## Reference 8. Study G-7

Title of Study: Anti-aggregatory Effect of Oral Cilostazol and Recovery of Platelet Aggregability In Patients with Cerebrovascular Disease
Publication: Arzneim-Forsch/Drug Res ; 35 (II) 5; 1189-1192; (1985).

Investigator and Sites:     Yasunaga, Y.; and Mase, K.;
Kansai Medical University, Osaka, Japan

Study Summary: No protocol, randomization codes or listing of adverse events were included within this paper.

This is a publication with some accompanying line listings. A total of 24 patients with a history of cerebrovascular disease (TIA, cerebral thrombosis, cerebro-embolism or cerebral athererosclerosis) that occurred at least one month prior to enrollment, were randomized to receive cilostazol at doses of either 50 mg (50 mg QD) , 100 mg (50 mg BID), 150 mg (50 mg TID) or 200 mg (100 mg BID). There were 17 male and 7 female patients with a mean age of 68.3 years. Seven of the patients had a history of TIAs, five had cerebro-atherosclerosis, nine with cerebral thrombosis and three had cerebral embolism.

Aggregation was tested with platelet rich plasma. The following were used as inducers of aggregation: ADP (2 uM), collagen (0.5 ug/ml); epinephrine (0.1 ug/ml); and arachidonic acid (100 ug/ml). Aggregation studies were performed after a single dose (only the 50 mg BID dose and the 100 mg BID dose). Aggregation studies were also performed before treatment and 3, 48 and 96 hours after 4 weeks of cilostazol treatment. Plasma concentrations, measured by          were done in concert with the platelet aggregation studies.

The plasma concentration data, per sponsor, is shown in Table 8.1. There, however, was missing data. Several subject's line listings were left blank and several others had a value of zero, particularly after the first dose at the 3 and 6 hour time points. The explanation for the missing data is not included. (It is very possible that the data that did not fit some preconceived estimate was left out. Since these subjects did have blood drawn for platelet aggregation studies at the appropriate time, I find it surprising that the concentration data were not included within this report.) The values below are taken from Table 3 of the publication. No statement is made about the loss of data.

Table 8.1 Plasma Concentration ng/ml of Cilostazol, After the First Dose and After 4 Weeks.

| Dose (mg) | First Dose | | | After 4-weeks | | |
|---|---|---|---|---|---|---|
| | 3 Hours | 6 Hours | | 3 Hours | 48 Hours | 96 Hours |
| 50 QD | | | | 650.3 | 31.3 | 13.5 |
| 100 mg (50 mg BID) | 483.3 | 275.3 | | 1025.2 | 54.8 | ND |
| 150 mg (50 TID) | | | | 1316.4 | 112.6 | 28.8 |
| 200 mg (100 mg BID) | 1529.6 | 1212.9 | | 1746.5 | 130 | 110.5 |

With respect platelet aggregation, I have reproduced sponsor's Table 2 as Table 8.2. There was some missing data for the 96 hour time point at week 4. All other points have all six patient's data available. There appears to be a trend at three hours at week 4 for a dose-related effect of cilostazol on inhibiting platelet aggregation.

Table 8.2 Effect of Inducers on Platelet Aggregation.

