STUDY 21-91-201F: PHARMACOKINETICS IN PATIENTS WITH INTERMITTENT CLAUDICATION

AN OPEN STUDY OF THE CHRONIC ADMINISTRATION OF CILOSTAZOL IN PATIENTS WITH INTERMITTENT CLAUDICATION SECONDARY TO CHRONIC OCCLUSIVE ARTERIAL DISEASE (PHARMACOKINETIC SUBSTUDY)

Reference: Volumes 68 to 70
Investigator:
Study Location:

Objective: To determine the pharmacokinetics of cilostazol in patients with intermittent claudication secondary to chronic occlusive arterial disease (COAD) following multiple oral dosing.

Study design:

This study was an open label pharmacokinetic substudy (amendment to a phase III, open-label, long-term extension study) in 26 male and female patients (of age 53 to 83 years) with moderately severe intermittent claudication secondary to chronic occlusive arterial disease. Pharmacokinetics were determined following a 100 mg single dose of cilostazol and after multiple dose of 100 mg q12 hours. Following a 10 day washout from cilostazol, patients were given cilostazol as a single 100 mg dose on day 0, 100 mg q12h on days 5 - 12, and another single 100 mg dose on day 13. All the dosing occurred in fasted state.

Cilostazol 100 mg tablets, lot # 6C73PA1

Blood samples for cilostazol and its metabolites analysis were drawn at 0 (pre-dose), 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72 and 96 hours after cilostazol dosing on days 0 and 13. Additional blood samples were collected before dosing on day 0 and at 96 hours after the last dose on day 13 for determination of cilostazol protein binding. PK parameters for cilostazol and its major metabolites were determined by noncompartmental methods. Regression analysis was used to assess relationships between pharmacokinetic and demographic data.

Results:

ASSAY PERFORMANCE: Assay conducted at

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:

OPC-13217:
Method used:
Range:
Linearity: Linear within the range.
QC samples:
Precision:

Accuracy:

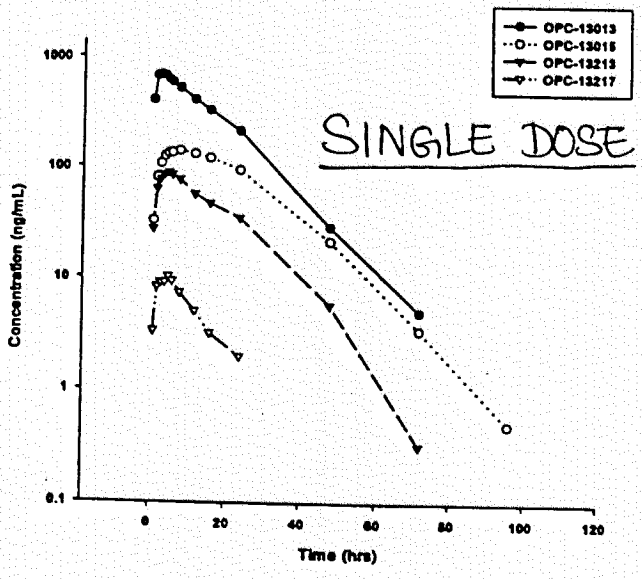
Specificity:

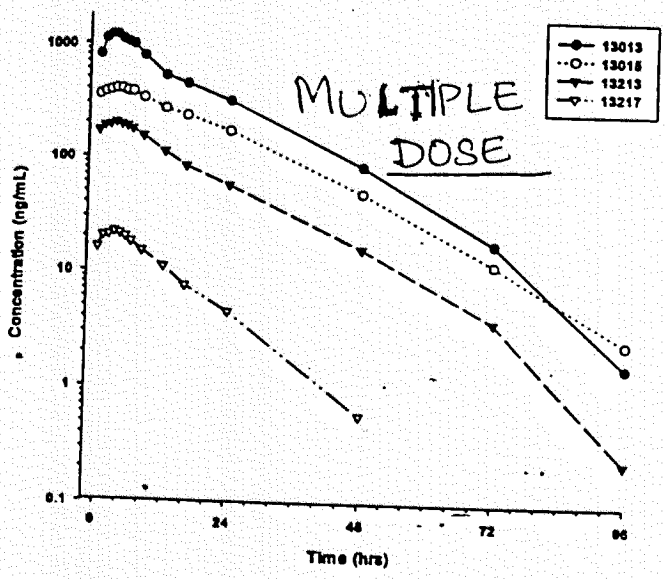
Assays were found to be acceptable with a % bias within ± 15% for all QC levels and a ± 20% at LOQ for both parent and metabolites. However, the protocol stated QC acceptance criteria of % bias of ± 20% for drug and ± 25% for metabolites is not acceptable. In future protocols, this acceptance criteria should be modified.

Mean pharmacokinetic parameters for cilostazol, OPC-13015, OPC-13217 and OPC-13213 following administration of single and multiple doses of cilostazol are shown in the following table.

