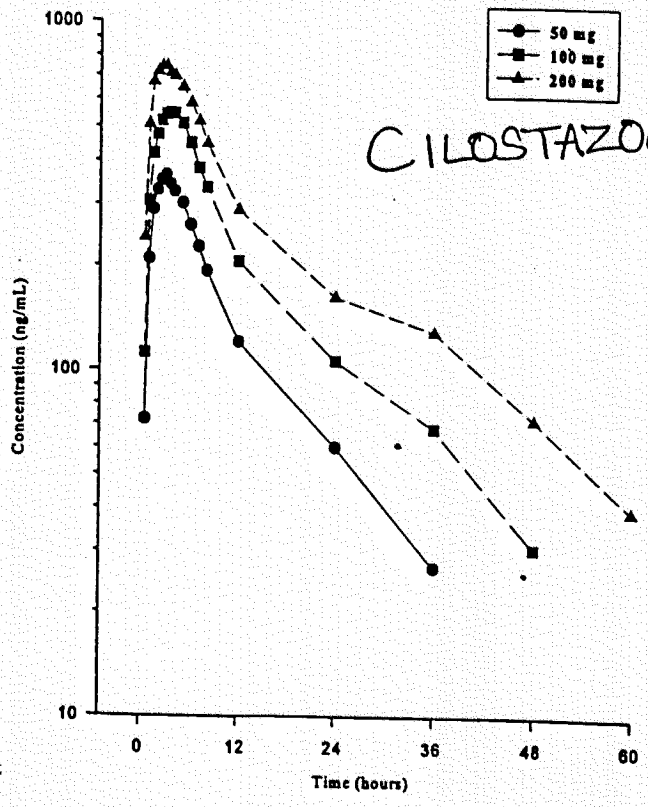
Mean pharmacokinetic parameters for cilostazol, OPC-13015 and OPC-13213 following administration of the 3 doses of cilostazol are shown in the following table.

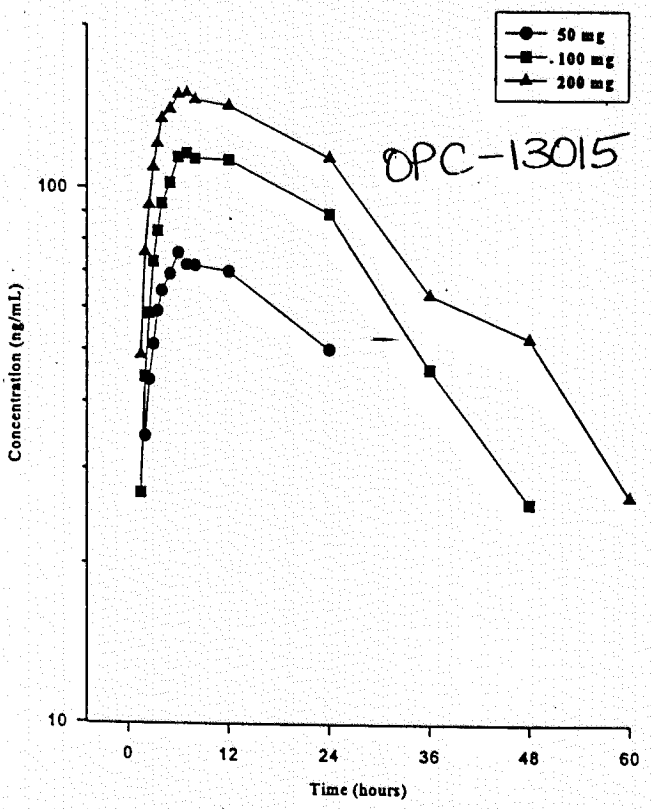
Dose Proportionality Pharmacokinetic Summary

