

STUDY 012542, report 010597 (IN VITRO ENZYME INHIBITION STUDY):

INHIBITION BY OPC-13013 OF DRUG-METABOLIZING ENZYME ACTIVITIES DERIVED FROM HUMAN CYTOCHROME P450 (CYP3A4, CYP2C9 AND CYP2C19) (II)

Reference: Volume 114

Investigator:

Objective:

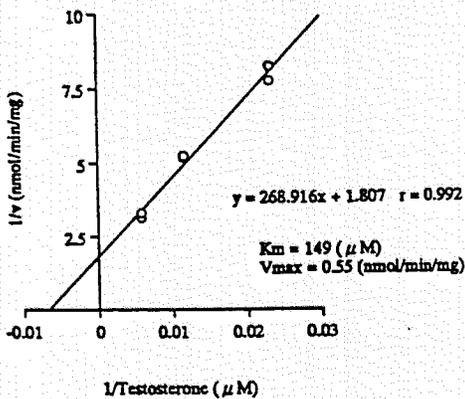
To investigate the in vitro effects of cilostazol on drug metabolizing enzyme activities in microsomes (CYP3A4, CYP2C9 and CYP2C19) transformed with B-lymphoblastoid cells expressing human cytochrome P450 (cDNA) and to determine the inhibition constants (K_i values) of cilostazol on CYP3A4-catalyzed testosterone 6β-hydroxylase activity, CYP2C9-catalyzed tolbutamide methyl-hydroxylase activity and CYP2C19-catalyzed S-mephenytoin 4'-hydroxylase activity.

Study design:

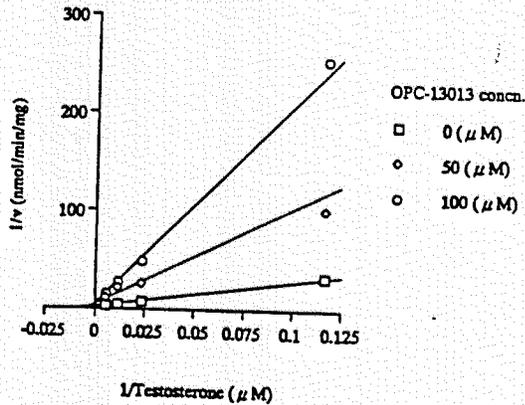
Human expressed CYP450 isoforms were incubated with various substrates [tolbutamide, (CYP2C9), testosterone, (CYP3A4) and S-mephenytoin, (CYP2C19)], reaction cofactors and cilostazol 0 to 200 μM for 30 minutes for CYP3A4 and CYP2C9 substrates and 60 minutes for CYP2C19. Quantitation of the specific metabolites formed by various isozymes of cytochrome P450 were performed using isolation and

Results:

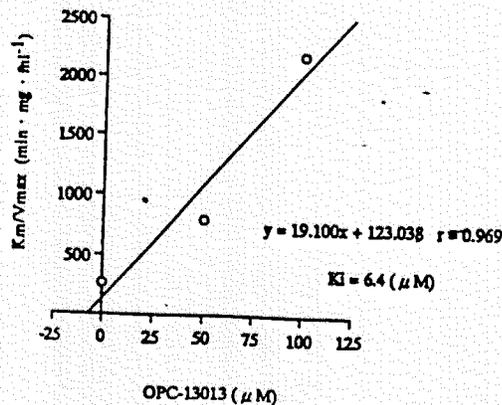
The effect of cilostazol on the CYP3A4 enzyme activity is shown in the following figures with the corresponding K_m, V_{max} and K_i values.



