NDA 20-863

REVIEW AND EVALUATION OF TOXICOLOGY DATA

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ORIGINAL NDA DATED: September 18, 1997
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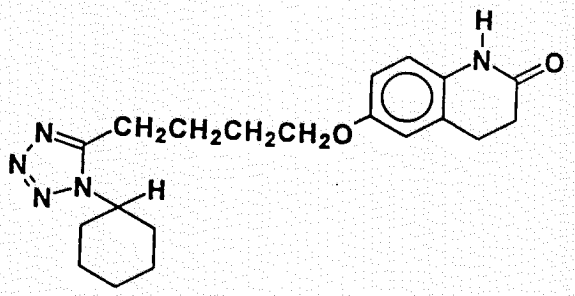
SPONSOR: Otsuka America Pharmaceutical, Inc.
2440 Research Boulevard
Rockville, MD 20850

DRUG PRODUCT: Pletal Tablets

DRUG: Generic name - Cilostazol
Code names - OPC-13013 and OPC-21



M.W. 369.47

FORMULATION: Pletal tablets containing 50 or 100 mg cilostazol are formulated with following inactive ingredients: corn starch, microcrystalline cellulose, carboxymethylcellulose calcium, hydroxypropyl methylcellulose 2910 and magnesium stearate.

PHARMACOLOGICAL CLASS: Phosphodiesterase III inhibitor

PROPOSED INDICATION: Intermittent claudication secondary to chronic arterial disease

PROPOSED DOSAGE REGIMEN: 50 or 100 mg bid

IND UNDER WHICH CLINICAL TRIALS WERE CONDUCTED:

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

SUMMARY OF TOXICOLOGICAL STUDIES

Acute Toxicity Studies

Single dose acute toxicity studies were conducted in JCL-ICR mice, JCL-SD rats and beagle dogs (both sexes) at the

(Study No. ML-222E; January - August, 1981).

Groups of rats and mice (5 weeks of age; 10/sex/group) were administered cilostazol (OPC-13013) as a suspension in 0.5% carboxymethycellulose by oral (po), intraperitoneal (ip) or intramuscular (im) routes at maximum feasible dose (MFD) levels (5000 mg/kg, po; 2000 mg/kg, ip and 1000 mg/kg, im. Dogs (9-11 months of age; 2/sex) received the MFD of 2000 mg/kg in gelatin capsules. No explanation is provided as to why any of these doses are considered to be maximum feasible doses.

All animals were observed 7 days post dose for clinical signs and mortality. Body weights were recorded on Days 3 and 7. At the end of the observation period, all animals were necropsied and examined macroscopically. No histopathology was performed.

No animals died or were found moribund during the study. Grayish or yellowish white feces was seen within 24 hours after oral dosing in all animals (all species). All rats dosed by the im route developed limping immediately after dosing, but returned to normal in 5 to 10 minutes. No limping was noted in mice. No significant effect on body weight was noted in any species. Macroscopically, in rats and mice which received ip dosing, the test compound was found attached to the visceral organs, mainly to the surface of the liver, while after im dosing, drug residue was observed in the muscles at the site of injection. No other notable findings were seen in any species.

Calculated LD50 values are given in Table 1. The data indicate that cilostazol exhibited very low acute toxicity in all species studied.

Single dose acute toxicity studies were similarly conducted in rats and mice with OPC-13015 and OPC-13213, the major human metabolites of cilostazol, using the maximum feasible im dose of 1000 mg/kg (again no explanation of why this is considered to be a MFD). There were no deaths during the 14 day observation period, and no clinical signs or gross pathological findings of toxicity were seen. The LD50 values are given in Table 2.

Table 1.

LD50 Values of Cilostazol in Acute Toxicity Studies

Animal (strain)	Sex	LD_{50} value (mg/kg)		
		p.o.	i.p.	i.m.
Mouse (ICR)	Male Female	>5000 >5000	>2000 >2000	>1000 >1000
Rat (SD)	Male Female	>5000 >5000	>2000 >2000	>1000 >1000
Dog (Beagle)	Male Female	>2000 >2000		

Table 2.