| Inducer | Dose | Before | First day | | | After 4-weeks | | |
|---|---|---|---|---|---|---|---|---|
| | | | 3 Hour | 6 Hour | | 3 Hour | 48 Hour | 96 Hour |
| ADP (2 uMol) | 50 | 72.7 ± 2.4 | | | | 60.8 ± 9.0 | 63.3 ± 9.6 | 75.3 ± 1.7 |
| | 100 | 72.1 ± 2.1 | 67.5 ± 4.5 | 67.5 ± 3.6 | | 56.8 ± 6.2* | 68.8 ± 4.5 | 70.8 ± 2.9 |
| | 150 | 70.5 ± 5.6 | | | | 46.0 ± 8.2* | 70.4 ± 5.3 | 73.7 ± 2.3 |
| | 200 | 78.8 ± 1.6 | 55.7 ± 8.6 | 57.3 ± 7.9* | | 43.8 ± 11.9* | 75.9 ± 3.7 | 71.3 ± 9.3 |
| Collagen 0.5 ug/ml | 50 | 69.5 ± 2.5 | | | | 54.7 ± 12.7 | 55.0 ± 11.7 | 71.3 ± 4.1 |
| | 100 | 67.3 ± 2.2 | 64.5 ± 6.7 | 62.4 ± 8.3* | | 60.7 ± 9.2 | 61.4 ± 7.0 | 63.2 ± 3.4 |
| | 150 | 68.8 ± 3.2 | | | | 43.7 ± 11.6* | 64.2 ± 10.7 | 71.3 ± 2.1 |
| | 200 | 79.0 ± 1.7 | 71.9 ± 3.2 | 54.8 ± 10.8 | | 14.0 ± 2.2* | 63.3 ± 8.8 | 63.4 ± 12.4 |
| Epinephrine 0.1 ug/ml | 50 | 71.7 ± 1.9 | | | | 63.6 ± 10.7 | 74.0 ± 3.7 | 74.6 ± 0.9 |
| | 100 | 71.0 ± 1.3 | 65.7 ± 8.6 | 68.7 ± 6.1 | | 62.5 ± 6.6 | 61.4 ± 8.1 | 67.0 ± 1.9 |
| | 150 | 70.0 ± 2.5 | | | | 41.0 ± 14.5* | 65.3 ± 8.7 | 71.2 ± 3.0 |
| | 200 | 77.7 ± 2.4 | 53.0 ± 14.6 | 60.4 ± 11.4 | | 30.7 ± 11.3* | 62.8 ± 8.1 | 62.0 ± 14.4 |
| Arachidonic Acid 100 ug/ml | 50 | 73.3 ± 2.4 | | | | 71.0 ± 6.6 | 76.8 ± 3.5 | 76.0 ± 2.4 |
| | 100 | 71.3 ± 1.6 | 60.0 ± 12.5 | 71.7 ± 4.2 | | 60.5 ± 12.4 | 71.1 ± 4.7 | 72.6 ± 2.3 |
| | 150 | 66.4 ± 4.0 | | | | 16.3 ± 10.1* | 33.2 ± 15.2* | 71.5 ± 2.7 |
| | 200 | 75.0 ± 2.3 | 47.0 ± 14.6 | 25.7 ± 14.8* | | 3.1 ± 1.6* | 56.2 ± 12.0 | 57.6 ± 18.3 |

^ P < 0.05

Safety: The paper contains the statement "there were no treatment-related adverse reactions encountered throughout this study" (The authors limits this statement to treatment-related adverse events and implies, with a certain degree of subjectivity, that other non-treatment related events may have occurred and were not reported).

Conclusion: There is some discrepancy between serum concentrations and dosing patterns. There appears to be dose related inhibition of platelet aggregation when the inducer was ADP, arachidonic acid, epinephrine and collagen only at hour 3 of the 4-

week regimen.  The effect wanes 48 hours after the final dose. The effect, however, at 50 and 100 mg was minimal. How fast the effect wanes is not assessable from the data.

Reference 9. Study G-8.

Title of Study: Effects of Cilostazol (OPC-13013) on Platelet Function (G-8).
Publication: Ipn Pharmacol Therp. 14; (30); 1537-1544; (1986).

Investigator and Sites: Uehara, S.; and Hirayama, T.; Tanan Hospital

No protocol, or CRFs were submitted. Selected line listings, however, were supplied.

Study Summary: The paper contains data both from an *ex-vivo* inhibition of platelet aggregation in normal individuals and an *in vivo* study of cilostazol followed by *ex vivo* determination of platelet effect in patients with a history of cerebral atherosclerosis or cerebral thrombosis (at least 1 month prior to the study).