Pharmacokinetic Parameter	Cilostazol Mean ± S.D.	OPC-13015 Mean ± S.D.	OPC-13213 Mean ± S.D.	OPC-13217 Mean ± S.D.
C_{max} (ng/mL)	738.7 ± 270.6	155.5 ± 46.8	92.8 ± 43.9	12.4 ± 4.5
T_{max} (hrs)	3.4 ±1.8	8.8 ± 5.1	5.9 ± 2.8	4.5 ± 2.1
AUC (ng*hr/mL) (up to ∞)	11902 ± 5220	4403 ± 2025	2234 ± 995	285 ± 151
AUC_{0-12} (ng*hr/mL)	6162 ± 2384	1330 ± 429	802 ± 371	93 ± 46
Cl/F/kg (mL/min/Kg)	0.15 ± 0.10	-	-	-
Cl/F_{sb} (mL/min/kg)	7.02 ± 4.12	-	-	-
Vd/F/kg (L/kg)	2.64 ± 2.79	-	-	-
$t_{1/2}$ (hrs)	11 ± 5.7	11.4 ± 2.4	14.8 ± 8.8	12.7 ± 13.6
The following table lists the mean ± S.D. for each pharmacokinetic parameter on Day 13:				
Pharmacokinetic Parameter	**Cilostazol Mean ± S.D.**	**OPC-13015 Mean ± S.D.**	**OPC-13213 Mean ± S.D.**	**OPC-13217 Mean ± S.D.**
C_{max} (ng/mL)	1331.5 ± 563.9	426.4 ± 158.5	224.0 ± 89.3	27.0 ± 8.6
T_{max} (hrs)	2.7 ± 1.4	5.2 ± 9	3 ± 1.8	3.4 ± 1.7
AUC (ng*hr/mL) (up to ∞)	21585 ± 12820	11318 ± 6487	4194 ± 1780	468 ± 198
AUC_{0-12} (ng*hr/mL)	10732 ± 5019	4148 ± 1712	1956 ± 810	213 ± 75
Cl/F (mL/min)	13.6 ± 14.6	-	-	-
Cl/F_{sb} (mL/min)	644.5 ± 582.4	-	-	-
Vd/F (L)	225.8 ± 302.1	-	-	-
$t_{1/2}$ (hrs)	10.5 ± 4.4	11.7 ± 3.8	13.3 ± 4.8	12.6 ± 6.6
AUC Day 0/AUC_{0-12h} Day 13	1.34 ± 0.26	1.11 ± 0.23	1.07 ± 0.32	1.13 ± 0.61
R	1.71 ±0.52	3.12 ± 1.37	2.54 ± 0.86	2.80 ± 2.04
The mean ± S.D. fraction unbound (%) for cilostazol was 2.02 ± 0.16.				

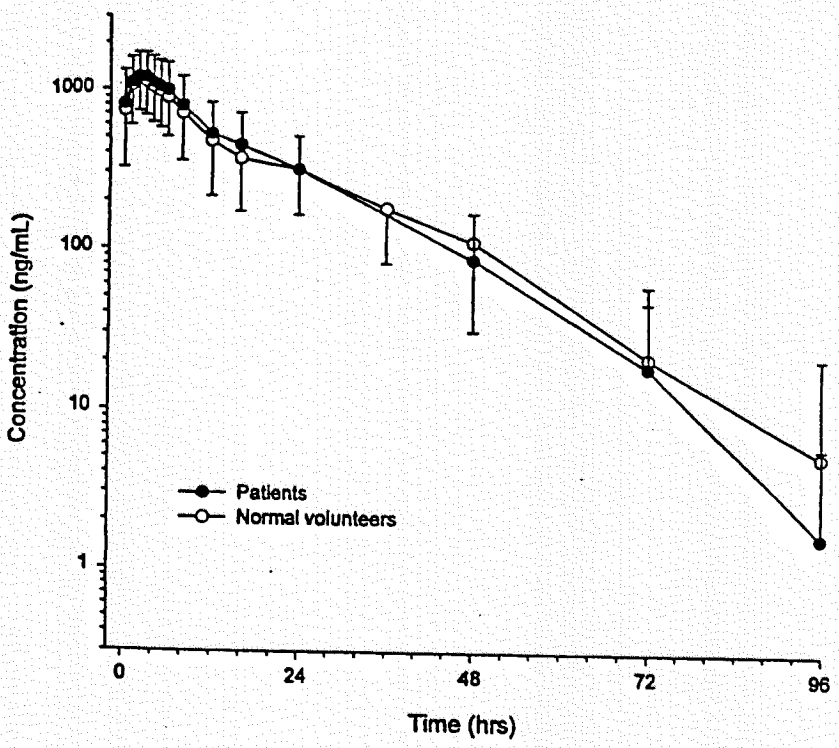
Mean plasma concentration-time curves for cilostazol and its metabolites after single and multiple cilostazol dosing are shown in the following figures.





Comparison of PK parameters (free fraction of cilostazol, oral clearance, oral unbound clearance, AUC, Cmax, half-life and volume of distribution) vs. age showed no correlation.

Since this study design was similar to study 21-93-202 (age and gender study) where the effects of age and gender on PK were assessed in normal volunteers, the PK in patients obtained in this study were compared to that of study 21-93-202. The figure below shows the plasma concentration-time profile of cilostazol following 100 mg cilostazol bid for 7 days in patients and normal volunteers (along with 95% confidence intervals).



Conclusions: The pharmacokinetics of cilostazol and its metabolites in patients are similar to those in normal volunteers.

