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APPENDIX 51

Introduction:

The following section contains summaries of 43 items. Of these, 41 are independent studies or publications, one item was found empty and one item was a duplicate of an already included study.

None of these studies included a protocol. Consequently, none of the data which were recorded were pre-specified as the primary measurement of the study. Most studies were open-labeled and/or baseline controlled. Few studies included sufficient information to independently verify the results contained within the publication or study report.

I counted a total of 450 patients who are described by the 41 studies (see Appendix A). The populations included within the data base are primarily subjects with cerebrovascular disease. There were, however, a substantial number of patients with peripheral vascular disease, diabetics and normals. The duration of exposure was generally short but there are a few patients who were exposed for 6-8 months. The largest studies, however, include data for only two to four weeks of cilostazol treatment. The maximum exposure in this data base was 300 mg/day divided TID. These doses are at the upper limit of the daily dose proposed for use in intermittent claudication.

Most studies explored the effect of cilostazol (or OPC-13013) on platelet aggregation, thrombosis, other clotting parameters and/or endothelial damage. Many of the studies limited the measurements to the *in vitro* effects of cilostazol when platelet aggregation was induced by either ADP, epinephrine, collagen or arachidonic acid. Several studies looked at similar studies on platelets derived from patients or subjects taking oral cilostazol.

Other studies looked at skin temperature changes in patients with vascular disease as a surrogate measurement of increased blood flow. These studies were small and baseline controlled and, in general, is not particularly convincing or useful in establishing a benefit of cilostazol in peripheral vascular disease.

None of the studies were large enough or proposed to measure clinically meaningful outcome parameters which reflect peripheral vascular disease.

A minority of the studies describe the outcome of patients treated with cilostazol. Descriptions of the safety profile of cilostazol are infrequent and may reflect only the studies with the best safety outcomes. When the safety information in a particular study is not included, it is unclear if there were no adverse events or if such events were ignored, if additional patients were recruited to substite for those who discontinued. The largest of the studies and the studies of longer duration are not terribly useful in establishing safety.

It is hard to tease out any data with respect to the effect of cilostazol on vital signs

(heart rate or blood pressure), or to its effect on ECGs.

In summary, this reviewer does not consider these studies particularly useful in defining the efficacy or safety of cilostazol.

Reference #1. Report No. 2567.

Title of Study: Tolerance and Pharmacodynamics of OPC-13013 in Healthy Human Volunteers.

Investigators and Site: A. P. McCraw; P. Hudson and A.D. Sim
Edenhall Hospital and Health Centre Port Seton
Musselburgh, Scotland

Protocol, Dates of Study, Line listings, CRFs and Formulations. No protocol is supplied. The study was carried out between 3 October 1982 and 16 November 1982. Line listings are available but only for selected information. No completed CRFs are presented. The formulation of OPC-13013 was not stated.

Study Summary: The primary end-points for this study was the effect of OPC-13013 on measurements of platelet aggregation. Prior to the study, the minimum concentration that would induce secondary or irreversible platelet aggregation for each inducing agent was determined. Subjects were to refrain from all medication for 14 days before enrolling.

There were 4 phases to the study. Each phase consisted of a single dose of OPC-13013. During phase 1, each subject received either placebo or 50 mg OPC-13013. During phase 2, they received either 50 or 150 mg of OPC-13013 or placebo. During phase 3, they received either placebo or 150 mg OPC-13013. During phase 4, they received either placebo or 300 mg of OPC-13013. There were approximately 2 weeks between each of the phases.

Table 1.1. Dosing During Each Phase.

Patient #	Phase 1	Phase 2	Phase 3	Phase 4
1	Placebo	50 mg	150 mg	300 mg
2	50 mg	Placebo	150 mg	300 mg
3	50 mg	150 mg	300 mg	Placebo
4	50 mg	150 mg	Placebo	300 mg
5	50 mg	Placebo	150 mg	300 mg
6	Placebo	50 mg	150 mg	300 mg

The procedures which were repeated during each phase are shown in Table 1.2.