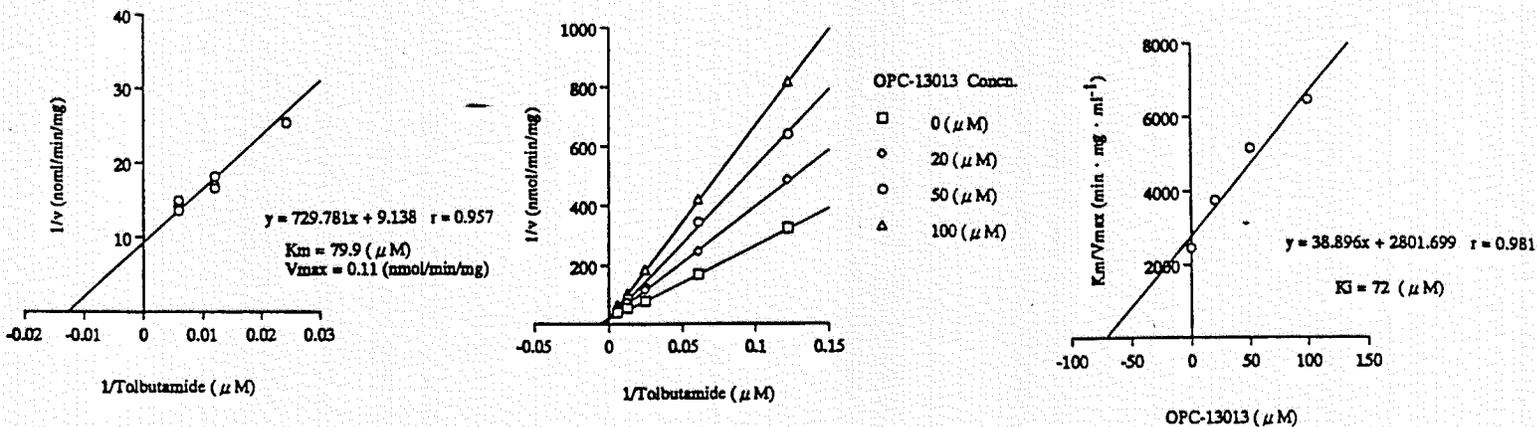
Double-reciprocal plot for the determination of apparent K_m and V_{max} on human CYP3A4 catalyzed testosterone 6β-hydroxylation. N=2. (Lineweaver-Burk plot)



Inhibitory effect of OPC-13013 on testosterone 6β-hydroxylation by human CYP3A4 (Lineweaver-Burk plot)



The effect of cilostazol on the CYP2C9 enzyme activity is shown in the following figures with the corresponding K_m , V_{max} and K_i values.

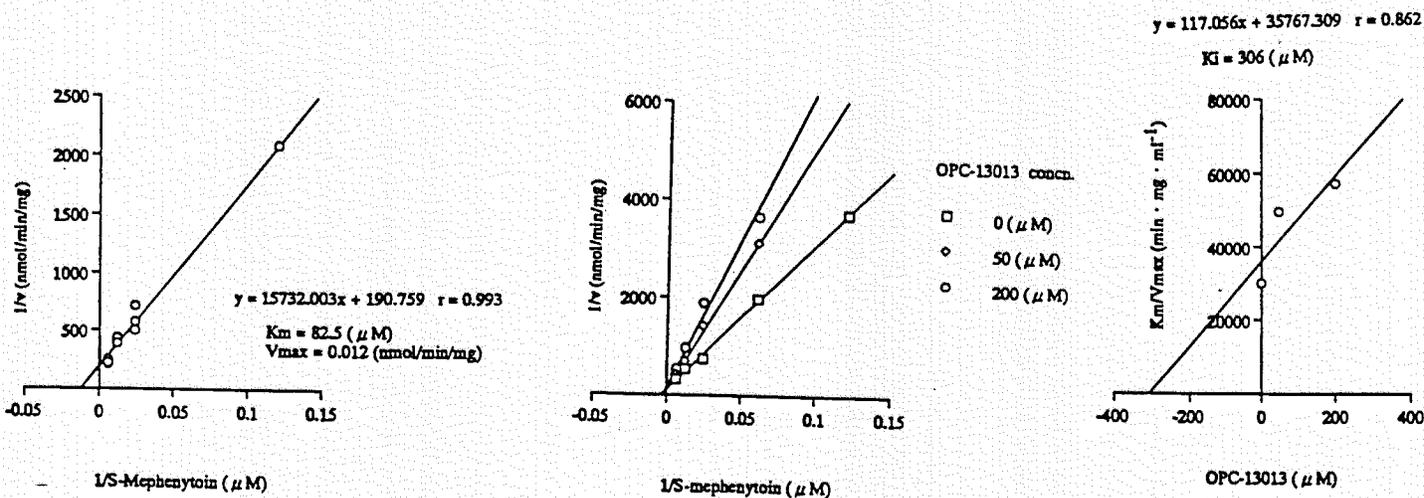


Double-reciprocal plot for the determination of apparent K_m and V_{max} in CYP2C9 catalyzed Tolbutamide 4-methylhydroxylation. N=3. (Lineweaver-Burk plot)