PROTEIN BINDING:

STUDY 003630 - THE IDENTIFICATION OF PROTEIN BINDING OF ^{14}C-CILOSTAZOL IN HUMAN PLASMA

Study ID: 003630
Volume: 1.114
Objective:

To determine the protein binding of cilostazol and to identify the protein to which cilostazol is highly bound.

Concentrations of cilostazol selected for this study were 0.25, 0.5, 1, 2.5 and 5 μg/mL. The C_{max} for cilostazol obtained in normal volunteers (600 ng/mL) is within the concentration range studied here.

Study Design:

Human plasma was obtained. An aliquot of radiolabeled drug (^{14}C) stock solutions in methanol was added to either blank human plasma or solutions of the test proteins in phosphate buffer solution (HSA 40 mg/ml, α_1-acid glycoprotein 0.9 mg/ml, α,β-globulin 17 mg/ml and γ-globulin 7 mg/ml, these are normal concentrations expected in plasma).

Protein binding of ^{14}C-cilostazol to human serum albumin (HSA), α_1-acid glycoprotein, α,β-globulin and γ-globulin was determined by method conducted at 37°C. Radioactivity was measured in both the and the original solution by

Protein binding rate was then determined. Binding parameter NK was calculated using the following equation: B = (NK)*F, where B = concentration of protein-bound cilostazol (μg/ml), F = concentration of free cilostazol (μg/ml), N = concentrations of binding sites in the protein, K = affinity constant and NK = binding capacity. B versus F graphs were plotted and NK was calculated for each protein from the slope of the curve.

Results:

% binding to plasma ranged from 91.8 to 95.8%. The NK value was 25.01 (see table below for details of binding). The % binding to HSA was 92.4 to 94%, to α_1-acid glycoprotein was 35.9 to 44.7%, to α,β-globulin was 47.9 to 52.8% and to γ-globulin was 16.7 to 20.2%. The binding capacity (NK) to HSA was 14.31 (57.2% that with plasma protein), for α_1-acid glycoprotein was 0.68 (2.7% that with plasma protein), for α,β-globulin was 0.91 (3.6% that with plasma protein) and for γ-globulin was 0.21 (0.8% that with plasma protein).

% protein binding of cilostazol in human plasma and other proteins

PROTEIN	Concentration of cilostazol (μg/ml) 0.25	0.5	1.0	2.5	5.0	NK
Plasma	91.8±1.11	93.4±0.6	95.8±0.4	95.9±0.1	95.9±0.1	25.01
HSA	92.4±0.8	94.0±0.1	93.3±0.2	93.9±0.2	93.4±0.1	14.31
α_1-AGP	44.7±1.3	43.1±2.1	42.7±0.6	40.9±1.8	35.9±0.4	0.68
α,β-globulin	52.8±1.0	48.5±1.5	49.5±1.3	48.8±1.6	47.9±1.5	0.91
γ-globulin	20.2±1.8	16.7±1.9	17.0±1.3	17.5±1.0	17.5±1.2	0.21

Conclusion: Human serum albumin was identified as the major binding protein. The protein binding of cilostazol to human plasma is very high (about 95%) and does not appear to be saturable at the concentrations studied. At a concentration of 5.0 μg/ml, binding to α_1-acid glycoprotein appears to be saturable.

Comment: Protein binding data on the major active metabolites of cilostazol has not been submitted. This information is extremely useful and should be obtained and submitted in the future.

PROTEIN BINDING INTERACTIONS

STUDY REPORTS X1243PB AND V1259PB: PROTEIN BINDING INTERACTIONS BETWEEN CILOSTAZOL, OMEPRAZOLE AND WARFARIN IN HUMAN PLASMA

Volume: 1.90

Objective:

1. To determine the in vitro protein binding of ^{14}C-cilostazol at 500 ng/ml and its possible displacement by omeprazole (0.5 and 1.0 μg/ml) or by warfarin (2 and 10 μg/ml) using ultrafiltration.
2. To determine the binding of warfarin in presence of cilostazol, OPC-13015 and OPC-13213 to plasma proteins.

Study Design:

The free fraction of cilostazol in human plasma was determined using ultrafiltration and the resulting samples (plasma and ultrafiltrate) were analyzed by
unbound = dpm in in plasma.

Results:

The mean % of free (unbound) cilostazol to human plasma proteins at a concentration of 500 ng/ml in the presence of 0, 0.5 and 1.0 μg/ml omeprazole are 1.856 ± 0.075%, 1.864 ± 0.036% and 2.149 ± 0.248%. The displacement of cilostazol by omeprazole is significant at the 1.0 μg/ml concentration only ($p<0.01$).

The mean % of free cilostazol in human plasma at a concentration of 500 ng/ml in the presence of 2 and 10 μg/ml warfarin are 2.201 ± 0.252% and 2.121 ± 0.254%. This displacement of cilostazol by warfarin is significant at the 2 and 10 μg/ml level ($p<0.01$).

