Chromosomal Aberration Study of Cilostazol (OPC-13013) in Cultured Chinese Hamster Ovary (CHO) Cells

Testing Facility:

Study Number: 275-108 (Contract Lab.'s Number)
012569 (Sponsor's Number)

Study Dates: October 2, 1996 to May 20, 1997

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

Lot Number of the Test Compound: 6A81M

Concentrations Tested: 50, 100 and 200 µg OPC 13013/ml (first experiment, with and without metabolic activation); 70, 100 and 140 µg/ml (second experiment, with S-9 fraction), and 70, 140 and 280 µg/ml (second experiment, without S-9 fraction). A study with delayed sampling times, without metabolic activation, was also performed (8.75, 12.5 and 17.5 µg/ml). [The doses for cytogenetic analyses were selected based on the cytotoxicity findings. The highest concentrations selected produced 45-75% reductions in cell counts.]

Solvent: DMSO

Positive Control Substances: 4-nitroquinoline (without metabolic activation) and cyclophosphamide (with metabolic activation)

Metabolic Activation System: Aroclor 1254 induced rat liver S-9 fraction

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The aberrant cells were categorized as follows:

1) cells with structural aberrations including gaps
2) cells with structural aberrations excluding gaps and
3) polyploid, endoreduplicated or hyperdiploid cells.

The data were analyzed using Fisher's exact test.

The assay was considered valid if the following criteria were met:

1) the proportion of cells with structural aberrations excluding gaps in negative controls fell within the normal range
2) the binomial dispersion test demonstrated acceptable heterogeneity between replicate cultures
3) at least 160 cells out of 200 were analyzable at each dose level and
4) the positive controls induced significant increases in the number of cells with structural aberrations.

The test article was considered positive if:

a) a statistically significant increase in the proportion of cells with structural aberrations (excluding gaps) occurred at one or more concentrations
b) the proportion of cells with structural aberrations at such doses exceeded the normal range and
c) the responses were reproducible

Increases in numbers of cells with gaps or increases in the proportions of cells with structural aberrations not exceeding the normal range or occurring only at very high or toxic concentrations were considered as equivocal.

Results: Number of cells with structural aberrations for different treatment regimens are presented in Tables 46-52.

Treatment of cultures for 20 hours with the test drug, in the absence of S-9, resulted in a statistically significant increase (compared to concurrent solvent control) in cells with structural aberrations at the highest dose level (200 µg/ml; Experiments 1 and 2, Tables 46 & 48). At this dose, the mitotic indices were 60 to 67% lower than the indices of solvent control. The percent of aberrant cells observed in these studies at 200 µg/ml exceeded the historical solvent control range for CHO cultures (Table 53). No significant aberrations were seen, in the absence of S-9, after treatment for 44 hours at 17.5 µg/ml (Table 50) or for 3 hours at 280 µg/ml (Table 52).

Treatment of cultures for 3 hours (with 17 hours of recovery) with OPC-21 in the presence of S-9 resulted in a significant increase in cells with aberrations at the highest concentration (200 µg/ml; Experiment 1, Table 47). The mitotic index was significantly reduced (about 85% reduction compared to solvent control), indicating increased cytotoxicity at this dose level. A significant (smaller) increase in aberrant cells was also seen at the high dose (140 µg/ml) in the repeat study (Table 49). No significant aberrations were seen, with S-9, after 3 hours of treatment followed by 41 hours of recovery (Table 51).

Increased incidence of numerical aberrations, in the presence of S-9, was observed after 3 hours of treatment followed by 17 hours of recovery. This finding was not dose-dependent.

Table 46.

OPC-21: cells with structural aberrations: Experiment 1

20 hour treatment -S-9, 0 hour recovery (20+0)

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	0	0		4.1
	B	100	5	4		4.9
	Totals	200	5	4		(4.5)
50	A	100	5	3		2.3
	B	100	4	3		1.9
	Totals	200	9	6	NS	(2.1)
100	A	100	3	2		3.8
	B	100	5	5		3.1
	Totals	200	8	7	NS	(3.5)
200	A	100	7	7		1.1
	B	100	11	10		2.5
	Totals	200	18	17	p ≤0.01	(1.8)
NQO, 0.25	A	25	14	14		
	B	25	13	11		
	Totals	50	27	25	p ≤0.001	

§ Statistical significance (Appendix 5a)

NS = not significant

Numbers highlighted exceed historical negative control range

[* Note: In Tables 46-52, OPC-21 = OPC-13013]

Table 47.