Inhibitory effect of OPC-13013 on tolbutamide 4-methylhydroxylation by human CYP2C9 (Lineweaver-Burk plot)

Secondary plot illustrating the tolbutamide—OPC-13013 interaction

The effect of cilostazol on the CYP2C19 enzyme activity is shown in the following figures with the corresponding K_m , V_{max} and K_i values.



Double-reciprocal plot for the determination of apparent K_m and V_{max} on human CYP2C19 catalyzed S-mephenytoin 4'-hydroxylation. N=3. (Lineweaver-Burk plot)

Inhibitory effect of OPC-13013 on S-mephenytoin 4'-hydroxylation by human CYP2C19 (Lineweaver-Burk plot)

Secondary plot illustrating the S-mephenytoin—OPC-13013 interaction

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Conclusions:

The apparent K_i of cilostazol for CYP3A4 mediated testosterone metabolism was 6.4 μM , for CYP2C9 mediated tolbutamide metabolism was 72 μM , for CYP2C19 mediated S-mephenytoin metabolism was 306 μM . At the expected therapeutic concentrations of cilostazol of 2 -3 μM , cilostazol is unlikely to inhibit the metabolism mediated by CYP2C9 and CYP2C19. However, this study, in contrast to other studies, indicates that a small increase in cilostazol concentrations can result in inhibition of CYP3A4 mediated drug metabolism.

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. Results from these studies are variable. Therefore, caution is recommended when cilostazol and CYP3A4 substrates are administered concomitantly.
2. The inhibitory effects of the cilostazol metabolites have not been investigated in this study.

REPORT 11083 - IN VITRO METABOLISM BY HUMAN LIVER MICROSOMES TO DETERMINE THE INTERACTION OF CILOSTAZOL WITH DRUGS METABOLIZED BY CYTOCHROME P450 ISOFORMS

Study ID: 11083; Volume: 1.114; Investigator:

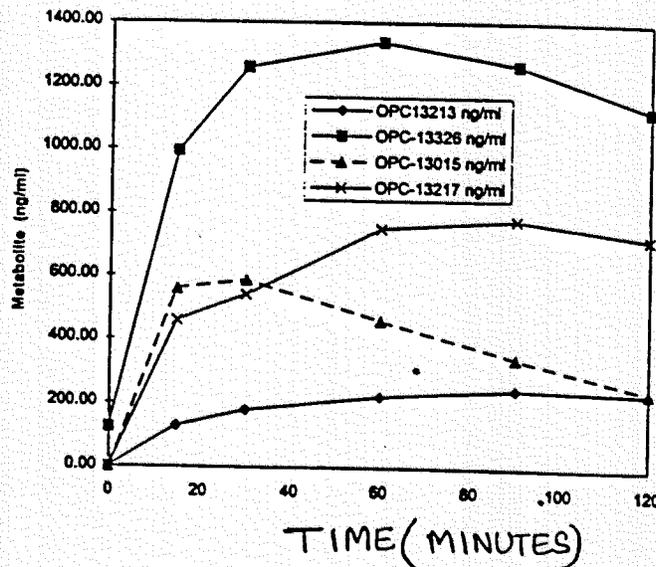
Objective: 1. To identify the human hepatic CYP450 isoforms that metabolize cilostazol using human liver microsomes. 2. To find out which metabolites of cilostazol are produced in vitro by human liver microsomes and characterize the metabolite kinetics. 3. To determine whether cimetidine inhibits cilostazol metabolism in vitro.

Identification of CYP isoforms that metabolize cilostazol: This was done using both chemical inhibitors and correlation analysis. The incubation conditions used to achieve linear production of cilostazol metabolites with the preparations of human liver microsomes (from 3 livers) were: Protein concentration 0.25 - 0.5 mg/ml, cilostazol concentration 25 μ M and time of incubation 15 - 30 minutes.