Cilostazol at concentrations of 1, 3, 10, 30 and 100 uM (the MW of cilostazol is 369 so these doses represent 3.69, 11.07, 36.9, 110.7 and 369 ug/l) was added to platelet rich plasma derived from normal individuals (See Figure 8.1 for results). For the *in vitro* studies trapidil hydrochloride, at test concentrations of 10, 30 , 100 , 300 and 1000 uM was included as a positive control (data not shown).
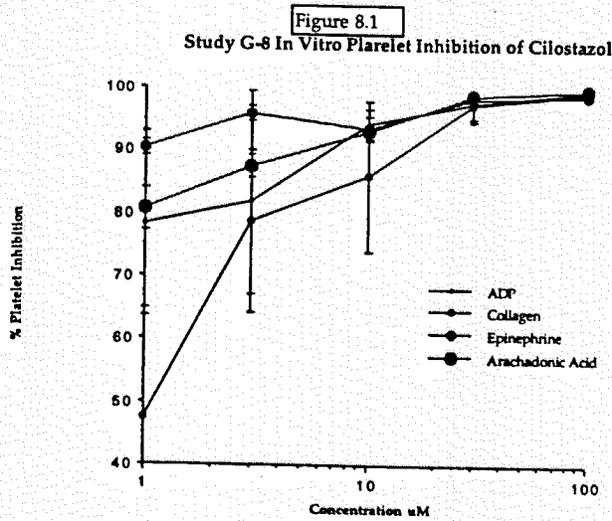


Figure 8.1
Study G-8 In Vitro Platelet Inhibition of Cilostazol

Table 8.1 Percent Inhibition of Platelet Aggregation After Oral Cilostazol  (50 mg BID)

| ADP | | Collagen 2.5 ug/ml | Epinephrine 1 ug/ml | Arachidonic Acid 200 ug/ml |
|---|---|---|---|---|
| 1.5 uM | 3 uM | | | |
| $41 \pm 7^*$ | $24 \pm 17^*$ | $16 \pm 10$ | $20 \pm 7$ | $-5.8 \pm 11$ |
| ^p <0.05 | | | | |

The *in vitro* data suggest a substantial inhibition of platelet aggregation with cilostazol, even at the lowest concentrations (1 uM).  Trapidil was less potent in inhibiting platelet aggregation.Trapidil at concentrations of approximately 3 to 30 fold more (depending of the inducer) than cilostazol were needed to generate the same degree of platelet inhibition.

In *in vivo* studies, 10 subjects with cerebrovascular disease received 50 mg BID of cilostazol. Platelet function was tested after 4 weeks of treatment. Inducers of platelet aggregation included: ADP (1.5 uM); collagen (3 uM); epinephrine (2.5 ug/ml); and arachidonic acid (200 ug/ml) (See table 8.1 for results). The *in vivo* data suggests minimal inhibition of platelet aggregation.

In addition to platelet aggregation, beta-thromboglobulin, and platelet factor 4 were measured by RIA. Bleeding time was also measured. The results are shown in Table 8.2

[Comment:The *in vivo* data should be interpreted with some caution. The time of assessment relative to dosing was not stated. The concentrations of cilostazol at the time the blood was drawn for platelet aggregation studies was also not stated. Lastly, concurrent anti-platelet therapies may have impacted both on baseline and on-therapy measurements. ]

Table 8.2 Platelet Factor-4 and Bleeding Time.

| | Beta-thromboglobulin | Platelet Factor-4 | Bleeding Time |
|---|---|---|---|
| Before (Mean $\pm$ SD) | $87 \pm 58$ | $46 \pm 33$ | $135 \pm 29$ |
| After (Mean $\pm$ SD) | $50 \pm 25$ | $37 \pm 35$ | $177 \pm 39$ |

## Safety:

No listing of adverse events were supplied.
Vital signs were essentially unchanged.

There were no striking laboratory abnormalities.

Reference 10. Study G-9

<u>Title of Study:</u> Effect of Cilostazol (OPC-13013) on Arachidonic Acid Metabolism.
<u>Publication:</u> <u>Jpn Pharmacol There.</u> <u>14</u> (10); 6319-6324; (1986) .

<u>Investigator and Site:</u>    Nagakawa, Y.; Konuki, Y.; Orimo, H.; Harasawa, M.;
University of Tokyo.