The mean % of free warfarin in human plasma at a concentration of 2000 ng/ml by itself, in the presence of 1300 ng/ml cilostazol, in presence of 1300 ng/ml cilostazol + 200 ng/ml OPC-13015 + 80 ng/ml OPC-13213, and in presence of 650 ng/ml cilostazol + 100 ng/ml OPC-13015 + 80 ng/ml OPC-13213 are 1.07 ± 0.06%, 1.24 ± 0.01%, 1.25 ± 0.03% and 1.34 ± 0.05%. These results indicate that the free fraction of warfarin at 2 μg/ml is statistically different ($p<0.01$) compared to that in presence of cilostazol and its metabolites.

Conclusion: Omeprazole in a concentration of 1.0 μg/ml causes significant displacement of cilostazol from protein binding sites (fraction unbound changes from 1.856 to 2.149%). Warfarin at concentrations of 2 and 10 μg/ml causes a significant displacement of cilostazol (fraction unbound changes from 1.856 to 2.201 and 2.121%). Cilostazol and its metabolites at various concentrations cause a significant displacement of warfarin from its protein binding sites.

IN VITRO METABOLISM:

STUDY 010572 - METABOLISM OF ^{14}C-OPC-21 BY RECOMBINANT 10 HUMAN CYTOCHROME P450 ISOFORMS EXPRESSED BY VACCINIA VIRUS

Study ID: 010572
Volume: 1.114

This study is not being reviewed since it is not clear what OPC-21 is and how it is related to cilostazol.

STUDY 010514 - IN VITRO METABOLISM OF OPC-13013 BY MICROSOMES DERIVED FROM HUMAN AHH-1 TK+/- CELLS EXPRESSING HUMAN CYTOCHROME P450s

Study ID: 010514
Volume: 1.114
Objective:

1. To identify the CYP isoforms involved in the metabolism of cilostazol by metabolizing enzymes derived from human AHH-1 TK+/- cells expressing human cytochrome P450s. 2. To investigate the inhibitory effects of cilostazol on CYP3A4-catalyzed testosterone 6β-hydroxylase activity and CYP2D6-catalyzed bufuralol 1'-hydroxylase activity.

Identification of CYP isoforms that metabolize cilostazol: A reaction mixture containing 2 mg/ml microsomal protein, 5 mM β-NADH and 100 μM cilostazol were incubated at 37°C for 2 hours. The supernatant samples were then analyzed for cilostazol and its metabolites using

Inhibitory effect on CYP3A4-catalyzed testosterone 6β-hydroxylase activity: A reaction mixture containing 0.7 mg/ml CYP3A4 microsomal protein, 5 mM β-NADPH, 2.5 mM β-NADH, 120 μM testosterone and either OPC-13013 or miconazole at 1 x 10^{-4}, 1 x 10^{-5} and 1 x 10^{-6} M were incubated at 37°C for 30 minutes. The supernatant was then analyzed using to measure the concentrations of 6β-hydroxytestosterone. The apparent Km and Vmax for CYP3A4-catalyzed 6β-hydroxytestosterone activity were calculated from the linear regression line obtained by Lineweaver-Burk reciprocal plot.

To determine Ki, the reaction mixture containing 0.7 mg/ml CYP3A4 microsomal protein, 5 mM β-NADPH, 2.5 mM β-NADH, testosterone and OPC-13013 at various concentrations (testosterone 40, 120 and 360 μM and OPC-13013 40 and 100 μM) were incubated at 37°C for 30 minutes. The supernatant was then analyzed using to measure the concentrations of 6β-hydroxytestosterone. The inhibition constant (Ki) for inhibition of CYP3A4-catalyzed 6β-hydroxytestosterone activity by OPC-13013 was calculated from the linear regression line obtained by the Dixon plot.

Inhibitory effect on CYP2D6-catalyzed bufuralol 1'-hydroxylase activity: A reaction mixture containing 1 mg/ml CYP2D6 microsomal protein, 5 mM β-NADPH, 2.5 mM β-NADH, 100 μM bufuralol and either OPC-13013 at concentrations of 1 x 10^{-4}, 1 x 10^{-5} and 1 x 10^{-6} M or quinidine at 1 x 10^{-5}, 1 x 10^{-6} and 1 x 10^{-7} M were incubated at 37°C for 30 minutes. The

supernatant was then analyzed using to measure the concentrations of 1'-hydroxybufuralol.

Results:

The catalytic properties of each human CYP isoform on the metabolism of cilostazol are shown in the following table. Results indicate that the primary CYP isoform involved in the metabolism of cilostazol is CYP3A4, while CYP1A2, CYP2B6, CYP2D6 and CYP2E1 are also involved in its metabolism.

	Activity (pmol/mg protein''/2hr)								
CYP	OPC-13015	OPC-13326	OPC-1533	OPC-13215	OPC-13211	OPC-13366	OPC-13371	OPC-13217	OPC-13213
Control Ms.									
(1A1)	—	—	—	—	—	—	—	—	—
CYP1A1	—	—	—	—	—	—	—	—	—
CYP1A2	71	68	—	—	—	—	—	—	—
CYP2A6	—	—	—	—	—	—	—	—	—
CYP2B6	—	—	—	—	—	—	—	—	—
CYP2C9	—	—	—	—	—	—	—	—	86
CYP2D6	288	385	—	—	—	45	—	31	—
CYP2E1	—	36	—	—	—	—	—	—	—
CYP3A4	996	1,349	460	217	309	72	60	301	—

The values indicated as — were not detected.