CORRELATION OF CILOSTAZOL METABOLISM WITH THE ACTIVITY OF CYP SPECIFIC PROBE SUBSTRATES: The rates of cilostazol metabolism were determined in a panel of 9 human hepatic microsomes. These rates were compared with those of selective CYP substrates to detect significant relationships between cilostazol metabolism and specific CYP forms.

CYTOCHROME P-450 SELECTIVE INHIBITORS: A series of inhibitors were incubated with cilostazol to determine which compounds could inhibit the metabolism of either or both compounds.

Results: The time course of appearance of cilostazol metabolites in human liver microsomes is shown in the following figure:



Based on the metabolites observed, the following metabolic profile of cilostazol in humans is proposed:

In vitro, the major metabolite found was OPC-13326 (hydroxylation of quinone), which is consistent with the major metabolite found in vivo (OPC-13015 which is formed from OPC-13326). The hydroxylation of the hexane moiety to OPC-13217 is the second most important metabolic route in vitro. In vivo the second most important metabolite is OPC-13213 which is an enantiomer of OPC-13217.

The mean Km for cilostazol metabolism was found to be 100.6 μM. Studies with chemical inhibitors indicate that ketoconazole is the most potent inhibitor of metabolism of cilostazol to OPC-13326 (see the 3 tables below).

LIVER ①

Inhibitor	OPC-13326	OPC-13217
1 μM Ketoconazole	0.00%	43.51%
0.1 μM Quinidine	89.62%	96.94%
10 μM Omeprazole	74.39%	94.47%
10 μM Furafylline	78.52%	91.68%

LIVER
②

Inhibitor	OPC-13326	OPC-13217
1 μM Ketoconazole	0.00%	65.00%
0.1 μM Quinidine	75.28%	85.05%
10 μM Omeprazole	92.74%	118.95%
10 μM Furafylline	60.25%	69.04%
100 μM DEDTC (2E1)	79.49%	76.59%
10 μM DEDTC (2B6)	76.75%	81.84%

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LIVER
③

Inhibitor	OPC-13326	OPC-13217
1 μM Ketoconazole	0.00%	6.00%
0.1 μM Quinidine	88.75%	116.48%
10 μM Omeprazole	60.58%	88.82%
10 μM Furafylline	80.07%	129.73%
100 μM DEDTC (2E1)	79.84%	88.33%
10 μM DEDTC (2B6)	84.49%	117.74%

→ 85.64%

This agrees with previous results that CYP3A4 is the primary isozyme involved in cilostazol metabolism.

Results of correlation analysis are shown in the following table:

Correlation Between Cilostazol Metabolism to OPC-13326 and CYP450 Isoform Probe Activity

Isoform	Correlation (RSQ)
3A	0.6728
2C19	0.4818
1A2	0.4722
2D6	0.4157
2E1	0.0044

Metabolism of cilostazol by a range of nine (9) human liver preparations. Data are expressed as the rate of OPC-13326 production as compared to the rate of metabolite in the following isoform specific probe reactions:

These results demonstrate a relatively strong correlation ($r^2 = 0.69$, $p < 0.01$) between cilostazol metabolism to OPC-13326 and metabolism of felodipine (a CYP3A4 probe).

Study done to test the effect of cimetidine on cilostazol metabolism demonstrated a concentration-dependent competitive inhibition of cilostazol metabolism with a K_i for cimetidine of 307.3 μM.

Conclusion: Results from this study indicate that CYP3A4 is the primary enzyme involved in cilostazol metabolism.

Comments: Potential interaction with CYP3A inhibitors like ketoconazole and erythromycin with cilostazol is possible, resulting in higher concentrations of parent cilostazol.

REPORT 11083 part II- IN VITRO METABOLISM BY HUMAN LIVER MICROSOMES TO DETERMINE THE INTERACTION OF CILOSTAZOL WITH DRUGS METABOLIZED BY CYTOCHROME P450 ISOFORMS

Study ID: 11083; Volume: 1.114; Investigator:

Objective:

To determine the ability of cilostazol to inhibit cytochrome P450 isoforms in whole human liver microsomes.