LD50 Values of OPC-13015 and OPC-13213, Major Metabolites of Cilostazol, in Acute Toxicity Studies

Animal (strain)	Sex	LD_{50} value (mg/kg)	
		OPC-13015 (i.m.)	OPC-13213 (i.m.)
Mouse (ICR)	Male Female	>1000 >1000	>1000 >1000
Rat (SD)	Male Female	>1000 >1000	>1000 >1000

Fifty-two-Week Oral (Gavage) Toxicity Study in Rats

Testing Facility:

Study Number:

001059 (Sponsor's No.)
001873 (Sponsor' Report No.)

Study Dates: January 28, 1982 to December 26, 1983

GLP Compliance: The study was conducted in compliance with GLP regulations.

Animals: JCL:SD strain rats 20/sex/group and housed individually in stainless steel cages, were about 5-6 weeks old (males 158-184 g and females 129-151 g) at the initiation of the study.

Dose Levels and Mode of Administration: 0, 6, 30 and 150 mg/kg/day. OPC-21 (Lot No.2C82M) was suspended in 0.5% sodium carboxymethyl-cellulose at required concentrations and was administered daily by oral gavage at a dose volume of 5 ml/kg. [It is stated that the doses were selected based on the results of a previous 13-week oral (gavage) toxicity study (0, 30, 150 and 1500 mg/kg/day) in rats. In that study, dose-related increases in body weight gain and liver weights (both absolute and relative) were seen at 150 and 1500 mg/kg/day. Hence, 150 mg/kg/day was selected as the highest dose for the present study. Thirty mg/kg/day was found to be the no toxic effect dose level for the 13 week study. In view of the duration of the present study, it is stated that a dose one-fifth of the non-toxic dose (6 mg/kg/day) was selected as the low dose, and 30 mg/kg/day was chosen as the mid dose (using a factor of five).]

Observations and Measurements: Animals were observed daily for clinical signs before and after (30 min, 1 and 4 hr) dosing. Body weights were recorded pretest, at weekly intervals during the treatment period and at necropsy. Food consumption was measured weekly at 3-4 day intervals. Urinalyses were done at 1, 3, 6, 9 and 12 months of treatment. Hematological evaluations [erythrocytes, leucocytes (total and differential) platelet and reticulocyte counts, hemoglobin, hematocrit, prothrombin time and activated partial thromboplastin time) and blood chemistry determinations (GOT, GPT, LDH, alkaline phosphatase, leucine aminopeptidase, cholinesterase, total protein, glucose, triglyceride, total cholesterol, phospholipids, urea nitrogen, creatinine, uric acid,

sodium, potassium, chloride, calcium, and protein fractions) were done at the termination of the study. Ophthalmoscopic examination (fundus of one eye per animal) was performed on animals from the high dose (during months 1, 3, 6, 9 and 12) and control (months 1 and 12) groups. Hearing response was tested in high dose and control animals at 6 and 12 months of treatment.

At the termination of the study, the animals were fasted 18 to 24 hours prior to necropsy. All organs were grossly examined and brain, pituitary, thyroids, thymus, heart, lungs, liver, kidneys, adrenals, spleen, testes, seminal vesicle, prostate, uterus, ovaries and submaxillary glands were weighed. In addition to the above organs, the following organs and tissues were removed and fixed in 10% neutral buffered formalin for histopathological examinations: pancreas, esophagus, stomach, duodenum, colon, urinary bladder, bone marrow of femur, aorta, mesenteric lymph nodes and all grossly abnormal tissues. Eyeballs were fixed in a mixture of 3% glutaraldehyde and 2.5% formalin. Tissues were processed by routine histological procedures and stained with H&E and Masson's trichrome. Liver and kidney sections were also stained with Sudan III for the demonstration of fat. Electron microscopic examination was performed on liver and kidney sections (2 rats/ sex/group).

Data were statistically analyzed using Student's t test.