No protocol, line listings or CRFs were supplied.

<u>Study Summary:</u> A total of six patients (four males and two females, mean age 64.7 years) with cerebrovascular disease were treated for two weeks with cilostazol at 50 mg BID. Platelet active drugs, such as aspirin or indomethacin were prohibited. Blood samples were collected at trough, both pre-treatment and after 2-weeks on therapy, from which platelet rich plasma was prepared.

Since the study intended to measure Thromboxane B2 (TxB2) generation, the concentration of the platelet inducer (ADP and collagen) was individualized for each patient and determined as follows: The minimum concentration of ADP which induced the secondary phase of platelet aggregation was employed for TxB2 generation studies. For collagen, the concentration required to reduce by more than 50% of the maximum optical density in collagen-induced platelet aggregation was employed for TxB2 generation studies. The average ADP concentration used was 4.6 ± 1.8 uM (mean ± SE). The average collagen concentration was 3.4 ± 1.5 ug/ml.

TxB2 was determined after the addition of 10 ug indomethacin and 5 mg dipotassium EDTA to blood collected in cooled citrated blood-sampling tube. TxB2 was measured by RIA after centrifugation of blood and ethyl acetate extraction of the supernatant.

TXB2 generation was determined from PRP, 10 minutes after the inducer (ADP or collagen) was added. The PRP was then centrifuged and extracted, as above with TXB2 determined by RIA.  PRP which was aggregated without including any inducers for the same duration of time and with the same extraction and assay procedures served as controls.

6-Keto-PGF1α and TxB2 (not generation of TxB2) were determined from whole blood that was collected, chilled and to which dipotassium EDTA and indomethacin were added. No inducer of platelet aggregation was added. The blood was centrifuged, TXB2 and 6-Keto-PGF1α were extracted and assayed by an RIA.

According to the sponsor TXB2 generation during ADP induced platelet aggregation dropped after cilostazol treatment, from 7.24 ± 2.20 pg/($10^7$ plt) at baseline to 3.45 ± 1.33 pg/($10^7$ plt). During collagen  induced platelet aggregation,

TXB2 decreased from $96.1 \pm 20.4$ to $62.1 \pm 22.4$ pg/($10^7$ plt).

ADP-induced platelet aggregation decrease from $55.2 \pm 4.6\%$ before, to $41.4 \pm 11.7\%$ after cilostazol treatment. For collagen induced aggregation the % aggregation decreased from $62.3 \pm 3.2$ % before, to $38.1 \pm 16.3\%$ after cilostazol treatment.

The plasma levels of TxB2 decreased from $147.8 \pm 29.4$ pg/ml before, to $102.6 \pm 14.5$ pg/ml and after cilostazol. The plasma concentration of 6-Keto-PGF1$\alpha$ increased from $26.8 \pm 3.8$ to $39.9 \pm 5.7$ pg/ml.

Safety: No safety discussion was presented.

Conclusion: The study is a baseline controlled study of patients with stable cerebrovascular disease who were treated with 2 weeks of cilostazol. There was an apparent decrease in plasma TxB2 and an increase in plasma 6-Keto-PGF1$\alpha$. During collagen or ADP induced aggregation, TxB2 production was apparently inhibited.

Reference 11. Study G-10
Title of Study: Antiaggregatory Effect of Cilostazol (OPC-13013) in Arteriosclerotic Disease.
Publication: Jpn Pharacol. Thera. 14 (93); 1531-1536, (1986).

Investigators and sites: Katsumura, T.; and Masaki, H.; Kawasaki Medical School

Study Summary: No protocol, or CRFs were supplied. Line listings were supplied for aggregation data, bleeding time data and vital signs.

A total of 13 patients were recruited. Eight of these patients had arteriosclerotic cerebrovascular disease and 5 had abdominal aortic aneurysms. Twelve were male and one was female. Subjects were treated with cilostazol for between two and six weeks with a dose of either 100 mg divided BID (n=4) or 200 mg divided BID (n=9). All those who received the 100 mg daily dose were treated for 6 weeks. Among those who received 200 mg divide BID, four were treated for 6 weeks, four were treated for 4 weeks and one for 2 weeks.