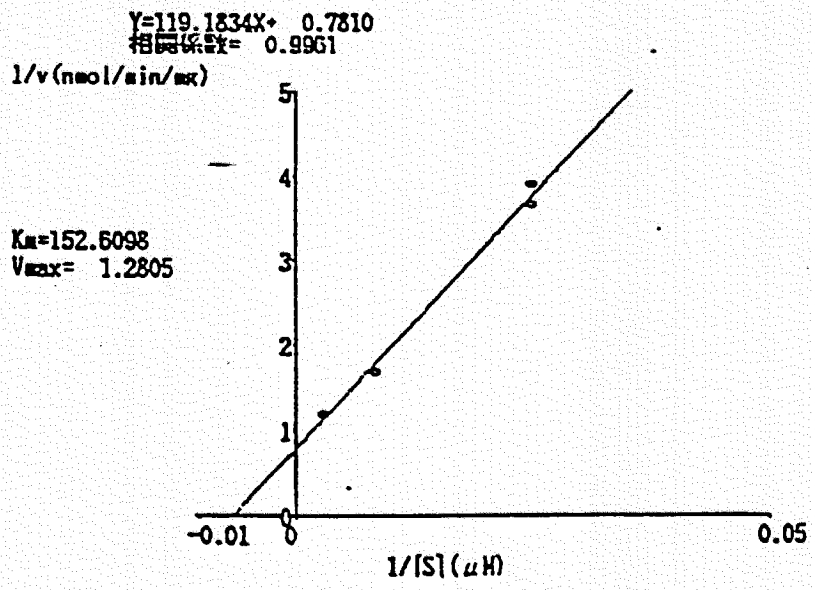
''Microsomal protein derived from human AHH-1 TK +/- cells expressing human cytochrome P-450s.

The inhibitory effects of cilostazol and miconazole on CYP3A4-catalyzed 6β-hydroxytestosterone activity are shown in the following table.

	Percent inhibition			
	Concentration (M)			
Compound	$1x10^{-4}$	$1x10^{-5}$	$1x10^{-6}$	$1x10^{-7}$
OPC-13013	82.1	25.3	3.4	—*
(±) Miconazole	—*	94.6	71.6	21.9

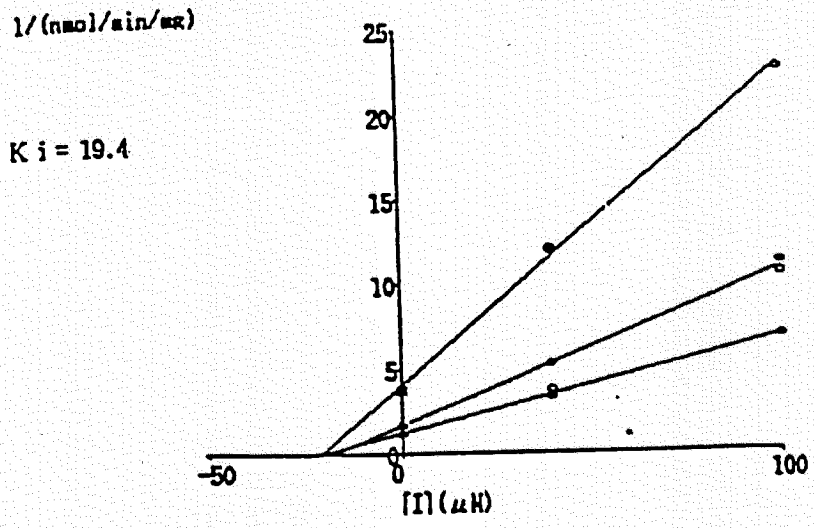
* : not done

The apparent Km and Vmax for CYP3A4-catalyzed 6β-hydroxytestosterone activity were 153 μM and 1.28 nmol/min/mg protein respectively (see figure below).



Double-reciprocal plot for the determination of apparent Km and Vmax on CYP3A4 catalyzed testosterone 6β-hydroxylase activity (Lineweaver-Burk plot)

These results suggest that cilostazol has an inhibitory effect on CYP3A4-catalyzed 6β-hydroxytestosterone activity, even though this is not as potent as miconazole. The apparent Ki was found to be 19 μM (see the following figure).



Inhibition of OPC-13013 on CYP3A4 catalyzed testosterone 6β-hydroxylase activity (Dixon plot)

The inhibitory effects of cilostazol and quinidine on CYP2D6-catalyzed bufuralol 1'-hydroxylase activity are shown in the following table. At the concentrations of cilostazol studied, no inhibition of CYP2D6-catalyzed activity was found.

	Percent inhibition or (stimulation)			
	Concentration (M)			
Compound	1x10^{-4}	1x10^{-5}	1x10^{-6}	1x10^{-7}
OPC-13013	3.3	0.5	(1.6)	—*
Quinidine	—*	92.1	52.2	9.1

* ; not done

Conclusion: Results from this study indicate that CYP3A4 is the primary enzyme involved in cilostazol metabolism while CYP2B6, CYP1A2, CYP2D6 and CYP2E1 may also possibly be involved. At therapeutically achievable concentrations (about 2 - 3 μM), cilostazol is a weak inhibitor of CYP3A4-catalyzed 6β-hydroxytestosterone activity based on a Ki of 19 μM and has no inhibitory activity on CYP2D6-catalyzed bufuralol 1'-hydroxylase activity.