Table 1.2. Procedures During Study

Procedure	Dose	UA/Hematology/ Clin Chemistry/ Medical Exam only phase 1 and 3	PT/ APTT	Vital Signs	ECG	Platelet Aggregation*	Platelet Adhe-siveness	Clot Retraction	Bleeding Time	Plasma Fibrinogen
pre-Trial		√				√				
0 Hour	√		√	√	√	√	√	√	√	√

1/2 Hour				√	√					
1 Hour				√	√	√				
3 Hours				√	√	√	√	√	√	
6 Hours				√	√	√				
12 Hours		—		√	√	√				
24 Hours			√	√	√	√	√	√	√	√
48 Hours				√	√	√				

* ADP, epinephrine, collagen and arachidonic acid . The concentrations used were : the critical concentration (defined pre-trial), 2 X the critical concentration and the standard concentration

According to the sponsor, platelet aggregation was only partially inhibited by OPC-13013, when either ADP or arachidonic acid was the inducer. There was no inhibition to aggregation when the inducer was epinephrine or collagen.

There was no prospective statistical plan to analyze the data presented in the report. Given the large number of end points, the number of subjects with missing data, the number of time points for each measurement and particularly for platelet aggregation, the number of stimuli and the number of doses of each stimuli, none of the results were particularly impressive.

Safety: There were no deaths or discontinuations.

There were four adverse events at 50 mg (two patients with headache one with feeling hot/faint and one with nausea), two at 150 mg (one patient with headache and one with lightheaded) and three patients with headache at 300 mg.

Vital signs were not recorded.

The sponsor claims that there were no differences in ECGs during the four phases. Data was shown for only one subject but the timing of several of the subject's ECGs is unclear. This subject had atrial ectopy at baseline and throughout the study.

Several subjects had hypernatremia (> 145 mM). Baseline for these patients was 135-145 mM.

Reference 2: Report # 10524

Title of Study: Comparative Study of Antiplatelet Drugs: Effects of platelet Activation (Ca^{++} Elevation, Aggregation, Release Reaction and TXA_2 Synthesis).

Investigator: Yūtaka Matsumoto
Department of Pharmacology
Tokushima Research Institute

No protocol, line listings or CRFs were supplied.

Study Summary. This was an *in vitro* study to define the dose response relationships of cilostazol on platelet function (platelet rich plasma) in response to a weak aggregating stimuli (ADP, 4 or 8 uM) and to a strong aggregating stimuli (thrombin, 0.05 U/ml). The study also compared the effect of cilostazol (1 to 100 uM) to PGE_1 (1 to 100 uM), aspirin (1 mm) and the Fab fragment c7E3 (Reopro®) on platelet function. The parameters measured in these figures are platelet aggregation, release of platelet factor 4 (PF4), the synthesis of TXB_2 and Calcium elevation. Platelets collected from five individuals were used.