OPC-21: cells with structural aberrations: Experiment 1

3 hour treatment +S-9, 17 hour recovery (3+17)

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	2	1		9.1
	B	100	5	5		7.0
	Totals	200	7	6		(8.1)
50	A	100	1	0		6.7
	B	100	6	3		8.2
	Totals	200	7	3	NS	(7.5)
100	A	100	4	4		6.4
	B	100	6	4		8.0
	Totals	200	10	8	NS	(7.2)
200	A	100	28	26		1.3
	B	100	39	37		1.0
	Totals	200	67	63	p ≤0.001	(1.2)
CPA, 25	A	25	24	23		
	B	25	14	14		
	Totals	50	38	37	p ≤0.001	

§ Statistical significance (Appendix 5a)

NS = not significant

Numbers highlighted exceed historical negative control range

Table 48.

OPC-21: cells with structural aberrations: Experiment 2

20 hour treatment -S-9, 0 hour recovery (20+0)

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	2	2		3.3
	B	100	6	5		2.7
	Totals	200	8	7		(3.0)
50	A	100	2	1		2.1
	B	100	3	0		1.9
	Totals	200	5	1	NS	(2.0)
100	A	100	3	2		1.2
	B	100	2	1		1.3
	Totals	200	5	3	NS	(1.3)
200	A	100	8	7		0.7
	B	100	15	10		1.2
	Totals	200	23	17	p ≤0.05	(1.0)
NQO, 0.25	A	25	10	10		
	B	25	11	10		
	Totals	50	21	20	p ≤0.001	

§ Statistical significance (Appendix 5b)

NS = not significant

Numbers highlighted exceed historical negative control range

Table 49.

OPC-21: cells with structural aberrations: Experiment 2

3 hour treatment +S-9, 17 hour recovery (3+17)

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	2	1		6.6
	B	100	2	1		6.6
	Totals	200	4	2		(6.6)
70	A	100	4	3		5.9
	B	100	7	4		9.7
	Totals	200	11	7	NS	(7.8)
100	A	100	4	4		4.9
	B	100	1	1		6.1
	Totals	200	5	5	NS	(5.5)
140	A	100	6	4		2.4
	B	100	12	9		2.6
	Totals	200	18	13	p ≤0.01	(2.5)
CPA, 25	A	25	21	21		
	B	25	23	23		
	Totals	50	44	44	p ≤0.001	

§ Statistical significance (Appendix 5b)

NS = not significant

Numbers highlighted exceed historical negative control range

Table 50.

OPC-21: cells with structural aberrations: Experiment 2

44 hour treatment -S-9, 0 hour recovery (44+0)

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	6	5		3.2
	B	100	4	2		4.5
	Totals	200	10	7		(3.9)
8.75	A	100	1	1		2.1
	B	55	1	1		2.6
	Totals	155	2	2	NS	(2.4)
12.5	A	100	3	3		1.9
	B	100	2	2		1.7
	Totals	200	5	5	NS	(1.8)
17.5	A	100	3	3		0.9
	B	100	2	1		1.0
	Totals	200	5	4	NS	(1.0)

Table 51.

3 hour treatment +S-9, 41 hour recovery (3+41)

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	4	2		3.6
	B	100	2	2		3.9
	Totals	200	6	4		(3.8)
140	A	100	5	3		3.7
	B	100	0	0		5.2
	Totals	200	5	3	NS	(4.5)

§ Statistical significance (Appendix 5c)

NS = not significant

Number highlighted exceeds historical negative control range

Table 52.

OPC-21: cells with structural aberrations: Experiment 2

3 hour treatment -S-9, 17 hour recovery (3+17)

Treatment (μg/mL)	Replicate	Cells scored	Cells with aberrations including gaps	Cells with aberrations excluding gaps	Significance §	Mitotic index (mean)
Solvent	A	100	2	2		4.4
	B	100	2	2		4.7
	Totals	200	4	4		(4.6)
70	A	100	0	0		6.1
	B	100	2	1		6.8
	Totals	200	2	1	NS	(6.5)
140	A	100	2	2		4.6
	B	100	1	1		4.6
	Totals	200	3	3	NS	(4.6)
280	A	100	3	2		4.5
	B	100	3	2		6.3
	Totals	200	6	4	NS	(5.4)

§ statistical significance
NS not significant

Table 53.