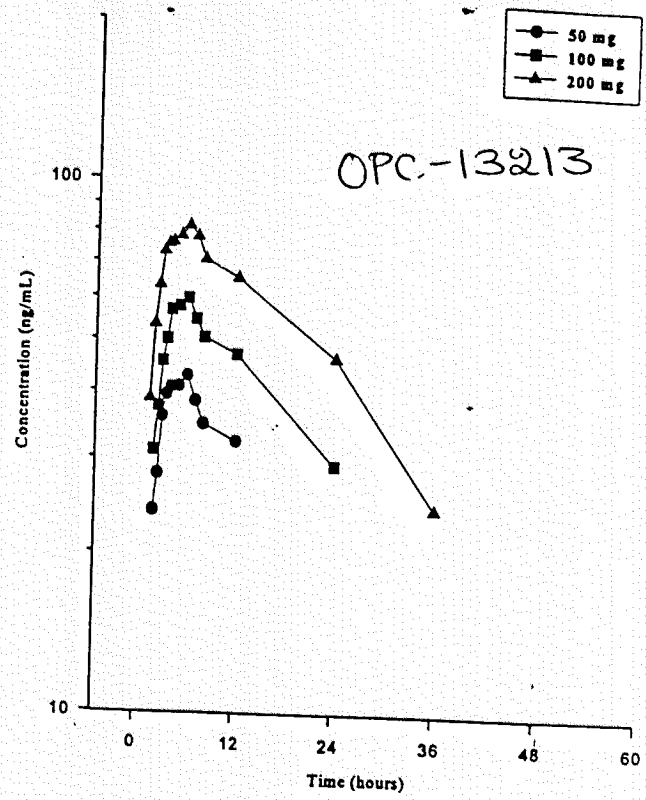
Analyte	Treatment/ Test Statistics*	C_{max} (ng/mL)	t_{max} (hr)	AUC_T (hr•ng/mL)	t½ (hr)	AUC (hr•ng/mL)
Cilostazol	50 mg	411.03	2.9	4334	11.0	4919
	100 mg	625.01	3.3	8087	15.1	8956
	200 mg	806.15	2.8	12943	13.4	13582
	p-value*	<0.001	0.604	<0.001	0.619	<0.001
	power*	0.636	0.823	0.266	0.134	0.379
OPC-13015	50 mg	77.99	5.6	1078	9.9	1522
	100 mg	122.42	6.1	2423	16.4	3153
	200 mg	162.00	7.1	3930	15.0	4551
	p-value*	<0.001	0.215	<0.001	0.110	<0.001
	power*	0.601	0.160	0.189	0.138	0.256
OPC-13213	50 mg	47.18	3.8	325	6.1	568
	100 mg	64.21	4.3	617	8.1	961
	200 mg	86.75	5.1	1451	14.0	1972
	p-value*	<0.001	0.003	<0.001	<0.001	<0.001
	power*	0.734	0.544	0.066	0.143	0.132

Treatment group means are least-squares means from dose proportionality analysis.

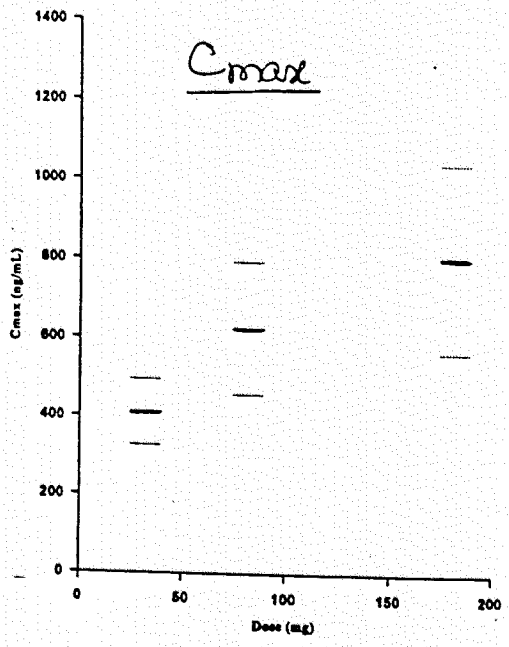
Mean plasma concentration-time curves for cilostazol, OPC-13015 and OPC-13213 following the 3 doses are shown in the following figures.