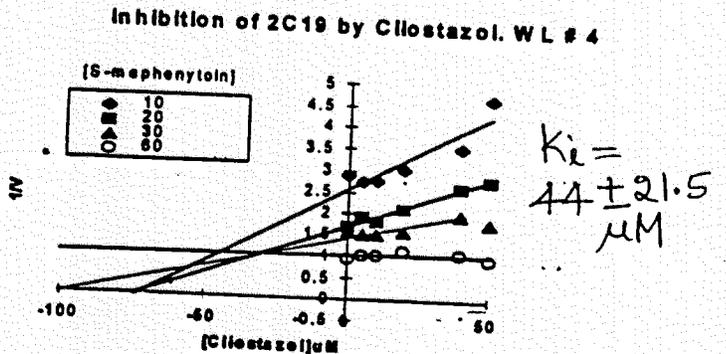
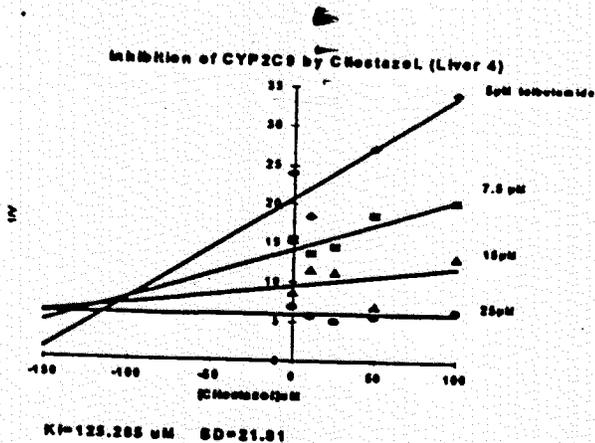
Study design:

Human liver microsome preparations were incubated with various substrates (see table below), under linear conditions of time and microsomal protein content, at a range of probe concentrations that spanned the Km for the specific probe, and a range of cilostazol concentrations between μM . Where inhibition by cilostazol was noted, the Ki was determined graphically using Dixon plots.

Cytochrome P450	Probe drug/metabolite	Concentration range
CYP1A2	Phenacetin/acetaminophen	μM
CYP2B6	S-mephenytoin/nirvanol	μM
CYP2C9	Tolbutamide/hydroxytolbutamide	μM
CYP2C19	S-mephenytoin/4'-hydroxymephenytoin	μM
CYP2D6	Dextromethorphan/dextrorphan	μM
CYP2E1	Chlorzoxazone/6-hydroxychlorzoxazone	μM
CYP3A	Dextromethorphan/3-methoxymorphinan	μM

Results:

The Kis for inhibition of CYP450 isozymes by cilostazol are shown in the following table. Dixon plots for inhibition of tolbutamide metabolism and S-mephenytoin metabolism are shown in the following two figures.



K_s for inhibition of Cytochrome P450 by Cilostazol.

Isoform	Liver	Cilostazol KI
1A2	2	No inhibition
1A2	5	No inhibition
1A2	8	No inhibition
2B6	2	No inhibition
2B6	4	No inhibition
2B6	9	No inhibition
2C9	2	23.8 ± 2.4 μM
2C9	4	125.3 ± 21.8 μM
2C9	9	108.4 ± 22.8 μM
2C19	2	No inhibition
2C19	4	44 ± 21.5 μM
2C19	9	No inhibition
2D6	2	No inhibition
2D6	4	No inhibition
2D6	9	No inhibition
2E1	2	No inhibition
2E1	8	No inhibition
2E1	9	No inhibition
3A	2	No inhibition
3A	4	No inhibition
3A	9	No inhibition

Conclusions:

Results indicate that cilostazol does not inhibit CYP1A2, CYP2B6, CYP2D6, CYP2E1 and CYP3A mediated metabolism. A small but consistent inhibition of CYP2C9 and CYP2C19 mediated metabolism by cilostazol was found. This indicates that clinically significant interactions are unlikely except for narrow therapeutic drugs metabolized by CYP2C9 and CYP2C19.

