possessing vasodilating and inotropic effects, are attributed to exaggerated pharmacodynamic effects, resulting in profound hemodynamic changes, such as sustained hypotension (results in decreased perfusion pressure) and tachycardia (results in decreased perfusion time and increased oxygen demand; Balazs, T. In: Balazs, T. (ed) Cardiac Toxicology, Vol.II. Boca Raton:CRC Press, 1981: 61-72.). A correlation between hypotension/tachycardia and subendocardial necrosis has been documented in dogs treated with minoxidil (a potassium channel opener) and other hypotensive agents (Herman et al. 1979. Toxicol. Appl. Pharmacol. 47:493).

Phosphodiesterase inhibitors act primarily by inhibiting the degradation of cAMP by type III phosphodiesterases, the predominant form of PDE found in both myocardium and vascular smooth muscle. These agents exert positive inotropic effects and enhance the contractile function of the heart, resulting in an increase in cardiac output and a reduction in systemic vascular resistance. Significant hypotension is seen at high doses.

With intravenous administration, cilostazol increased contractility of dog heart by 25% at a dose of 0.45 mg/kg (ED25); the incidence of left ventricular lesions in dogs after one week of iv administration was 75% at 3 mg/kg (7 times the ED25) and 100% at 10 mg/kg (22 times the ED25).

The oral doses and durations of treatment associated with CV lesions in the dog are given for several PDE inhibitors/ vasodilators on page 129.

Minimum Oral Dose and Duration of Treatment Reported to be Required for PDE inhibitors/Vasodilators to Induce Cardiovascular Lesions* in the Dog

Compound	Dose (mg/kg/day p.o.)	Duration of Treatment	Lesion Site		
			Left Ventricle	Right atrium	coronary artery
cilostazol	450	2 weeks	+	+	+
cilostazol	30-150	13 weeks	+	-	+
cilostazol	6-12	52 weeks	+	-	+
flosequinan	10	8 days	+	+	+
amrinone	32	2 weeks	+	-	-
amrinone	8	52 weeks	+	-	-
milrinone	0.4 - 2	13 weeks	+	-	-
milrinone	2	52 weeks	+	-	-
minoxidil	1	3 days	+	+	+
hydralazine	24	2 days	a	+	+
hydralazine	10	5 days	+	+	+

+ = lesions present a = lesions absent - = not reported

* includes focal endocardial fibrous thickening with hemorrhage in the left ventricle, hemorrhage of the right atrial wall, intimal thickening of the coronary artery, and coronary arteritis and periarteritis.

The dog is considered to be very susceptible to inotropic/vasodilating agent-induced CV lesions. It is reported that treatment with these agents produce an increased range of movements of the papillary muscles as a result of increased contractility, with subsequent rise in mechanical stresses applied to the vessel walls. The coronary arterial lesions, observed only in dogs following PDE III inhibitor administration, are attributed to the anatomical differences in the coronary circulation in this species. The coronary supply of the dog is dominated by the left coronary artery, whereas in cynomolgus monkey and pig, it resembles the situation in humans, with a more symmetrical coronary supply from both right and left coronary arteries (Isaacs et al. 1989).

The vasodilator-induced CV lesions in the dog do not seem to have any clinical relevance (Mesfin et al. 1989. Toxicol. Pathol. 17:164.). Although minoxidil produced CV lesions in the dog, it has been reported that there was no evidence of cardiovascular lesions in human patients treated with minoxidil. Autopsies conducted in about 200 investigational patients who died during or following the administration of minoxidil did not reveal cardiac lesions similar to those seen in animals (Sobota, 1989. Toxicol. Pathol. 17:193).

A thirteen-week oral toxicity study in male cynomolgus monkeys (0, 300, 900 and 1800 mg/kg/day) revealed left ventricular endocardial hemorrhage and intimal thickening of the coronary artery in all treated and control groups. Myocardial hemorrhage (low dose), myocardial fibrosis (mid dose) and right atrial hemorrhage (high dose) were seen only in treated groups; however, no dose dependency was found for these lesions. There was no treatment-related coronary arteritis in this study. The lowest dose that produced myocardial lesions (300 mg/kg/day) was about 30 times the MRHD on a mg/m^2 basis.

An increase in heart rate was seen in the monkey study at 900 mg/kg/day in treatment weeks 5 and 12 (10-31%) and at 1800 mg/kg/day in week 12 (36%). Shortening of the QT interval was observed at both dose levels. No other notable findings were observed in this study.