Platelet aggregation studies using platelet rich plasma were performed prior to treatment and at 2, 4 and 6 weeks after the start of treatment. The inducers of platelet aggregation (and their concentrations) were ADP (10 uM), collagen (200 ug/ml) and epinephrine (1 ug/ml ).

There was no appreciable effect on platelet aggregation induced by either ADP, epinephrine or collagen among the 4 patients who were treated with cilostazol at 100 mg divided BID. Among those who were treated with 200 mg divided BID of cilostazol, there was a decrease in the amount of aggregation at two weeks (before any patient seems to have been lost). Since atherosclerotic patients were the majority of those who received 200 mg/day cilostazol, the effects of drug on platelet aggregation in this subgroup is similar to the group as a whole (i. e. the abdominal aortic aneurism group did not sufficiently differ to change the conclusion). Bleeding time for the 200 mg dose was unchanged.

Table 11.1 Platelet Aggregation Among Those Who Received 200 mg Divided BID

|  | Predrug | 2 Weeks | 4 weeks | 6 weeks |
|---|---|---|---|---|
| ADP (10 uM) | 82 .2 ± 6.5 (n=9) | 62.0 ± 15.5 (n=9)* | 56.3 ± 12.4 (n=8)** | 46.5 ± 20.7 (n=4)* |
| Collagen (200 ug/ml) | 78.4 ± 8.8 (n=9) | 60.1 ± 10.0 (n=9)* | 53.1 ± 11.8 (n=8)** | 58.5 ± 11.4 (n=4)+ |
| Epinephrine (1 ug/ml) | 80.0 ± 7.4 (n=9) | 76.1 ± 10.7 (n=8) | 71.1 ± 5.1 (n=8)* | 68.3 ± 12.2 (n=4) |

+ P <0.1.        * p<0.05;        **p< 0.01

Safety: Vital signs at two weeks showed a 9.5 mm Hg drop in SBP (t-test p value < 0.05) an increase of 2 mm in DBP and an increase of 4.9 BPM (t-test p value < 0.05) in heart rate.

There was no discussion of discontinuations or dropouts.

<u>Conclusion</u>: This was a small study in patients with arterio-sclerotic cerebrovascular disease and abdominal aortic aneurysms who received 100 or 200 mg daily divided BID. Based on change from pretreatment value, only the 200 mg dose group had an effect on platelet aggregation.

Reference 12. Study G-11

<u>Title of Study</u>: Long-Term Effect of Cilostazol on Cerebral Blood Flow in Chronic
Cerebral Infarction.
<u>Investigators and Sites:</u> Kobayashi, S.; Yamaguchi, S.; Katsube, T.; Kitani, M.; Okada, K.;
and Tsunematsu, T.; Shimane Medical University ; Shimane, Japan.
<u>Publication:</u> <u>Arzneim-Forsch/Drug Res.</u> <u>35</u> (II); 1193-1197 (1985).

<u>Study Summary.</u> No protocol was supplied. A total of 14 patients with cerebrovascular
disease (6 patients with RIND and 8 with cerebral thrombosis) were studied both
before and after 100 mg BID cilostazol. Regional blood flow was determined by the
$^{133}$xenon inhalation method. Cerebral blood flow was measured for the hemisphere
effected by the stroke as well as the non-effected side. Twelve patients (two normals,
five with cerebral infarcts and five with neurological disorders) served as controls for
the reproducibility of cerebral blood flow measurements.

The sponsor claims that there was a mean increase in cerebral blood flow both
in the infarcted and non-infarcted sides by 10.8 and 13.4%, respectively. For six of those
with infarctions, the increase in blood flow exceeded the variability (2 standard
deviations) of the control group.

<u>Safety:</u> No safety information is included.

<u>Reference 13. Study G-12</u>

<u>Title of Study:</u> Effect of OPC-13013, An Anti-platelet Aggregating Drug, On Human – –
Cerebral Circulation-A Study in Chronic Ischemic Cerebrovascular Disorders
<u>Publication:</u> <u>Kekkan</u> (Blood Vessels) <u>9</u> (2); 89-94; (1986).