Comment:

1. So far, three studies were conducted to evaluate the inhibition potential of cilostazol on CYP3A4 mediated metabolism of drugs. Two utilized recombinant systems and one utilized human liver microsomes. While there is no major indication that inhibition of CYP3A4 mediated metabolism is likely to occur at therapeutic concentrations of cilostazol, in one study the K_i value was found to be about 6 μM (twice the therapeutically achievable concentrations). Therefore, caution is recommended when CYP3A4 substrates are administered with cilostazol.
2. The inhibitory effects of the cilostazol metabolites have not been investigated in this study.

REPORT 11083 part III- IN VITRO METABOLISM BY HUMAN LIVER MICROSOMES TO DETERMINE THE INTERACTION OF CILOSTAZOL WITH CYTOCHROME P450 ISOFORMS: *INTERACTION BETWEEN CILOSTAZOL AND S-WARFARIN*

Study ID: 11083; Volume: 1.114; Investigator:

Objective:

To identify and quantitate the interaction between cilostazol and S-warfarin in human liver microsomes.

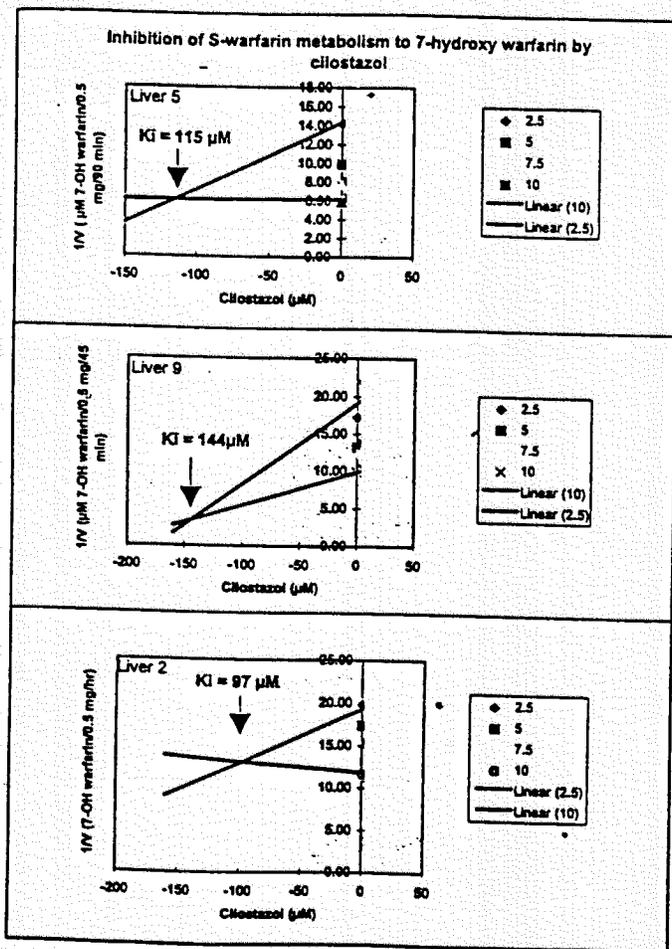
Study design:

Human liver microsome preparations were incubated under linear conditions of time (45 to 90 minutes) and microsomal protein content, at a range of S-warfarin concentrations, 2.5, 5, 7.5 and 10 μM that spanned the K_m for the specific probe, and a range of cilostazol concentrations between 0 to 20 μM . Samples were analyzed for 6 and 7-hydroxywarfarin using validated methods. The kinetic data obtained were analyzed in order to determine the K_i using Dixon plots that allow estimation of inhibition of S-warfarin metabolism by cilostazol.

Results:

The K_i s for inhibition of S-warfarin metabolism by cilostazol are shown in the following figures.

Dixon Plots



Conclusions:

Results from this study agree with the previous study which indicates that cilostazol inhibits metabolism mediated by CYP2C9. This data is consistent with competitive inhibition of warfarin metabolism by high concentrations of cilostazol. Clinical interaction between cilostazol and S-warfarin, based on K_i values, is likely to occur only at very high concentrations of cilostazol (about 50 fold higher than the therapeutic range).