According to the sponsor, an estimated 725,000 patients have received cilostazol since its first approval for marketing in Japan in 1988. In U.S. clinical trials, it is stated that the overall incidence of adverse events was similar for cilostazol (100 mg bid) and placebo groups. Although the cilostazol group had a higher number of myocardial infarctions than the placebo group, trend analysis showed no statistically significant dose-response relationship. There have been no reports of a treatment-related increased incidence of CV lesions in patients treated with

cilostazol.

A one year toxicity study in Sprague-Dawley male and female rats (0, 6, 30 and 150 mg/kg/day, administration by gavage) revealed dose-related increases in serum total protein, total cholesterol and phospholipid levels in mid and high dose male groups. Dose-dependent increases in both absolute and relative liver weights were observed in mid and high dose males and females; however, light microscopic or electron microscopic examinations revealed no liver pathology. Increased incidences of nodular hyperplasia and adenoma of the pituitary gland were seen in all treated male groups. A non-toxic dose level for pituitary pathology was not established for males in this study. The non-toxic dose for the females was found to be 6 mg/kg/day. [The drug-relatedness of these pituitary findings is, however, doubtful, as there was no pituitary pathology in a two-year (dietary administration) study in F-344 rats.]

A 4-week toxicokinetic study in Sprague-Dawley rats (administration by gavage) showed dose-dependent increases in Cmax and AUC values for both sexes at the doses administered in the one year experiment (6, 30 and 150 mg/kg/day). The Cmax and AUC values were higher in females than in males at each dose level.

It is noted that cardiovascular lesions, associated with cilostazol administration in dogs, were not observed in rats at doses up to 1500 mg/kg/day (about 74 times the MRHD on a mg/m^2 basis) for 5 or 13 weeks, or up to 150 mg/kg/day (about 7 times the MRHD on a mg/m^2 basis) for 52 weeks.

For the two-year dietary carcinogen bioassay in F-344 rats (0, 50, 150 and 500 mg/kg/day), both sponsor's and FDA's analyses showed significant positive linear trends for dose-related mortalities for both males and females ($p<0.05$). However, pairwise comparisons showed statistically significant mortality only for the high dose female group compared to concurrent control ($p<0.05$).

According to sponsor's analysis, a statistically significant ($p<0.05$) increased incidence of adrenal medullary adenoma was seen for the high dose male group (control 8%, low dose 6%, mid dose 20% and high dose 22%). FDA statistical evaluations (which used, for trend test, a significance level of 0.005 for common tumors and 0.025 for rare tumors) revealed a statistically significant trend ($p=0.016$) only for uterine leiomyoma in female rats (conrol 0%, low dose 0%, mid dose 0% and high dose 6%). However, pairwise comparison of high dose and control groups showed that the difference was not statistically significant ($p=0.13$). When the incidences of leiomyomas and leiomyosarcomas of both uterus and

uterine cervix were combined (control 4%, low dose 2%, mid dose 2% and high dose 10%), no significant trend (p=0.034) or significant difference between high dose and control groups (p=0.36) was noted.

The laboratory historical control range for leiomyomas and leiomyosarcomas (uterus or uterine cervix) in female F-344 rats is 0-4%.

The highest dose employed in this study (500 mg/kg/day) is about 25 times the MRHD on a mg/m^2 basis and 1 and 3 times the MRHD (for males and females, respectively) on the basis of systemic exposure (AUC for parent drug).

Based on the significant positive linear trends for dose-related mortalities for both male and female rats, it is considered that maximum tolerated dose levels were achieved, if not exceeded, for both sexes in this 2-year bioassay.

In the two-year dietary carcinogenicity study in B6C3F1 mice (0, 100, 300 and 1000 mg/kg/day), a significant dose-related trend for mortality was seen for males (p<0.01; sponsor's and FDA's analyses) with pairwise comparisons showing a significantly higher than control incidence for the high dose males (p<0.05). No significant trend for mortality was seen in female mice.

Sponsor's life table analysis (age-adjusted) revealed that the incidence of hepatocellular adenoma was higher than control in males treated with 300 or 1000 mg/kg/day; however, their trend test just failed to attain a level of statistical significance (p=0.051). The incidental tumor analysis showed a statistically significant difference from control only at 300 mg/kg/day. In females, although the incidence of hepatocellular adenoma showed a significant positive trend with dosage in both life table and incidental tumor tests, pairwise comparisons with control failed to attain statistical significance. No significant differences were seen in the incidence of carcinoma in either sex. When the incidences of adenomas and carcinomas were considered together, the results of the analyses followed the same pattern as in the case of adenomas.