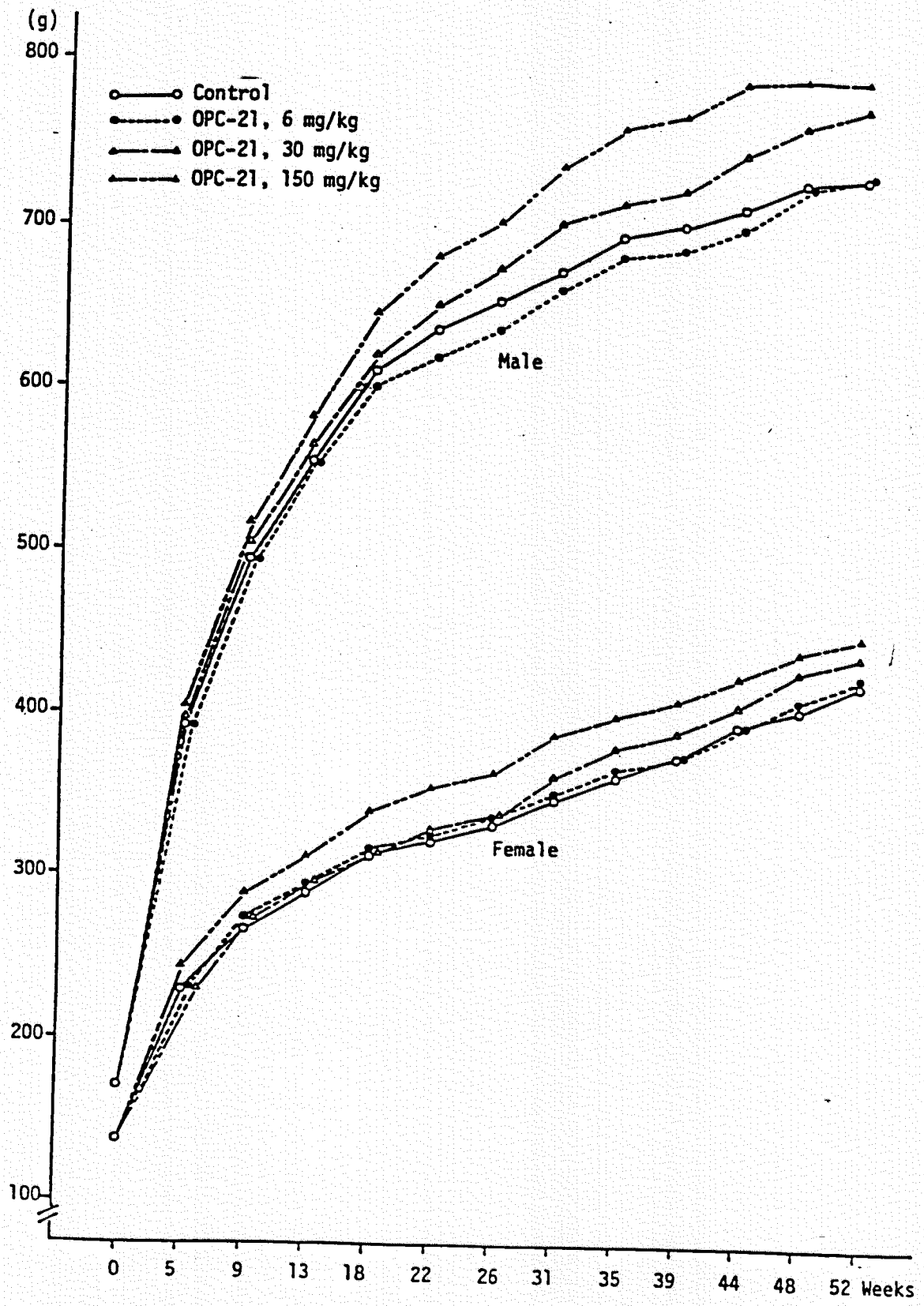
Results: No notable clinical signs were seen in the study. The number of animals that died or were killed in extremis during the study is given below.

Treatment Group	Male	Female
Control	2	1
6 mg/kg/day	3	0
30 mg/kg/day	1	2
150 mg/kg/day	0	2

N=20/group

All deaths or sacrifices occurred after 18 weeks of treatment except for one control female which was sacrificed on Day 11. None of the deaths was attributed to drug treatment.

Fig. 1 Body Weights (g) of Rats: 52-Week Study



The body weight data is presented graphically in Figure 1. The mean body weights of high dose male and female groups were significantly higher than concurrent control beginning with treatment weeks 11 and 4 for males and females, respectively, and continuing throughout the study. Though not statistically significant, the mean body weights of the mid dose male and female groups were also higher than concurrent control after the first two weeks of treatment. No significant effect on body weight was seen for the low dose group.

Dose-dependent increases in food consumption were observed in mid and high dose male and female groups (statistically significant at the high dose during the second half of the study). No significant treatment-related effect on water consumption or urine volume was seen.