STUDY 21-95-204: (DRUG INTERACTION STUDY WITH WARFARIN)

A PHARMACOKINETIC AND PHARMACODYNAMIC STUDY OF THE POTENTIAL DRUG INTERACTION BETWEEN CILOSTAZOL AND WARFARIN IN HEALTHY SUBJECTS

Reference: Volumes 88 to 91

Investigator:

Study Location:

Objective:

To determine the pharmacokinetics and pharmacodynamics of commercially available racemic warfarin when administered as a single oral 25 mg dose, with or without concomitant administration of cilostazol.

Study design:

This is a double blind (warfarin doses were not blinded), single-center, two-way crossover study of single doses of warfarin (25 mg) with multiple dosing of either cilostazol 100 mg bid or a matching placebo in 20 healthy male volunteers of age 18 to 45 years (23 were enrolled and 15 completed the study, 4 subjects withdrew due to adverse events of rash, urticaria and vomiting with either cilostazol only or placebo treatment). During screening, each subject received a single 25 mg priming dose of warfarin. Thirteen days after the warfarin priming dose, subjects received either 100 mg cilostazol bid or matching placebo bid for 13 days for two treatment periods, with an 8 day washout between treatment periods. Subjects also received a single 25 mg dose of warfarin on day 7 of each treatment period (a schematic diagram of schedule of dosing and assessments is provided below). Each dose was administered with 240 ml of water.

Batch #s: Cilostazol 100 mg tablet: batch # 4K79PA1

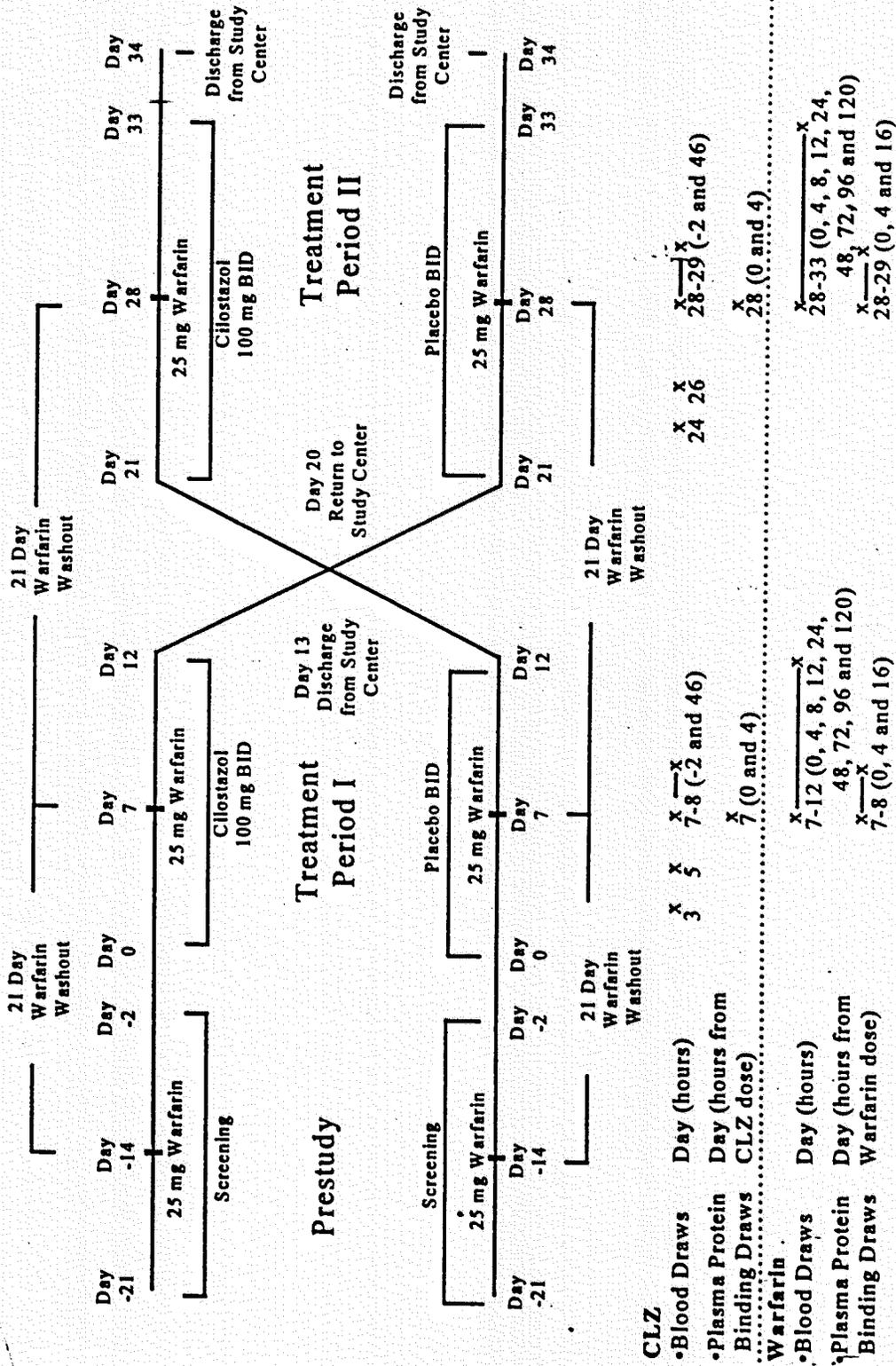
Matching placebo tablet: batch# 4L75P100