Comments:

1. The concentrations used in this study are about 50 fold higher than therapeutically expected plasma concentrations. If compared to unbound concentrations expected after a therapeutic dose, these concentrations used in this study are several fold higher. In future, the sponsor should use therapeutically relevant concentrations in the in vitro metabolism studies.
2. Since this drug is metabolized by 3A4, it may potentially interact with drugs like ketoconazole and erythromycin.
3. The inhibition potential of cilostazol metabolites has not been studied.

STUDY 011114, report 009526 - IN VITRO METABOLISM STUDY OF OPC-13013 USING MICROSOMES FROM HUMAN AHH-1 TK+/- CELLS THAT EXPRESS SINGLE FORMS OF HUMAN CYTOCHROME P450s

Study ID: 011114; **Volume:** 1.114

Objective: To investigate whether cilostazol metabolism is catalyzed by the human cytochrome P450 isoforms 2C19 and 2C8, which are derived from human AHH-1 TK+/- cells that express these isoforms.

Identification of CYP isoforms that metabolize cilostazol: A reaction mixture containing 2 mg/ml microsomal protein (microsomes that express human CYP2C8 and CYP2C19 individually obtained from Gentest Corporation), 5 mM β-NADPH, 2.5 mM β-NADH and 100 μM cilostazol were incubated at 37°C for 2 hours. The supernatant samples were then analyzed for cilostazol and its metabolites using

Results: The catalytic properties of each human CYP isoform on the metabolism of cilostazol are shown in the following table (this includes data on CYP2C8 and CYP2C19 obtained from this study along with other isozymes obtained from the previous study). Results indicate that the CYP2C19 but not CYP2C8 is involved in cilostazol metabolism.

	Activity (pmol/pmol of P450/2 hr)								
CYP	OPC-13015	OPC-13326	OPC-1533	OPC-13215	OPC-13211	OPC-13366	OPC-13371	OPC-13217	OPC-13213
Control	--	--	--	--	--	--	--	--	--
1A1	<1.52	<1.02	<2.03	<1.02	<1.02	<1.02	<1.02	<1.02	<1.02
1A2	2.46	2.35	<1.38	<0.69	<0.69	<0.69	<0.69	<0.69	<0.69
2A6	<0.70	<0.47	<0.93	<0.47	<0.47	<0.47	<0.47	<0.47	<0.47
2B6	<0.41	<0.27	<0.55	<0.27	<0.27	<0.27	<0.27	<0.27	1.17
2C9	<3.23	<2.15	<4.30	<2.15	<2.15	<2.15	<2.15	<2.15	<2.15
2D6	18.58	24.84	<2.58	<1.29	<1.29	2.90	<1.29	2.00	<1.29
2E1	<0.36	0.44	<0.48	<0.24	<0.24	<0.24	<0.24	<0.24	<0.24
3A4	42.75	57.90	19.74	9.31	13.26	3.09	2.58	12.92	<0.86
2C8	<1.36	<0.91	<1.82	<0.91	<0.91	<0.91	<0.91	<0.91	<0.91
2C19	<2.73	<1.82	<3.64	<1.82	<1.82	<1.82	<1.82	37.27	<1.82

Conclusion: Results from this study indicate that CYP2C19 catalyzed the production of OPC-13217, whereas CYP2C8 has no catalytic activity on cilostazol metabolism.

Comments: 1. The concentrations used in this study are about 50 fold higher than therapeutically expected plasma concentrations. If compared to unbound concentrations expected after a therapeutic dose, these concentrations used in this study are several fold higher. In future, the sponsor should use therapeutically relevant concentrations in the in vitro metabolism studies.

2. Since this drug is metabolized by CYP2C19, it may potentially interact with drugs like omeprazole, phenytoin and diazepam.

(77)

STUDY 012060, report 010227 (IN VITRO ENZYME INHIBITION STUDY):

INHIBITION BY OPC-13013 OF DRUG-METABOLIZING ENZYME ACTIVITIES DERIVED FROM HUMAN CYTOCHROME P450

Reference: Volume 114
Investigator:
Objective:

To investigate the in vitro effects of cilostazol on drug metabolizing enzyme activities in microsomes prepared from human AHH-1 TK+/- cell line expressing human cytochrome P450.

Study design:

Human expressed CYP450 isoforms were incubated with various substrates [theophylline, (CYP1A2): 500 μM; tolbutamide, (CYP2C9): 50 μM; debrisoquine, (CYP2D6): 250 μM, testosterone, (CYP3A4): 50 μM, chlorzoxazone, (CYP2E1): 50 μM and S-mephenytoin, (CYP2C19): 50 μM], reaction cofactors and cilostazol 0, 0.01, 0.1, 1, 10 and 100 μM for 30 minutes (positive controls, furafylline for CYP1A2, sulfaphenazole for CYP2C9, tranylcypromine for CYP2C19, diethyldithiocarbamate for CYP2E1, quinidine for CYP2D6 and miconazole for CYP3A4 were also employed). Quantitation of the specific metabolites formed by various isozymes (1A2, 2C9, 2D6, 2C19, 2E1 and 3A4) of cytochrome P450 were performed using methods. .