FDA analyses revealed a significant trend (p=0.002) in female mice for hepatocellular adenoma, but it is stated in the statistical review that the "trend is difficult to interpret because both the low and mid doses had numerically lower incidence rates than the control" (control 12%, low dose 4%, mid dose 10% and high dose 25%). Although the incidence was higher in the high dose group than in the concurrent control (and exceeds the laboratory historical control range of 0-22%), the difference was not statistically significant (p=0.064). In male mice, no significant trend was seen

for hepatocellular adenoma. In pairwise comparisons, only the mid dose group appeared to have a significantly higher incidence of this tumor than the control ($p<0.001$). It is considered that the significance of this finding is uncertain since no significantly increased incidence of liver tumor was noted in the high dose group. Combining the incidence of hepatocellular adenoma with hepatocellular carcinoma showed a similar result.

It appears that the mouse study was conducted at appropriate dosage levels. Although a significant positive trend in dose-related mortality was seen only for males, increasing the dose beyond 1000 mg/kg/day in females would have provided no appreciable increase in systemic exposure (conclusion based on results of 13 week dose finding study).

The highest dosage employed in this study (1000 mg/kg/day) is about 25 times the MRHD on a mg/m^2 basis and almost equal to the MRHD on the basis of systemic exposure (AUC for parent drug).

The Executive Carcinogenicity Assessment Committee, at their June 9, 1998 meeting, concluded that there is no evidence for a tumorigenic potential for cilostazol either in the rat or mouse.

A fertility study in JCL:SD strain rats (0, 30, 150 and 1000 mg/kg/day) showed no significant dose-dependent treatment related effects on reproductive parameters except for an increase in the incidence of fetuses with 14 ribs in drug-treated groups compared to concurrent control, the difference being statistically significant at 1000 mg/kg/day (which is about 49 times the MRHD on a mg/m^2 basis.)

In a rat developmental toxicity study (0, 30, 150 and 1000 mg/kg/day), significantly decreased fetal weights (female) and increased incidence of external anomalies (postaxial polydactyly of the right hind limb, unilateral anophthalmia and general edema) were seen at the high dose. A dose-related increase in number of fetuses with major visceral malformations (increased incidence of ventricular septal defect in all treated groups and absence of the aortic arch, aberrant right subclavian artery and presence of right aortic arch in the high dose group) was seen in cilostazol treated groups (statistically significant at mid and high doses). Skeletal anomalies (nodulated ribs and hypoplasia of the fibula and tibia) and variations (due primarily to increased incidence of 14th rib) were significantly increased at the high dose. The incidences of unossified or incompletely ossified bones were increased in mid and high dose groups. In F1 offspring (from spontaneous delivery group), a dose-dependent increased incidence of visceral abnormalities (statistically significant at the high dose), due

primarily to severe dilatation of the renal pelvis, was observed in treated groups. The doses that produced significant visceral malformations or ossification abnormalities, 150 and 1000 mg/kg/day, were about 7 and 49 times the MRHD on a mg/m^2 basis, respectively.

In a rabbit developmental toxicity study (0, 30, 150 and 1000 mg/kg/day), no treatment-related effects on reproductive parameters or external, visceral or skeletal anomalies were seen except for an increased incidence of delayed ossification of the sternum observed in all treated groups (statistically significant at 1000 mg/kg/day which is about 100 times the MRHD on a mg/m^2 basis).

A peri-and post-natal study in rats (0, 30, 150 and 1000 mg/kg/day) showed significantly decreased birth weights for the high and mid dose F1 offspring and increased incidence of stillborn in the high and mid dose groups (statistically significant at high dose). The number of stillborn in the high dose F2 offspring was also higher than in control. The 150 and 1000 mg/kg/day doses were about 7 and 49 times the MRHD on a mg/m^2 basis, respectively.

All reproductive toxicity studies appeared to be adequately performed.