Warfarin (coumadin) 10 mg tablets, lot # JK320A and warfarin 5 mg tablets, lot # KA009A

Plasma samples for the assay of R(-) and S(+) warfarin were drawn on day 7 and 28 at 0, 4, 8, 12, 24, 48, 72, 96 and 120 hours after warfarin dosing. Plasma samples for determination of trough cilostazol and its metabolites concentrations were collected pre-dose on days 3, 5, 7, 8, 24, 26, 28 and 29. Plasma protein binding of cilostazol and warfarin were determined at suitable times during the study. In addition, the following PD parameters were measured: prothrombin time (PT), activated partial thromboplastin time (aPTT) and ivy bleeding time.

Analysis of plasma and urine samples for cilostazol and its metabolites was carried out using a _____ method at _____ R and S-warfarin concentrations were determined at _____ using _____ detection. Protein binding of cilostazol in plasma samples was determined by _____ of the radioactivity of ¹⁴C-cilostazol.

Schematic of Schedule of Assessments for Warfarin Dosing, Cilostazol and Placebo Dosing, Pharmacokinetic Blood Draws, and Coagulation Parameters



Pharmacokinetic parameters were determined by non-compartmental methods. The natural log of cilostazol and its metabolite trough concentrations were analyzed with an ANOVA for a repeated measures design. For PK parameter estimates of R and S-warfarin except t_{max} , the natural logarithms were analyzed with ANOVA. The natural log of the PD parameters were analyzed with an ANOVA for a repeated measures design. The 90% confidence limits for PK and PD parameters, with and without cilostazol, were calculated.

Results:

ASSAY PERFORMANCE: Assay for cilostazol and its metabolites was conducted at
Assay for R(+) and S(-) warfarin was conducted at

Plasma samples:

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:

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metabolites, chromatograms acceptable.

S(-) warfarin:

Method used:

Range:

Linearity: Linear within the range,

QC samples:

Precision:

Accuracy:

Specificity:

R(+) warfarin:

Method used:

Range:

Linearity: Linear within the range.

QC samples:

Precision:

Accuracy:

Specificity:

Assays were found to be acceptable.

Table below provides a summary of the main pharmacokinetic parameters (mean \pm SD) for R(+) warfarin given with or without cilostazol.

Parameters	With Cilostazol	With Placebo	Geometric mean ratio	90% C.I.
Tmax, hrs	4.3 \pm 1.0	4.0 \pm 0.0		
Cmax, ng/ml	1321 \pm 281	1384 \pm 248	0.948	0.89 - 1.00
t _{1/2} , hrs	38.7 \pm 8.0	38.0 \pm 9.4	1.022	0.92 - 1.13
AUC ₀₋₆ , ng-hr/ml	60113 \pm 13517	61640 \pm 13640	0.974	0.94 - 1.01
AUC _{0-∞} , ng-hr/ml	69080 \pm 19285	70600 \pm 18651	0.975	0.92 - 1.03
Cl/F, ml/hr/kg	2.41 \pm 0.60	2.36 \pm 0.60	1.027	0.97 - 1.09
Vz/F, ml/kg	130 \pm 21	124 \pm 21	1.048	0.99 - 1.11