Results:

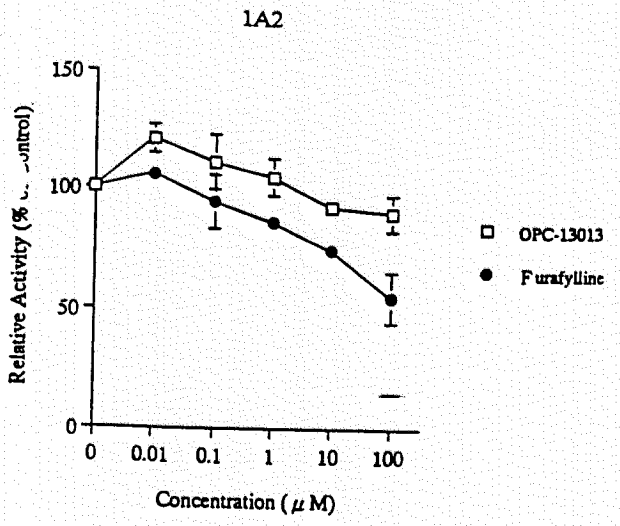
The effect of cilostazol on the enzyme activity of each human cytochrome P450 are shown in the following table and figures.

Inhibitory effect of OPC-13013 on enzyme activity of P450s

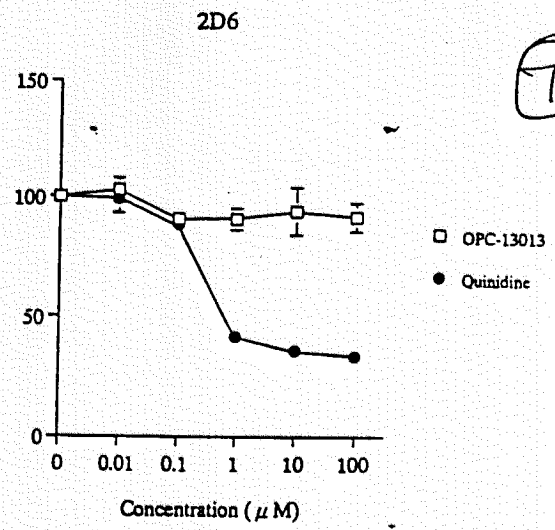
OPC-13013 Concentration	Activity (% of control)					
	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP2E1	CYP3A4
0	100.00	100.00	100.00	100.00	100.00	100.00
10^{-8} M	120.34±6.05	86.21±4.04	90.89±4.77	103.34±5.08	99.36±7.56	87.13±1.49
10^{-7} M	110.90±11.62	83.85±7.80	89.66±11.79	91.39±0.90	94.05±5.92	68.42±12.33
10^{-6} M	104.98±8.01	73.80±5.31	80.40±4.57	91.32±4.78	95.06±19.96	51.60±4.13
10^{-5} M	93.04±3.44	62.15±3.12	67.29±0.80	94.55±9.94	90.60±11.37	35.87±9.96
10^{-4} M	90.41±7.75	20.71±7.58	43.94±4.81	91.97±6.16	88.79±16.42	11.66±7.41

Inhibitor Concentration	Activity (% of control)					
	Furafylline	Sulfafenazole	Tranylcypromine	Quinidine	Diethyldithiocarbamate	Miconazole
0	100.00	100.00	100.00	100.00	100.00	100.00
10^{-8} M	105.26±0.42	90.94±3.11	85.76±4.21	99.51±5.91	89.82±14.56	63.19±10.55
10^{-7} M	94.12±11.05	73.64±5.50	73.79±4.09	88.88±4.36	89.18±19.98	43.78±7.99
10^{-6} M	85.80±3.39	24.72±3.01	64.62±7.66	42.09±2.27	76.47±10.00	19.56±5.56
10^{-5} M	74.83±4.25	4.98±0.34	45.36±6.43	35.58±2.42	73.64±16.92	8.44±7.19
10^{-4} M	55.43±10.42	2.02±0.46	18.37±1.88	33.55±3.30	52.64± 6.66	6.27±5.87

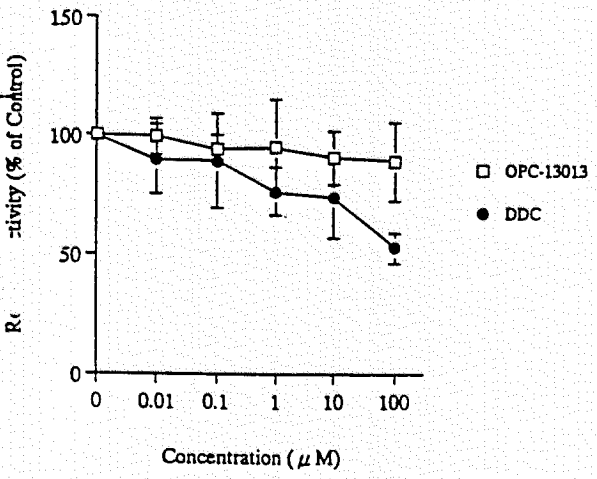
The values show mean±S.D. N=3.