Cilostazol was not found to be genotoxic in bacterial gene mutation (Salmonella/E.coli), bacterial DNA repair (Bacillus subtilis), biochemical induction (E.coli incorporated with λ-lac z gene), cell to cell communication (Chinese Hamster V79 cell), mammalian cell gene mutation (mouse lymphoma L5178Y cell) and *in vivo* chromosomal aberration (mouse bone marrow) test systems. Cilostazol, however, was associated with a significant increase in chromosome aberrations in the *in vitro* Chinese Hamster Ovary Cell assay. It is noted that these chromosome aberrations were seen at doses which produced 60 to 85% reduction in mitotic indices, compared to solvent control, indicating increased cytotoxicity.

OPC-13015 and OPC-13213, the two major human metabolites of cilostazol, tested negative in bacterial gene mutation and bacterial DNA repair assays. However, it is noted that in the bacterial gene mutation test, precipitation occurred at all or most concentrations (156 µg/plate and/or above, for both compounds) with all tester strains. The sponsor was asked to address the validity of the test data. According to the sponsor, in the dose finding experiments, no precipitation was observed at lower concentrations (50 and 100 µg/plate) at which no evidence of mutagenicity was observed. They consider the data to be valid "because the study was conducted in accordance with the OECD Guidelines for Testing of Chemicals (1983) and consistent with recent ICH Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for

Pharmaceuticals." The ICH document recommends the following strategy for the testing of relatively insoluble compounds: "If no cytotoxicity is observed, then the lowest precipitating concentration should be used as the top concentration but not more than 5 mg per plate for bacterial tests." Based on the ICH guidelines, the assay is considered to be adequately performed.

In conclusion, it is considered that there are no approvability issues for cilostazol resulting from the animal toxicity testing program.

This review does not address the pharmacodynamic or pharmacokinetic studies conducted with cilostazol. Those studies are addressed in a separate review by Dr. John Koerner.

THIS SECTION WAS DETERMINED **NOT** TO BE RELEASABLE

4 pages.

The use of Pletal in pregnant women has not been studied.

Xavier Joseph, D.V.M.
July 30, 1998

Attachment:

Review and evaluation of carcinogenicity studies in rodents

cc.
Orig.NDA
HFD-110
HFD-110/CSO
HFD-110/JKoerner
HFD-110/XJoseph
HFD-345

accepted by CAR on 8-6-98

MEMORANDUM

Date: October 20, 1997

To: Henry J. Malinowski, Ph.D.
Mehul U. Mehta, Ph.D.

Through: Patrick Marroum, Ph.D., Team Leader P.M.

From: Venkata Ramana S. Uppoor, Ph.D. 10/20/97

Subject: Filing meeting for NDA 20,863 for Pletal® (Cilostazol) tablets, 50 and 100 mg strengths, 1S NDA, submitted on September 18, 1997 by Otsuka America Pharmaceuticals, Inc., Rockville, MD 20850

Pletal tablets contain cilostazol, a platelet aggregation inhibitor and vasodilator, in a dosage strength of 50 and 100 mg. This is indicated for the amelioration of symptoms in patients with intermittent claudication. Cilostazol prevents platelet aggregation through inhibition of cyclic AMP phosphodiesterase and enhances the effects of PGI_2, the endogenous vessel wall prostaglandin that causes vasodilation. Cilostazol improves the flow of blood to the extremities in patients having intermittent claudication. The recommended oral dose is 100 mg twice a day.

The to-be marketed formulation is same as the one used in the Phase III clinical trials. The only difference is in the shape of the 50 mg strength. Clinical trials used a round 50 mg tablet while the proposed tablet is triangular in shape. Dissolution testing has been conducted to demonstrate that these two tablets (different shapes) are equivalent. The safety and efficacy of Pletal has been evaluated in several clinical trials.

PHARMACOKINETIC / BIOAVAILABILITY STUDIES

This NDA contains several clinical and pharmacokinetic (27 *in vivo* and 10 *in vitro*) studies. Several pharmacokinetic studies were submitted that characterize the following:

I. single and multiple dose pharmacokinetics
ii. dose proportionality
iii. mass balance
iv. metabolic pathway
v. in vitro metabolism of the drug
vi. inhibition characteristics of cilostazol
vii. effect of age, gender
viii. food effect
ix. relative bioavailability and bioequivalence
x. drug interaction studies with aspirin, warfarin, erythromycin, quinidine and omeprazole

xi. study in patients (population pharmacokinetics)
xii. PK in renal impairment
xiii. PK in hepatic impairment
xiv. protein binding
xv. analytical methods used and quality control data
xvi. dissolution data.
xvii. several PK studies with an ointment formulation and a slow-release oral formulation.