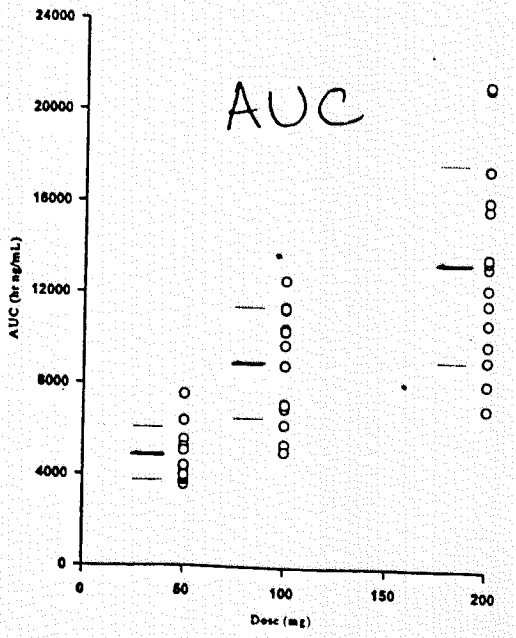


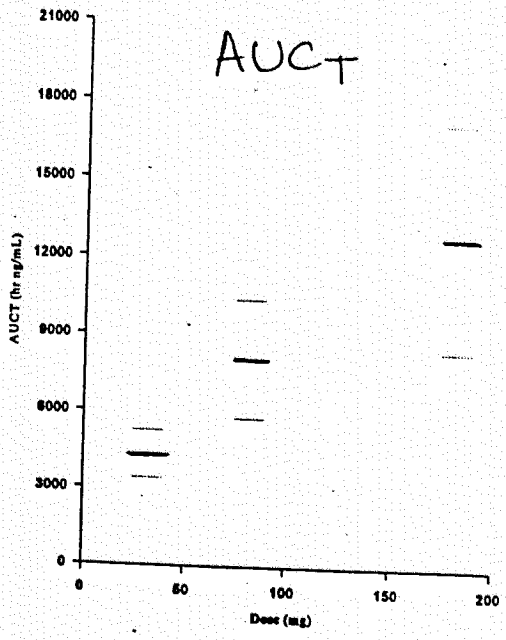




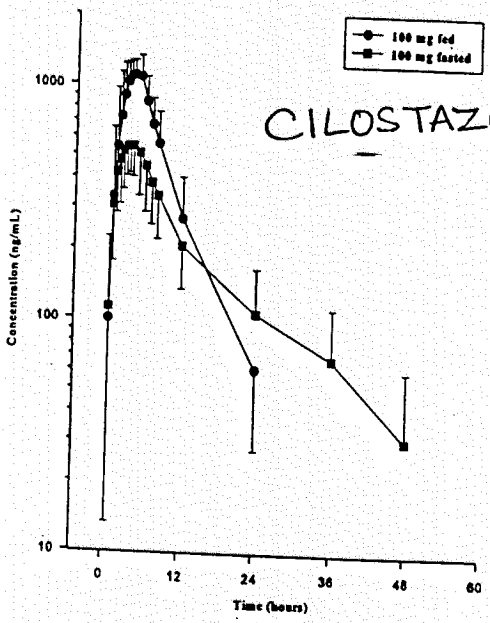
The relationship between cilostazol Cmax vs. dose and AUC and AUC_T vs. dose are shown in the following three figures:

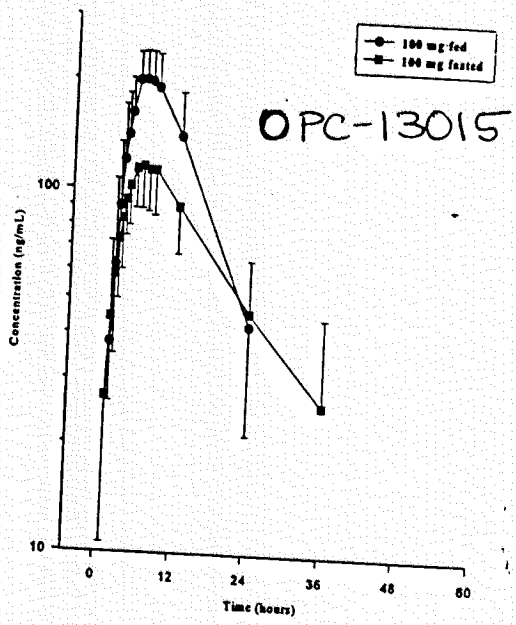


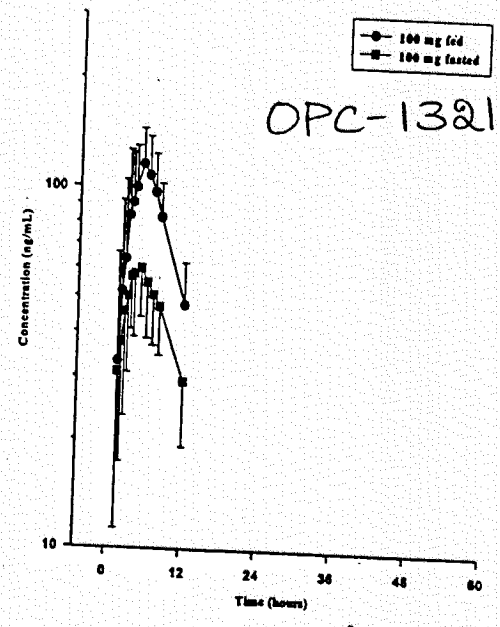




Mean plasma concentration-time curves for cilostazol and its metabolites when cilostazol was administered under fed and fasted conditions are shown in the following three figures.







The mean ± stdev pharmacokinetic parameters of cilostazol and its metabolites when given with and without food, along with the 90% confidence intervals are summarized in the following table.