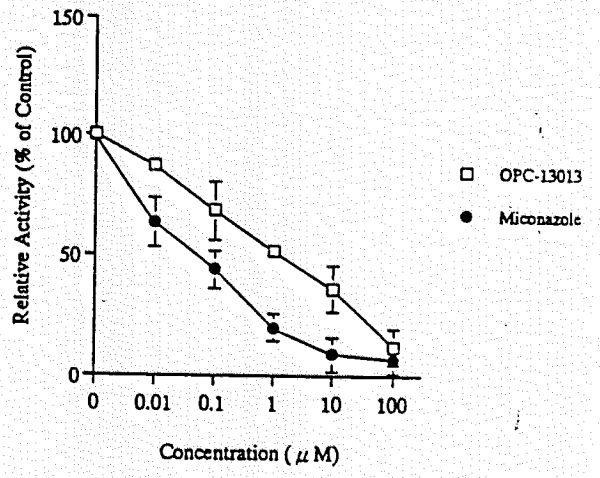
Effect of OPC-13013 on the CYP1A2-catalyzed Theophylline metabolism. Furafylline, especial inhibitor for CYP1A2, was used as positive control.



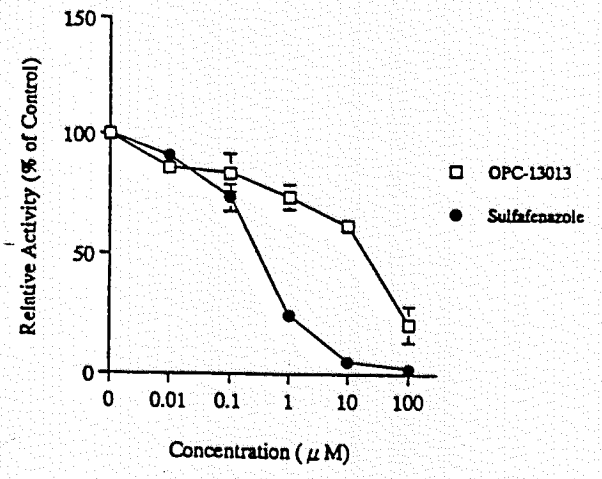
ct of OPC-13013 on the CYP2D6-catalyzed Debrisoquine ıbolism. Quinidine, especial inhibitor for CYP2D6, was l as positive control.



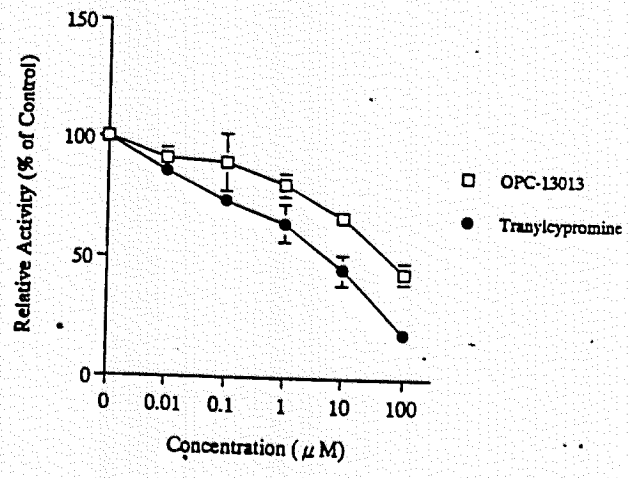
Effect of OPC-13013 on the CYP2E1-catalyzed Chlorzoxazone metabolism. Diethyldithiocarbamate (DDC) , especial inhibitor for CYP2E1, was used as positive control.



Effect of OPC-13013 on the CYP3A4-catalyzed Testosterone metabolism. Miconazole, especial inhibitor for CYP3A4, was used as positive control.



Effect of OPC-13013 on the CYP2C9-catalyzed Tolbutamide metabolism. Sulfafenazole, especial inhibitor for CYP2C9, was used as positive control.



Effect of OPC-13013 on the CYP2C19-catalyzed S-Mephenytoin metabolism. Tranylcypromine, especial inhibitor for CYP2C19, was used as positive control.

Cilostazol did not significantly inhibit CYP1A2-mediated theophylline metabolism, CYP2D6-mediated debrisoquine metabolism and CYP2E1-mediated chlorzoxazone metabolism.

Addition of cilostazol at 10 and 100 μM indicated significant inhibition of CYP3A4, CYP2C9 and CYP2C19 mediated metabolism. The next study evaluated the Ki values for this inhibition.

Conclusions:

Cilostazol did not inhibit cytochrome P450 1A2, 2D6 and 2E1 catalyzed metabolism. Cilostazol, however, inhibited CYP2C9, CYP3A4 and CYP2C19 mediated metabolism in vitro. This suggests that inhibition of CYP2C9, CYP3A4 and CYP2C19 mediated metabolism of drugs is possible upon concomitant administration with cilostazol.

Comment:

1. These results represent a contrast to what was seen in study 010572, which indicated that while CYP3A4 mediated metabolism is inhibited by cilostazol, this is unlikely to occur at therapeutically achievable concentrations. Further analysis of Ki values needs to be conducted (in next study).
2. The inhibitory effects of the cilostazol metabolites have not been investigated in this study.