Serum triglyceride, total cholesterol and phospholipid levels were increased in high and mid dose males (dose dependent except for triglycerides). Serum total protein was increased in males and females receiving 30 or 150 mg/kg/day (dose-dependent in males).

Ophthalmologic and audiometric examinations showed no significant findings.

Dose-dependent significant increases in the absolute (13-20%) and relative (8-11%) liver weights were seen in both males and females of the 30 and 150 mg/kg/day dose groups.

No treatment-related gross lesions were seen in the study.

Histologically, increased incidences of nodular hyperplasia and adenoma of the pituitary gland were seen in treated male groups. The incidences of these findings in the male groups are given below.

	Dose Groups			
	Control	Low	Middle	High
Pituitary:				
Chromophobe cell adenoma	0	1	3	3
Nodular hyperplasia of chromophobe cells	0	1	1	1
Nodular hyperplasia of basophilic cells	0	1	0	2
Adenoma of anterior lobe	0	0	0	1
N =	18	17	19	20

For females, the incidences of the pituitary findings were comparable in treated and control groups.

Electron microscopic examination of liver and kidney sections showed no treatment-related findings.

The 6 mg/kg/day dose was considered, by the sponsor, to be the non-toxic dose for the test compound. However, the pituitary pathology data indicate that a non-toxic dose may not have been established for males in this study.

A separate 4-week toxicokinetic study, with OPC-13013 administered by oral gavage, was conducted in male and female Sprague-Dawley rats at doses of 6, 30, 150 and 1500 mg/kg/day to determine the systemic exposure to cilostazol in repeat dose toxicity studies conducted at the same doses by the same mode of administration.

The Cmax, Tmax and AUC values for this gavage study are given below.

Toxicokinetics study of OPC-13013 in rats : Four-week repeated oral administration
Item : Parameter - OPC-13013 -
Sex : Male

Dose(mg/kg)	6	30	150	1500 (Day : 27)
Cmax (μg/ml)	0.4	1.6	5.1	12.8
Tmax (hr)	2.00	2.00	2.00	8.00
AUC_{0-24hr} (μg·hr/ml)	1.0	7.7	59.0	185.4

Sex : Female

Dose(mg/kg)	6	30	150	1500 (Day : 27)
Cmax (μg/ml)	13.5	30.9	50.9	54.0
Tmax (hr)	2.00	2.00	2.00	2.00
AUC_{0-24hr} (μg·hr/ml)	97.1	274.0	625.4	560.1

A dose-dependent increase in Cmax and AUC values was observed for males of all groups and for females from 6, 30 and 150 mg/kg/day groups (day 27 of treatment). There were no notable differences in Cmax or AUC values between the 150 and 1500 mg/kg/day female groups. The Cmax and AUC values were higher in females than in males at each dose level.

Carcinogenicity Studies in Rodents

Two year carcinogenicity studies in mice and rats and the respective 13 week dose range finding studies were reviewed under (please see attachment). Supporting toxicokinetic studies in mice and rats are summarized below.

Four-Week Dietary Toxicokinetic Study in Mice

[This study was conducted at same dose levels (100, 300 and 1000 mg/kg/day) as used for the mouse carcinogenicity study, in order to determine the systemic exposure to cilostazol in that experiment.]

Testing Facility:

Study Number: 012270

Study Dates: June 5, 1996 to December 16, 1996

GLP Compliance: The study was conducted in compliance with GLP regulations.

Animals: B6C3F1, SPF mice 12/sex/ group and housed individually in stainless steel cages, were about 6 weeks old (body weight ranged from 22.2 to 25.6 g for males and 18.0 to 22.7 g for females) at the initiation of the study.

Dose Levels and Mode of Administration: OPC-13013 (Lot No.6A81M) was mixed with CRF-1 powdered diet (obtained from at appropriate concentrations to provide an average daily intake of 100, 300 and 1000 mg/kg. The drug-diet mixture was found to be stable for 2 weeks when stored at room temperature protected from light.

Observations/Measurements: Body weight was recorded pretest and weekly during the treatment period. Food consumption was recorded weekly; and the actual drug intake was determined based on the mean body weight, food consumption and the concentration of the test drug in the dietary admixture. About 1 ml of blood was collected at 10.00, 14.00 and 22.00 hours on Day 28 and at 02.00 hours on Day 29 (3 lowest numbered animals/sex/group/sampling time; different animals at each sampling time) for serum drug level determinations. Animals were discarded after blood collection.