Historical solvent control data for CHO cultures*

	- S-9		+ S-9	
Aberrant cells per 100 scored	Mean	Defined normal range	Mean	Defined normal range
Cells with structural aberrations including gaps	2.5		2.9	
Cells with structural aberrations excluding gaps	1.0		1.4	
Cells with numerical aberrations	1.8		2.1	

* Calculated on the basis of data from 42 or 38 (- and + S-9 respectively) negative control cultures

Cytogenetic Test of Cilostazol (OPC-13013) in Mice

Testing Facility:

Study Numbers: 000494 (Sponsor's number)
CH-012 (Contract Lab.'s number)

Study Dates: December 1, 1980 to June 30, 1981

GLP Compliance: It is stated that since the laboratory where the study was done was in the process of developing the GLP Compliance program during the early part of the study in question, only the latter part of the study was in compliance with GLP regulations.

Animals: Eight-week-old BDF1 male mice (hybrid of C57BL/6 and DBA/2 strains) - Body weight 24-25 g.

Lot No. of the Test Compound: 1B76

Doses Tested: 0 (vehicle control), 1500, 3000 and 6000 mg OPC-13013/kg, po. The test drug was suspended in 0.5% carboxymethyl-cellulose aqueous solution and was given by oral intubation at a dosing volume of 0.5 ml/25 g body weight.

(Based on an acute oral dose range finding study, 6000 mg/kg was selected as the highest dose for the cytogenetic test.)

Positive Control: Mitomycin C, 5 mg/kg, ip

Results: Incidences of chromosomal aberrations are presented in Table 54. No significant differences in the percent of cells with chromosomal aberrations or the type of aberrations between drug treated and control mice were observed. Chromatid gap or break, but not chromatid exchange was seen in both drug-treated and control animals. A significant increase in chromosomal aberrations was seen in positive control mice.

Table 54.

Chrocosome aberrations in mouse bone marrow cells after oral administration of OPC-21*

Chemical & dose (mg/kg)	Animal number	Cells analysed	Cells with aberrations (%)	Number of aberrations (per cell)	Types and numbers of chromosome aberrations					
					cdg	icdg	cdb	icdb	f	cdx
Control (0.5% CMC)	1	100	2	2	2	0	0	0	0	0
	2	100	2	2	2	0	0	0	0	0
	3	100	1	1	1	0	0	0	0	0
	4	100	3	3	2	0	1	0	0	0
	5	100	2	2	2	0	0	0	0	0
Total	5	500	10(2.0)	10(0.020)	9	0	1	0	0	0
OPC-21 1500	1	100	1	1	1	0	0	0	0	0
	2	100	1	1	1	0	0	0	0	0
	3	100	1	1	0	0	1	0	0	0
	4	100	1	1	1	0	0	0	0	0
	5	100	0	0	0	0	0	0	0	0
Total	5	500	4(0.8)	4(0.008)	3	0	1	0	0	0
OPC-21 3000	1	100	2	2	2	0	0	0	0	0
	2	100	3	3	2	0	0	0	1	0
	3	100	0	0	0	0	0	0	0	0
	4	100	0	0	0	0	0	0	0	0
	5	100	1	1	1	0	0	0	0	0
Total	5	500	6(1.2)	6(0.012)	5	0	0	0	1	0
OPC-21 6000	1	100	2	2	2	0	0	0	0	0
	2	100	3	4	3	0	1	0	0	0
	3	100	0	0	0	0	0	0	0	0
	4	100	1	1	1	0	0	0	0	0
	5	100	0	0	0	0	0	0	0	0
Total	5	500	6(1.2)	7(0.014)	6	0	1	0	0	0
Positive control (mitomycin C 5	1	100	18	25	12	0	6	0	7	0
	2	100	37	66	22	3	23	1	15	2
	3	100	44	88	30	0	28	1	27	2
	4	100	44	75	28	0	22	0	20	5
	5	100	1	1	0	0	0	0	1	0
Total	5	500	144(28.8)**	255(0.510)	92	3	79	2	70	9

** Significant difference to control($P < 0.05$)

Abbreviations: cdg; chromatid gap, icdg; iso-chromatid gap, cdb; chromatid break, icdb; iso-chromatid break, f; fragment, cdx; chromatid exchange

* OPC-21 = OPC-13013

SUMMARY AND EVALUATION

Cilostazol (OPC-13013), a quinolinone derivative with platelet anti-aggregating and vasodilating properties, is being developed for use in the amelioration of ischemic symptoms in patients with intermittent claudication secondary to chronic arterial occlusive disease of the limbs. Although the exact mechanism for the amelioration of symptoms of intermittent claudication is not fully understood, it is thought that the test drug improves the blood flow in the limbs through a combination of inhibition of platelet aggregation and increased vasodilation. The antiplatelet and vasodilatory effects of cilostazol are believed to be due to the inhibition of cyclic AMP phosphodiesterase (PDE III), thereby increasing the cAMP levels in platelets and blood vessels, and also due to the potentiation of the effects of prostaglandin I_2 (PGI_2), an endothelial cell-derived substance having an inhibitory effect on platelet aggregation and vasodilating properties.