<u>Investigators and Sites:</u> Nimi, T.; Sawada, T. and Kuriyam, Y.; National
Cardiovascular Center; Osaka, Japan.

<u>Study Summary:</u> No protocol or CRFs were supplied. A total of 12 patients (7 males
and 5 females; mean age 64.8 years) with cerebrovascular disease (1 patient with TIAs
and 11 with cerebral infarctions, occurring > 1 month before study entry) were
enrolled. The subjects were treated with 200 mg (100 mg BID) or 300 mg (100 mg TID)
daily for 2 weeks. Cerebral blood flow was measured by the argon gas inhalation
method. Cerebral blood flow was determined by argon clearance, comparing the argon
concentrations in the cerebral artery to that in the cerebral vein.

In addition to cerebral blood flow, the following parameters were also measured
at baseline and after 2-weeks of treatment.
- regulation of cerebral circulation,
- autoregulation (change in cerebral blood flow when arterial pressure was
decreased by increase in tilt angle) and
- chemical auto-regulation (response to cerebral 5% $CO_2$ for 3 minutes).

According to the sponsor, cerebral blood flow increased from the pre-dose level
of $46.4 \pm 10.2$ to $51.1 \pm 9.0$ ml/100 g of brain/min (mean $\pm$SD). Among those treated
with 200 mg of cilostazol, the increase in cerebral blood flow was 10.9% (from $49.4 \pm$
$10.0$ to $58.8 \pm 9.2$ ml/100 g of brain/min. For the 300 mg dose, the increase was 13.2%
from $43.4 \pm 10.2$ to $48.3 \pm 8.7$ ml/100 g of brain/min.

There was a concomitant 4.9 mm Hg drop in mean arterial pressure, with no
change in the 200 mg dose group and a drop of 11.8 mm Hg for the 300 mg dose.

There was a slight (7.7%), but not statistically significant increase in cerebral
oxygen consumption (from $3.1 \pm 0.6$ to $3.34 \pm 0.44$ ml/100 g of brain/min).

Cerebrovascular resistance for the group as a whole decreased from $2.82 \pm 0.92$ to
$12.39 \pm 0.69$ mm Hg/ml/100 g of brain/min. Neither the 200 mg or 300 mg dose groups
differed from before to after cilostazol treatment.

Plasma concentration of the drug was $1316 \pm 757$ for the 200 mg dose and $1532 \pm$
$302$ for the 300 mg dose (not dose proportional). There did not appear to be a  · ·
correlation between drug concentration and changes in cerebral hemodynamics.

With respect to autoregulation, upon tilting the "effective" blood pressure decreased 11.8 mm Hg but there did not appear to be any effect in cerebral blood flow (what is "effective" blood pressure?) at baseline. During treatment despite equivalent blood pressure decreases (approximately 12 %) there was only a 2% drop in cerebral blood flow.

With respect to chemical sensitivity, the slope of the change in cerebral blood flow per unit of $CO_2$ partial pressure increased 26%, but this did not quite attain statistical significance.

Safety: No discussion of safety is presented.

Conclusion: This was an open label, baseline controlled study. No strong conclusions can be derived from this study.

Reference 14

Title of Study: Hemodynamic Effects of the Antithrombotic Drug Cilostazol in Chronic Arterial Occlusion of the Extremities.
Publication: Arzneim-Forsch/Drug Research 35 (II); 1201-1203 (1985).

Investigator and Site:        Kamiya, T.; and Sakaguchi, S.
                              Hammatsu University, Hamamatsu, Japan.

Study Summary: No protocol, CRF or Line listings are supplied.

There were a total of nine male patients with peripheral vascular disease who were enrolled. Seven of these patient had arteriosclerotic obliterans and two had thromboangiitis obliterans. Three of those with arteriosclerotic obliterans had both limbs compromised, one of the two with thromboangiitis obliterans had both limbs effected. The sites of occlusion were: between the abdominal aorta and iliac artery (4 patients, 6 limbs); the superficial femoral artery (4 patients, 5 limbs); and the popliteal artery (1 patient, 2 limbs).