Food-Effect Pharmacokinetic Summary

Analyte	Treatment/ Test Statistic*	C_{max} (ng/mL)	t_{max} (hr)	AUC_T (hr ng/mL)	t½ (hr)	AUC (hr ng/mL)
Cilostazol	Fast	625±168	3.3±1.2	8087±2299	15.1±14.5	8956±2441
Mean±SD	Fed	1216±211	3.8±1.0	10150±2009	5.4±2.0	10433±2112
	90%CI*	166.7-200.7	NA	119.1-148.5	-34.5- 90.5	106.3-137.4
OPC-13015	Fast	122±28	6.13±1.41	2423±754	16.4±9.8	3153±1188
Mean±SD	Fed	218±43	6.47±2.03	2981±746	8.3±5.9	3396±863
	90%CI*	161.2-187.9	NA	122.7-149.7	26.7-95.6	109.0 -137.5
OPC-13213	Fast	64±16	4.33±1.06	617±229	8.1±2.4	961±216
Mean±SD	Fed	122±36	4.77±0.82	974±270	5.2±2.4	1288±326
	90%CI*	143.1-173.2	NA	113.5-167.9	46.7-85.8	105.9-150.4

Cilostazol is absorbed with a t_{max} of approximately 3 hours. It was eliminated with a half-life of about 11 hours. Half-life estimates were variable due to secondary peaks in the PK profile. For the dose range of 50 to 200 mg tested, the plasma concentrations increased less than proportionally to dose level. With increase in dose, a lower dose adjusted Cmax and AUC were observed. The metabolites OPC-13015 and OPC-13213 also showed the same dose-dependent trends as the parent cilostazol, although the effect was not as prominent for OPC-13213. This indicates that, with increase in dose of cilostazol, a lower fraction of cilostazol may be absorbed.

When 100 mg cilostazol was administered in fed and fasted states, a 2-fold increase in Cmax and a 20% increase in AUC of cilostazol was observed when taken with food.

Conclusions: Following single oral doses of 50 to 200 mg, the plasma concentrations of cilostazol and its metabolites, OPC-13015 and OPC-13213 increased less than proportionally to the dose. This non-linearity was more pronounced for Cmax than AUC. A four-fold increase in dose resulted in only a two-fold increase in cilostazol peak concentrations.

Upon coadministration with food, the Cmax of cilostazol increased significantly (2-fold) and the AUC increased by 24%. Therefore, patients should take cilostazol tablets in a fasted state.

STUDY 011808 (report 009969): FOOD EFFECT STUDY ON 50 MG CILOSTAZOL TABLET

PHASE I STUDY OF CILOSTAZOL: THE EFFECT OF A MEAL ON THE PHARMACOKINETICS OF CILOSTAZOL IN HEALTHY MALE VOLUNTEERS

Reference: Volume 1.64
Investigator:
Study Location:

Objective:
To characterize the effect of a meal on the pharmacokinetics of cilostazol and its metabolites.

Study design:
This is a randomized, single-center, open-label, 2-way crossover study of single doses of 50 mg cilostazol tablets taken either with or without food. 6 healthy male volunteers of age 20 to 39 years participated in the study. The two assessment periods were separated by a one week washout period.

Study drug: Cilostazol 50 mg tablet, single dose, batch #s not provided.

Subjects reported to the study site the evening before the dosing day. The subjects fasted at least 10 hours before dosing administration. At about 8 a.m. on day 1, after at least a 10 hour fast (fasting treatment) or within 30 minutes of taking a standardized breakfast (fed treatment), each subject received 50 mg cilostazol tablet along with 150 ml of water according to one of the 2 treatment sequences shown below:

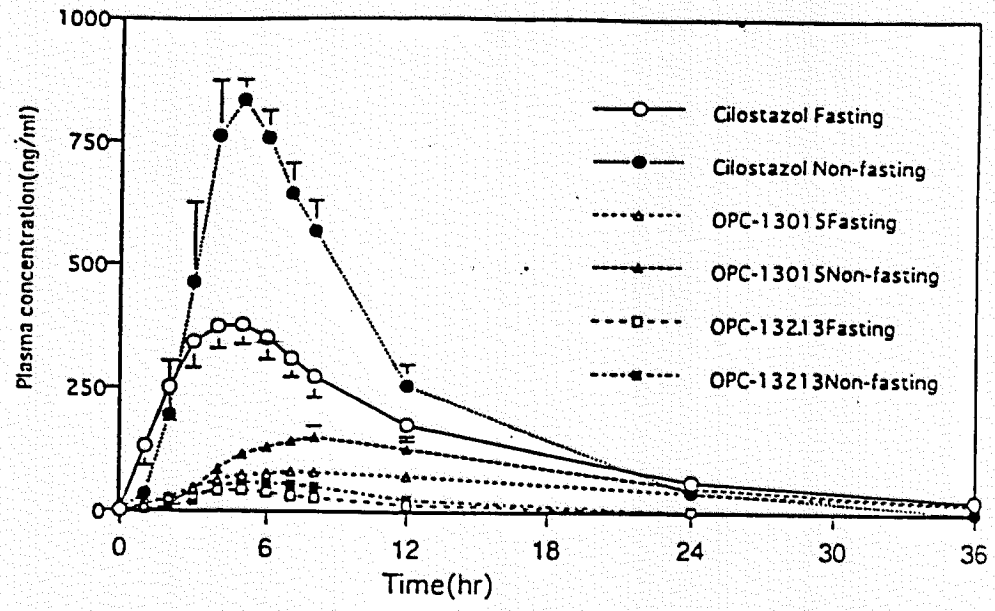
Sequence 1: Fed treatment, then washout, then fasting treatment.
Sequence 2: Fasting treatment, washout and then fed treatment.

Details of meal content have not been provided.

Blood was collected for determination of plasma concentrations of cilostazol and its metabolites, OPC-13015, OPC-13213 and OPC-13217, at 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24 and 36 hours after each dose. Assays were conducted using methods. Pharmacokinetic analysis of data was performed using techniques.

Results:
ASSAY PERFORMANCE: Details not provided.

Mean plasma concentration-time curves of cilostazol and its metabolites after administration of 50 mg cilostazol tablets under fasted conditions and fed conditions are shown in the following figure



Plasma concentration of cilostazol and metabolites following p.o administration of cilostazol at dose of 50mg/person in healthy volunteers in the fasting or non-fasting state

Results of the pharmacokinetic analysis (mean ± S.E.) are summarized in the following two tables:

Fasting

Parameters	cilostazol	OPC-13015	OPC-13213
Cmax(ng/ml)	412±32	85±9	42±7
Tmax(hr)	4.0±0.5	7.7±1.6	4.2±0.3
AUC_{0-24h}(ng·hr/ml)	4551±543		
$AUC_{0-\infty}$(ng·hr/ml)	5040±659	1660±350	368±107
$AUC_{inf.}$(ng·hr/ml)	5491±833	2461±636	514±91
$T_{1/2}(hr)\alpha$	5.7±0.9	14.9±3.3	6.9±0.9
$T_{1/2}(hr)\beta$	9.6±1.3 *		
$MRT_{inf.}$(hr)	12.69±2.16		
$VRT_{inf.}$(hr^2)	144.81±47.94		

* : n=4

Non-fasting

Parameters	cilostazol	OPC-13015	OPC-13213
Cmax(ng/ml)	932±53	150±22	61±10
Tmax(hr)	4.5±0.4	7.3±0.4	5.2±0.5
AUC_{0-24h}(ng·hr/ml)	7354±705		
$AUC_{0-\infty}$(ng·hr/ml)	7574±776	2553±584	528±107
$AUC_{inf.}$(ng·hr/ml)	7522±826	2983±660	629±83
$T_{1/2}(hr)\alpha$	4.0±0.4	9.2±0.9	5.6±0.6
$MRT_{inf.}$(hr)	8.58±0.55		
$VRT_{inf.}$(hr^2)	32.05±5.37		

Ratio of each metabolite to cilostazol PK is shown in the following table:

Parameters	cilostazol	OPC-13015		OPC-13213	
		Fasting	Non-fasting	Fasting	Non-fasting
Cmax	1	0.21	0.16	0.10	0.07
$AUC_{0-\infty}$	1	0.33	0.34	0.07	0.07
AUC_{0-t}	1	0.45	0.40	0.09	0.08

Cmax and AUC of cilostazol were 2.3 times and 1.4 times higher after a meal than in the fasted state. However, it was lower after a meal during 1 to 2 hours after dosing showing delayed absorption of cilostazol after a meal.

Comments:

1. Food significantly affects the pharmacokinetics of cilostazol and its metabolites following administration of cilostazol tablets with or without food.
2. The meal composition and details of assay performance should be provided in the study report.

Conclusion: Based on the data provided, a significant food effect is found on the bioavailability of cilostazol and its metabolites following administration of 50 mg cilostazol tablets. Therefore, based on the pharmacokinetic data, patients should take cilostazol tablets in fasted state (without meals). Data obtained in this study is in agreement with the pivotal food effect study 21-93-204.