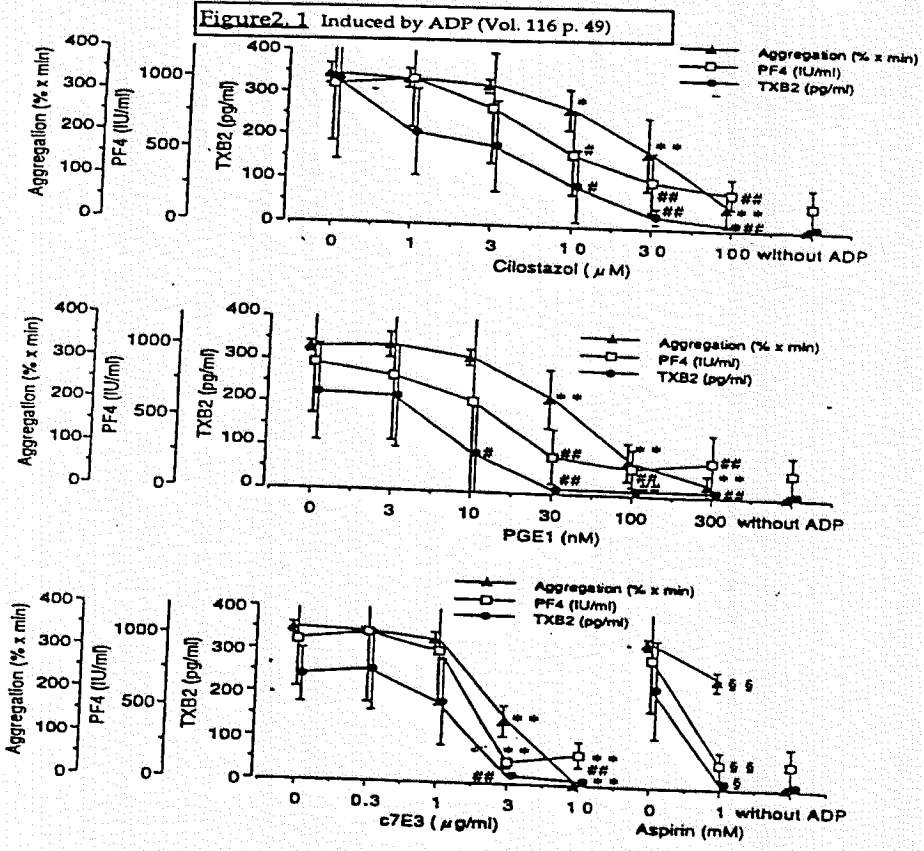


Fig.1 Effects of cilostazol, PGE_1, c7E3 and aspirin on aggregation, release reaction (PF4) and TXA_2 synthesis (TXB_2) in human PRP activated by ADP

Mean±SD (n=5)
: Significantly different from control (0) (closed testing procedure t-test)
* : Significantly different from control (0) (Dunnett test)
§ : Significantly different from control (0) (t-test)
#, *, § : p<0.05, ##, **, §§ : p<0.01

I have recreated the sponsor's display of the data. Figure 1 represents the effect of a the drugs after activation by ADP. Figure 2 after activation by thrombin. The data

Figure 2.2 Induced by Thrombin (Vol. 116 p. 50)