Patients were treated with 150 mg (50 mg TID) for 2 weeks, with measurements of the distal extremity, ankle blood flow (determined by strain-gauge plethysmography, the flow was measured by the venous occlusion method at a cuff pressure of 30-50 mm Hg after reactive hyperemia) and ankle pressure index (doppler ultrasound flowmeter divided by arm pressure) done 3 hours after dose.

In the 13 abnormal limbs there was an increase of ankle blood flow from $3.15 \pm 0.32$ to $3.66 \pm 0.41$ ml/min/100 g tissue (an increase in 16% $p<0.05$). In the four non-affected limbs the increase was 13%. There was a 2.5% increase (p=NS) in the ankle pressure index.

Safety. The paper notes no reports of adverse events.

Conclusion: This was a small open-labeled, baseline controlled study. Peripheral blood flow was increased above baseline.

Study # 15.

Title of Study: Hemodynamic Effect of Cilostazol on Increasing Peripheral Blood Flow in Arteriosclerosis Obliterans
Publication: Arzneim-Forsch/Drug Res. 35 (9 II); 1198-1200 (1985).

Investigator and Sites: Yasuda, K.; Sakuma, M. and Tanube, T.; No study site is mentioned.

Study Summary: The only submitted information was a one-page study synopsis.

A total of four male patients with atherosclerotic heart disease were enrolled and treated for two weeks with 200 mg/day (divided BID) of cilostazol. The measurements reported included, systolic ankle pressure (which increased above baseline 9.1%); mean flow velocity (which increased 36.6%) and peak flow velocity in the femoral artery (which increased 21.7%) and the segmental blood flow in the leg (which increased 19.9%).

No adverse events were reported.

Conclusion:There was insufficient information to assess this study.


Reference # 16 Study (G-15)

Title of Study: Thermographic Evaluation of the Hemodynamic Effect of the Antithrombotic Drug Cilostazol in Peripheral Arterial Occlusion (G-15).
Publication: Arzneim-Forsch/Drug Res. 35 (II), 1203-1208 (1985).

Investigator and Sites:    Ohashi, S.; Iwatani, M.; Hyakuna, Y.; and Moriaki, Y.; Tokyo, Japan.

Study Summary: No protocol, CRFs, or line listings were supplied. A total of 10 patients with peripheral artery disease (seven with thromboangiitis obliterans and three with arteriosclerosis obliterans) were treated for six weeks with either 100 mg/day (2 patients) and 200 mg/day (7 patients). One patient was treated with 100 mg for only 4 weeks.

Skin temperature and skin blood flow were measured by thermography before and after 2, 3, 4, and 6 weeks of treatment. The sponsor claims an increase of 0.7 degrees centigrade for legs (from $31.0 \pm 0.2$ to $31.7 \pm 0.2$ °C and 1.2 degrees for feet (from $29.6 \pm 0.5$ to $30.8 \pm 0.6$ °C) only at the 6-week time point (despite the fact that one patient only received treatment for 4 weeks!!)

<u>Safety:</u> Adverse events were seen in 4 of 14 patients taking 200 mg/day. Three of these were headaches and one was abdominal distention.

<u>Conclusion.</u> This was a baseline controlled, open label study that did not appear to have a underlying protocol. Patients were treated for different lengths of time and it was unclear if a specific date for final measurements was prespecified. No credible conclusions can be drawn from this study.

<u>Reference # 17 Protocol PUS94001</u>

<u>Title of Study:</u> Study on the Relationship between Plasma Cilostazol Concentration and the Concomitant Symptoms (Headache and Dull Headache).

<u>Study Summary.</u> Investigator was Kanamura, M. Only a study synopsis was supplied. This was a randomized placebo-controlled crossover study involving 16 normal subjects. The treatment regimens were 50, 75 and 100 mg of cilostazol or placebo. Each dose was given once, with a one-week washout period between dosing.