DETAILS:

A. Drug physicochemical properties: 6-[4-(1-Cyclohexyl-1H-tetrazol-5-yl)butoxyl]-3,4-dihydro-2(1H)-quinolinone
Molecular weight: 369.42
Solubility: Highly insoluble in water, soluble in acetic acid and chloroform

B. Formulation:
Strength: 50 and 100 mg, both are not compositionally proportional and have different shapes, triangular and round respectively.
Dosage form: Immediate release tablet
Excipients: corn starch, microcrystalline cellulose, carboxymethylcellulose calcium, and magnesium stearate.
Formulation composition details have been submitted.

C. Radiolabeled ADME studies: 73.8% in urine and 20.4% in feces. Less than 1% of dose is excreted in urine as unchanged drug or OPC-13015. 20% of dose in urine is OPC-13213. 56% of dose in plasma is cilostazol, 15% is OPC-13015 (3,4-dehydro-cilostazol, 3 - 7 time more potent than cilostazol), 4% is OPC-13213 (4'-trans-hydroxy-cilostazol, 2 - 3 times less potent than cilostazol for platelet aggregation) and 25% as conjugates of the metabolites. OPC-13015 contributes about 63% of overall activity for phosphodiesterase inhibition and 41% of the activity for platelet aggregation inhibition.

D. Human pharmacokinetics:
I. Single dose PK: Half-life of cilostazol and OPC-13015 = 11 hours, for OPC-13213 = 14 hours.

ii. Multiple dose PK: Accumulation index = 1.71 for cilostazol, 3.12 for OPC-13015 and 2.54 for OPC-13213.

iii. Dose proportionality: Less than proportional increase in Cmax up to 200 mg doses. Dose proportional with respect to AUC.

iv. Food effect: Food increased cilostazol Cmax by 91% and AUC by 24%.

v. Bioequivalence: Relative bioavailability is 100% relative to suspension.
Dosage form equivalence: 2 x 50 mg is bioequivalent to 1 x 100 mg strength (based on

cilostazol and its 2 active metabolites) per sponsor's interpretation. Cmax for cilostazol does not meet 80 -125% confidence intervals.

vi. Special populations:

a. Elderly: No age effect on PK.

b. Patients: Pharmacokinetics similar to normals.

c. Renal impairment: No dosage adjustment in mild to moderate patients. In severe impairment, caution since clearance of OPC-13213 (metabolite) is decreased.

d. Hepatic impairment: No dose adjustment.

e. Gender: No gender effect on PK of cilostazol and its metabolites.

f. Smokers: Cilostazol exposure 18% lower in smokers.

F. In vitro metabolism: CYP3A4, CYP1A2, CYP2D6 and CYP2C19.

G. In vivo drug interactions: Effect of coadministration of cilostazol and aspirin on PT, APTT, bleeding time or platelet aggregation --- no effect.
No effect of cilostazol on PK or PD of warfarin.
No effect of quinidine on PK of cilostazol or its metabolites.
Erythromycin (CYP3A4) increased AUC of cilostazol and OPC-13213 by 73 and 141%.
Omeprazole (CYP2C19) increased AUC of OPC-13015 by 69%.

H. Analytical methods: method in all studies except one where method was used. Validation data provided.

I. Protein binding: >95% binding of cilostazol predominantly to albumin.

J. Dissolution testing:

K. Population PK: Non-parametric analysis.

L. PK-PD: Exploratory analysis for PK-PD relationships was done between cilostazol PK and inhibition of platelet aggregation induced by arachidonic acid.

M. Other studies: 3 PK studies with ointment and 3 with slow-release oral formulation were conducted. These will not be reviewed for this NDA since the formulations are not relevant.

COMMENTS: Details of development of dissolution methodology have not been presented in this section. Also, the comparative dissolution data between 50 mg round tablet and triangular tablet has not been provided. Need to check with the chemist.

RECOMMENDATION: The Human Pharmacokinetics and Bioavailability section of this NDA is organized, indexed, and paginated in a manner to initiate review. Hence, the submission is fileable from OCPB point of view.

G. Beuhler
MAY 20 1998

NDA 20-863 Cilostazol For Intermittent Claudication

Miscellaneous Studies/Clinical Pharmacology

Reviewed by Abraham M. Karkowsky, M.D.; Ph.D. _____

Amended (Typographical Corrections) June 10, 1998

CC: NDA 20-863, File
HFD-110; AKarkowsky, GBeuhler (CSO), SRodin