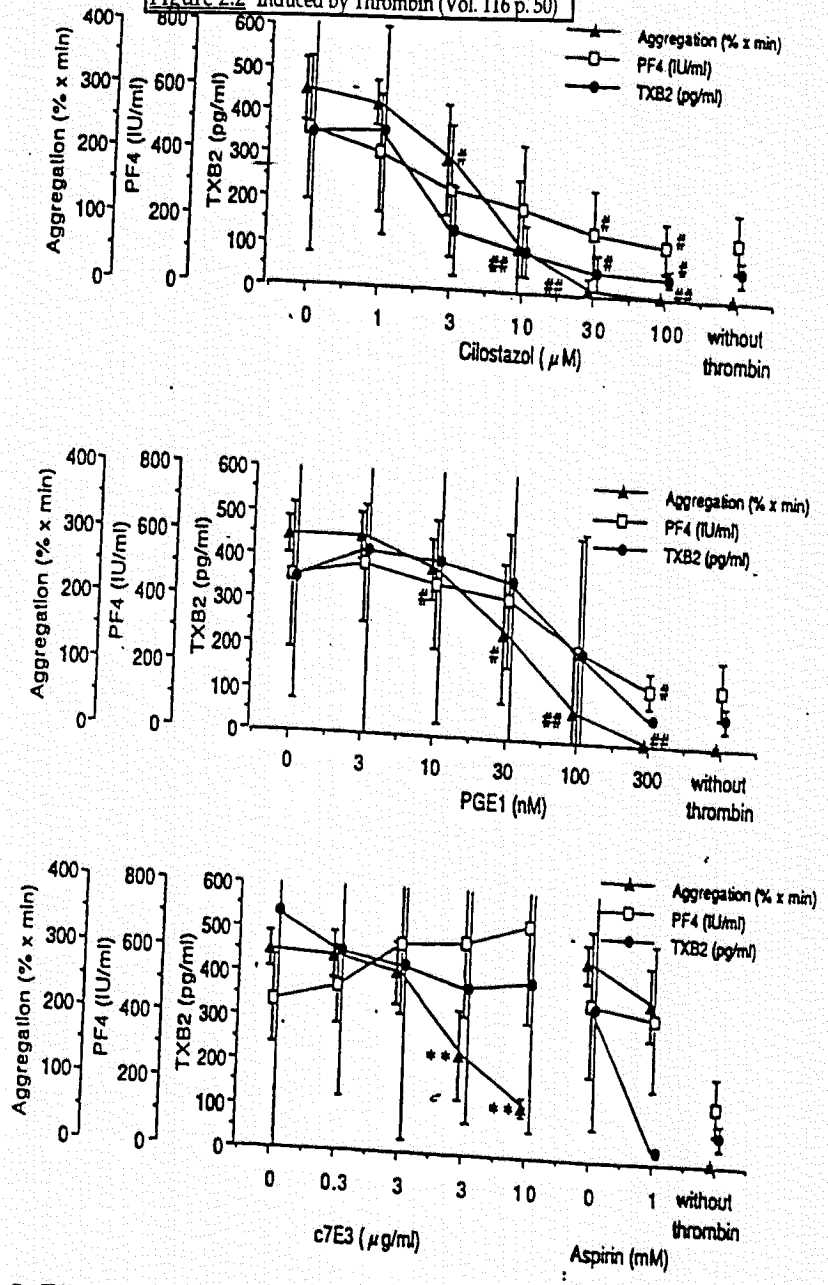


Fig.2 Effects of cilostazol, PGE_1, c7E3 and aspirin on aggregation, release reaction (PF4) and TXA_2 synthesis (TXB_2) in human washed platelets activated by thrombin

Mean±SD (n=5 : Aggregation, PF4, n=4 : TXB2)
#: Significantly different from control (0) (closed testing procedure t-test)
*: Significantly different from control (0) (Dunnett test)
#, * : p<0.05, ##, ** : p<0.01

was analyzed first by linear regression, and if positive, the individual concentrations were compared to placebo by a t-test. If there was no linear trend two-point comparisons by Cunnett's method was performed (sponsor's analysis).

The effect of cilostazol appears similar when the aggregating inducer is either ADP, a weak platelet activator or thrombin, a strong platelet activator. There is a statistically significant drop in % AUC of aggregation aggregation, PF4 release and TXB_2 at concentrations of cilostazol between approximately 3-30 uM.

The profile for PGE1 is similar to that of cilostazol with ADP stimulation (the weak inducing stimulus). At concentrations between 10-30 nM there was significant inhibition of aggregation, PF4 release and TXB2. When thrombin (the strong stimulus) is the inducer, concentrations between 10-30 uM also yield significant inhibition of platelet aggregation.

With respect to

inhibition of platelet function by c7E3, after ADP stimulation, there was substantial inhibition of platelet aggregation, PF4 release and TXB2 production. With thrombin as the stimulus, only platelet aggregation was inhibited (at a concentration of 3 ug/ml). Neither PF4 release or TXB2 production was inhibited at concentrations up to 10 ug/ml.

After washed platelets are exposed to thrombin, calcium levels increase (data not shown). This increase was inhibited by cilostazol, with statistical differences evident at concentrations ≥10 uM. The maximum inhibition leveled off at approximately 60%. For PGE1, inhibition of calcium increase was noted at concentrations > 10 nM with no obvious leveling off of effect at increasing concentrations of PGE1.

Neither Reopro ® nor aspirin effected calcium concentrations.

Conclusions: This study suggests that cilostazol at concentrations of > 3 uM modifies platelet function. The modification does not attain substantial effect until 10 uM. These concentration appears to be somewhat greater than those generated in *vivo* with cilostazol at doses of 100 mg/day. Moreover, the in *vivo* generated concentrations are highly protein bound so that the "free" concentrations of cilostazol reached *in vivo* are even lower.

PGE1 differs from cilostazol in that it more completely inhibits calcium elevations from washed platelet preparations.

Reference # 3. Study G2.

Title of Study: Phase I Study of a New Synthetic Platelet Suppressant OPC-13013 (6-[4-(1-Cycloheyl-1H-Tetrazole-5-yl) Butoxy]-3,4-Dihydro-2(1H)-Quinolinone).

Investigator and Sites: Abe, T.; Kazaka, M.; Kinoshita, T.; Naito, I.; Nakamura, K.; Matsuda J.; Kawasugi K.; and Yoshimura Y.
Teikyo University School of Medicine

Formulation: Lot #1F96-50

Protocol, CRFs and Line Listings. No protocol was supplied. Line listings, however, were available. No CRFs were supplied.

Study Summary. Normal male subjects were recruited from the sponsor's work-force. The study was carried out in several stages. The first stage was single dose exposure to cilostazol. There were three separate groups exposed to 50, 100 and 150 mg, respectively.