The results of the study both pharmacokinetic and headache are tabulated below:

Table 17.1 Pharmacokinetic and Symp[toms of Cilostazol

|  | Placebo | 50 mg cilostazol | 75 mg cilostazol | 100 mg Cilostazol |
|---|---|---|---|---|
| AUC 0-24 ng.hr/ml | NA | 4862 ± 1698 | 6142 ± 2065 | 7074 ± 2243 |
| Tmax (hr) | NA | 4 ± 2 | 4 ± 1 | 4 ± 1 |
| Cmax (ng/ml) | NA | 512 ± 166 | 643 ± 200 | 743 ± 214 |
| Headache | 1/16 | 5/16 | 9/16 | 6/16 |

Pharmacokinetics showed extensive variability. It is unclear if the apparent deviation from linearity (particularly for Cmax) is real or due to the excessive variability. Headache is greater with treatment but whether there is a dose response relationship to headache among the active doses is unclear.

<u>Reference 18.</u>

<u>Title of Study:</u> Bronchodilator and Bronchoprotective Effects of Cilostazol in Humans *in vivo*.

Publication: <u>Am J. Respir Crit. Care Med; 151;</u> 222-225, 1995.Fujimura, M.; Kamio, Y.; Saito, M.; Hashimoto, T., and Matsuda, T.;  Kanzawa University; Kanzawa, Japan.

<u>Study Summary.</u> Only the publication was submitted. A total of eight subjects (all female) were randomized to receive single doses of cilostazol (200 mg) or placebo, followed after three hours with a methacholine challenge. After a 5 day washout period the subjects received the alternate treatment.

The methacholine challenge consisted of sequentially doubled doses of methacholine, administered by nebulization. Two ml of methacholine solution were administered at each dose. The initial concentration of methacholine 0.04 mg/ml was doubled sequentially to a maximal concentration of 160 mg/ml. The dose of methacholine which caused a decrease FEV1 by 20% was shifted to higher methacholine concentrations among those on cilostazol when compared to placebo
    The sponsor also claims that the maximum expiratory flow rate at isovolume of 40% FVC rate above residual volume was greater on cilostazol than placebo.

Reference 19

Title of Study: Clinical Evaluation on Combined Administration of Oral Prostacyclin Analogue Beraprost and Phosphodiesterase Inhibitor Cilostazol.
Publication: Pharmaceutical Res. 31 (2); 121-125; (1995).
Investigators and Sites:   Fujitani, K.; Kambayashi, J.; Murata, K.; Yano, Y.; Shinozaki, K.; Yukawa, M.; Sakon, M.; Murata, T.; Kawasaki, T.; Shiba, E.; Mori, T.
Osaka University Medical School, Osaka, Japan

Study Summary: Only the publication was supplied. This was study looking at the interaction of Cilostazol (at a dose of 200 mg/day) in combination with Beraprost (BPT, a prostacyclin analogue) in 12 healthy individuals. The timing of the dosing and observations is shown below.

Table 19.1 Schedule of Doses

| Day | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | |
| 40 ug BPT | | | | | | 120 ug/day p.o BPT | | | | | | | | |
| | | 200 mg/day Cilostazol | | | | | | | | | | | | |
| **Assessment Chronology** | | | | | | | | | | | | | | |
| A    B | | | | | | C    D | | | | | | | E | |

A single dose of BPT is given on day 1 with assessments before = (A)   and 1 hour after =(B) the dose of BPT
Cilostazol is started on Day 3 and continued through day 14. BPT is again restarted at a dose now of 120 ug/day PO on day 7. Analysis is done on Cilostazol and before BPT on day 7 = C   and 1 hour after BPT is restarted = D and Day 14= E.

    The sponsor notes no change in vital signs during the five (A-E) observation periods. Platelet aggregation studies were performed with platelet rich plasma samples. Aggregation of platelets was induced by ADP (2 and 5 uM) or collagen (0.5, 1.0 and 2.0 ug/ml) . None of the aggregation studies was affected during the cilostazol alone treatment C (Table 19.2).