Results: One female from the 1000 mg/kg/day group was found dead on Day 2. The cause of death was not determined.

The actual drug intake (weekly group means) in males and females ranged between 81 and 97 mg/kg/day for the 100 mg/kg/day group, 240 and 284 mg/kg/day for the 300 mg/kg/day group and between 792 and 930 mg/kg/day for the 1000 mg/kg/day group.

The kinetic parameters for the study are given below.

Toxicokinetic study of OPC-13013 ; Four-week administration via diet in mice
Item : Kinetic parameter
Sex : Male

			Stage : Day28
Dose (mg/kg)	100	300	1000
Cmax (μg/ml)	0.3	0.6	1.2
Tmax	02:00	02:00	02:00
AUC_{0-24hr} (μg·hr/ml)	2.6	6.8	16.6

Sex : Female

Dose (mg/kg)	100	300	1000
Cmax (μg/ml)	0.2	0.5	0.8
Tmax	22:00	22:00	22:00
AUC_{0-24hr} (μg·hr/ml)	2.0	6.8	13.0

The above data indicate that maximum concentrations and AUC values on Day 28 increased dose-dependently in both males and females. No significant gender difference was noticed for any parameter. The serum concentrations reached maximum levels at 2.00 hours in all treated male groups and at 22.00 hours in all female groups.

Four-Week Dietary Toxicokinetic Study in Rats

[This study was conducted using the same dose levels as used for the rat carcinogenicity study (50, 150 and 500 mg/kg/day), in order to determine the systemic exposure to cilostazol in that experiment.]

Testing Facility:

Study Number: 012269

Study Dates: June 5, 1996 to December 16, 1996.

GLP Compliance: The study was conducted in compliance with GLP regulations.

Animals: F-344, SPF rats 12/sex/group and housed individually in stainless steel bracket cages, were about 5 to 6 weeks old (body weight ranged from 112 to 132 g for males and 90 to 104 g for females) at the initiation of the study.

Dose Levels and Mode of Administration: OPC-13013 (Lot No.6A81M) was mixed with CRF-1 powdered diet (obtained from at appropriate concentrations to provide an average daily intake of 50, 150 and 500 mg/kg. The diet-drug mixture was found to be stable for 2 weeks when stored at room temperature protected from light.

Observations/Measurements: The body weight was recorded pretest, and weekly during the treatment period. Food consumption was recorded weekly and the drug intake was determined based on the mean body weight, food consumption and concentration of the test substance in the dietary admixture. Blood samples were collected at 10.00, 14.00 and 22.00 hours on Day 28 and at 2.00 hours on Day 29 (3 lowest numbered rats/sex/group/sampling time; different animals at each sampling time) for serum drug level determinations. The animals were discarded after blood collection.

Results: No mortality was noted in the study.

The mean drug intake (weekly group means) in males and females during the study ranged between 48 and 50 mg/kg/day for the 50 mg/kg/day group, 144 and 152 mg/kg/day for the 150 mg/kg/day group and between 486 and 508 mg/kg/day for the 500 mg/kg/day group.

The kinetic parameters for the study are given below.

Toxicokinetic study of OPC-13013 ; Four-week administration via diet in rats
Item : Kinetic parameter
Sex : Male Stage : Day28

Dose (mg/kg)	50	150	500
Cmax (μg/ml)	0.13	0.27	0.77
Tmax	22:00	10:00	22:00
AUC_{0-24hr} (μg·hr/ml)	1.69	5.27	13.82

Sex : Female Stage : Day28

Dose (mg/kg)	50	150	500
Cmax (μg/ml)	1.92	4.80	4.17
Tmax	22:00	22:00	22:00
AUC_{0-24hr} (μg·hr/ml)	32.58	62.40	63.79

The Cmax and the AUC values increased dose-dependently in males. In females, although the Cmax and AUC values in the 150 mg/kg/day group were higher than in the 50 mg/kg/day group, no notable differences in these values were seen between the 150 and 500 mg/kg/day groups. Additionally, the Cmax and AUC values at all dose levels were higher in females than in males (5 to 19 times). The serum drug concentrations reached maximum levels at 22.00 hours in all male and female dose groups except for the 150 mg/kg/day male group, in which the maximum concentration was seen at 10.00 hours.