The second stage of the study was a four day exposure to cilostazol. There were five individual groups exposed to doses of: 50 mg QD, 100 mg QD, 50 mg BID, 100 mg BID and 100 mg TID. With the exception of the last group, there were 4 subjects/group; the last group had 3 subjects/group. There appeared to be 5 subjects among those who received multiple cilostazol doses whose initials, weight and age were the same as those who received single doses.

The listing of procedures during the single dose phase is shown as Table 3.1 and those for the multiple dose studies are shown as Table 3.2.

Table 3.1 Single Dose Phase: Listing of Procedures

Time post dose	pre	0	3 hr	6 hr	8 hr	12 hr	24 hr	1 week	1 month
Dose		√							
Subjective and Objective Symptoms, Vital Signs	√		√	√	√	√	√	√	√
Hematology, Chemistry, Urinanalysis, Stool Exam, Chest X-ray, Fundoscopy, ECG	√						√		
Plasma Concentration	√		√	√	√	√	√		
Bleeding Time, Clot Retraction Time,	√		√				√		
Platelet aggregation	√		√	√	√	√	√		
Platelet Adhesiveness	√		√	√			√		
Beta-thromboglobulin	√		√	√			√		

Table 3.2 Multiple Dose Phase: Listing of Procedures

	Days 1-4						Day 5		
Time post dose	pre	0	3	6	8	12		1 week	1 month
Time of Day	8	9	12	15	17	21	8		

Dose		[redacted]							
Subjective Symptoms,	√	[redacted]	√	√	√	√	√	√	√
Objective Symptoms Vital Signs	√	[redacted]	√		√*		√		
Hematology, Chemistry, Urinanalysis,	√**	[redacted]					√	√	√
ECG	√	[redacted]	√***	√****					
Fundoscopy, CXray	√**	[redacted]					√		
		[redacted]							
Stool Exam	√**	[redacted]					√		
Plasma Concentration	√	[redacted]	√***	√****			√		
Clot Retraction Time***	√**	[redacted]	√	√	√****				
Bleeding Time *****	√**	[redacted]	√***				√		
Platelet aggregation	√	[redacted]	√***	√****			√		
Platelet Adhesiveness	√**	[redacted]	√***	√‡			√		
Beta-thromboglobulin	√	[redacted]	√	√			√		

* only for 100 mg BID group. ** day 1 only *** not examined in M-6 **** Only in M6
but there is no M-6 group??? *****Not examined on Day 3 ‡Day 4 only fir M-6 group only

Platelet aggregation Platelet rich plasma (PRP) was prepared at the specified time. Four inducers of platelet aggregation were separately used as stimuli for aggregation: 1) ADP, 2) collagen, 3) epinephrine and 4) arachidonic acid. The concentration of the inducers was determined for each individual. The lowest concentration of ADP (1, 2, 4 and 8 uM); collagen (1, 2, 4, and 8 ug/ml); epinephrine (0.0625, 0.125, 0.25, 0.5, 1 ug/ml) and arachidonic acid (100 and 200 ug/ml) that irreversibly induced platelet aggregation was used for the platelet studies.

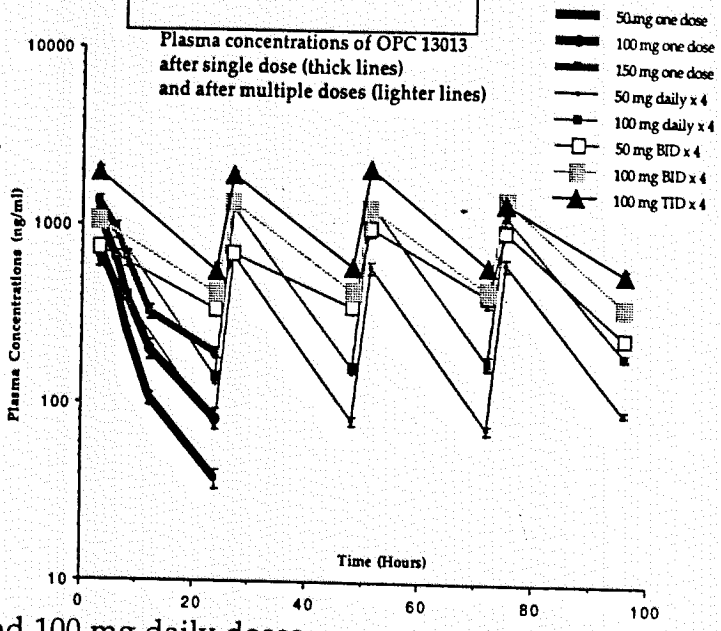
Maximum aggregation rate was defined the highest level of aggregation within 5 minutes of adding the test inducer of platelet aggregation. (Note the curves look like there is some reversibility of aggregation). Also measured was AUC of aggregation.