An oral dose of 100 mg b.i.d is proposed for the amelioration of symptoms in patients with intermittent claudication.

Toxicity studies with cilostazol, including acute and chronic studies, genotoxicity, and reproductive and developmental toxicity studies are summarized and evaluated below.

Single dose acute toxicity studies were conducted in mice, rats and dogs. There were no deaths in these studies. The oral LD50 values were found to be >5000 mg/kg in rodents and >2000 mg/kg in dogs. In rodents, the intramuscular LD50 values for cilostazol and its two major human metabolites (OPC 13015 and OPC 13213) were >1000 mg/kg.

A one year oral toxicity study in beagle dogs (0, 6, 12, 30 and 150 mg/kg/day) revealed cardiovascular (CV) lesions which included focal endocardial fibrous thickening with hemorrhage in the left ventricle, intimal thickening of the coronary artery, coronary arteritis and periarteritis, and swelling of the tunica intima and/or media of the coronary artery. A non-toxic dose level for CV lesions was not established in females; in males, 6 mg/kg/day was considered as non-toxic. The peak mean plasma concentrations at 12 mg/kg/day for males and 6 mg/kg/day for females (the lowest doses that produced CV lesions in this one year dog study) were 142 and 704 ng/ml, respectively, compared to a steady state Cmax value of 1332 ng/ml in patients at the maximum recommended human dose (MRHD) of 100 mg b.i.d., and the lowest doses which induced CV lesions in this dog study were only about 1-2 times the MRHD on a mg/m^2 basis.

There were no significant serum enzyme elevations in the one year

dog study. Tachycardia and ST-segment depression were noted in one of four high dose males, and atrioventricular block in one of four high dose females. There were no treatment-related changes in R-R, P-P, PQ, QRS and QT intervals. The plasma drug levels were consistently higher in females than in males at all dose levels tested.

Cardiovascular lesions, similar to those found in the one year dog study, were also seen in a 13-week oral toxicity study in dogs at 0, 30, 150 and 750 mg/kg/day dosages. Although a non-toxic dose for cardiotoxicity was not established for males in this study, 30 mg/kg/day was considered to be the non-toxic dose level in females. The lowest doses that produced lesions (30 mg/kg/day for males and 150 mg/kg/day for females) were about 5 and 25 times (males and females, respectively) the MRHD on a mg/m^2 basis.

Heart and coronary artery lesions, which included thickening of the tunica intima, swelling of the tunica intima and/or media of the coronary artery, hemorrhage of the coronary arterial wall, necrosis of the smooth muscle cells of the coronary artery, and hemorrhage of right atrial and left ventricular walls, were also observed in a 5-week oral toxicity study (with 5-week recovery period) in dogs (0, 30, 150 and 750 mg/kg/day). The above lesions, except for the thickening of the coronary artery in one of the four high dose animals, were not seen after the recovery period. The non-toxic dose was found to be 150 mg/kg/day for this 5-week study. The dose that produced CV lesions (750 mg/kg/day) was about 123 times the MRHD on a mg/m^2 basis. (Note: Since doses between 150 and 750 mg/kg/day were not tested, it is uncertain whether such a wide safety margin really exists in this study.)

A 2-week oral toxicity study (450 mg OPC-13013/kg/day) also revealed coronary arterial lesions and hemorrhage of the right atrium and left ventricle in male beagle dogs.

Oral administration of OPC-13015, a main human metabolite of OPC-13013, for 2 weeks at 600 mg/kg/day, produced similar cardiovascular lesions in dogs.

Cardiovascular lesions (subendocardial necrosis of the left ventricle, right atrial hemorrhagic lesions and coronary arterial injuries) have been reported in the dog after the administration of phosphodiesterase (PDE) inhibitors, positive inotropic agents and/or vasodilating agents (Isaacs, et al. 1989. Toxicol. Pathol., 17: 153.; Harleman, et al. 1986. Arch. Toxicol., 59:51.; Mesfin, et al. 1989. Toxicol. Pathol. 17:164; Herman et al. 1979. Toxicol. Appl. Pharmacol. 47:493.) These CV lesions, induced by several agents (with different structures and mechanisms of action)