Other platelet function tests included: platelet adhesiveness, bleeding time, clot retraction time and β–TG, these were measured by standard methodologies.

Results:

Pharmacokinetics: The concentration of OPC-10313 were measured at 3, 6, 8, 12 and 24 hours, after single doses and at 3 and 24 hours daily for those who were treated for 4 days. The maximum concentration was observed at the first sampling time and suggests that peak cilostazol was missed. The concentrations for the multiple day samplings are also shown. For the multiple dose regimens, the trough concentration of 100 mg TID are equivalent to the 3 hour (peak?) concentration of the 50 mg single dose. There did not appear to be significant accumulation of cilostazol over time.

Figure 3.1 Pharmacokinetics of OPC13013



Platelet aggregation: The effect of the various stimuli on platelet aggregation both for the single, aeute dose and during the 4 day course of cilostazol treatment are shown in the Figures 3.2-3.9. The data do not appear to be easily interpreted. Invariably the effects are not entirely consistent when single versus are compared to multiple doses. The inhibitory efffect on platelet aggregation is much greater after the single doses. Furthermore, neither a dose response relationship nor an effect on platelet aggregation at the interdosing interval is convincingly demonstrated. There was less inhibition of platelet aggregation for the BID than the QD dose for both the 50 and 100 mg daily doses.

When ADP is used as the platelet aggregation stimulus, after a single dose of

Figure 3.2 Single dose ADP induced platelet Aggregation

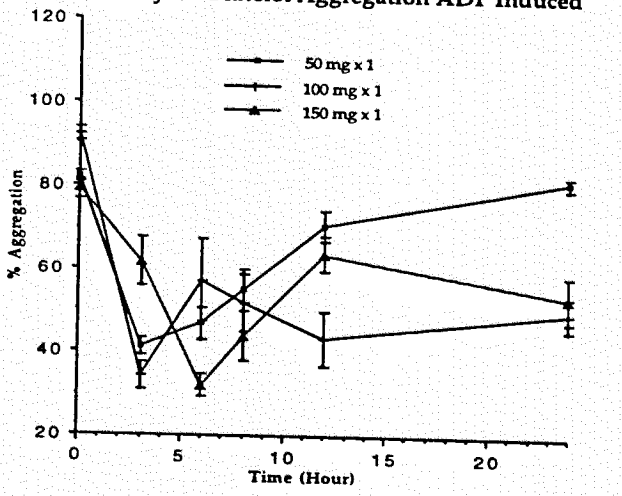
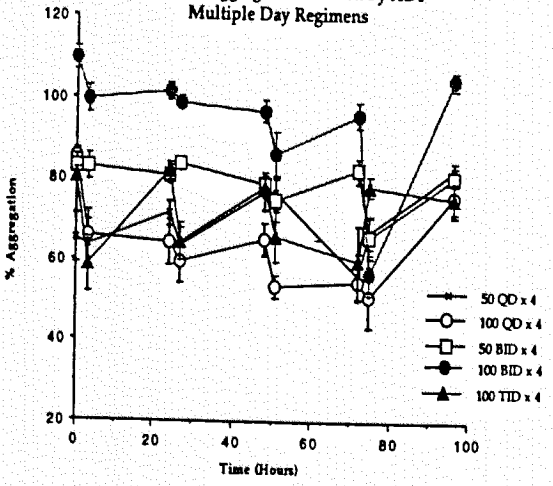


Figure 3.3 Multiple Dose Platelet Aggregation



cilostazol, there is a rapid inhibition of aggregation. The maximum effect is seen at 3-6 hours. During the 4 day course of cilostazol, however, if there is an effect in preventing aggregation it is quite small. The effect at 24, 48, 72 and 96 hours (i.e

Figure 3.4

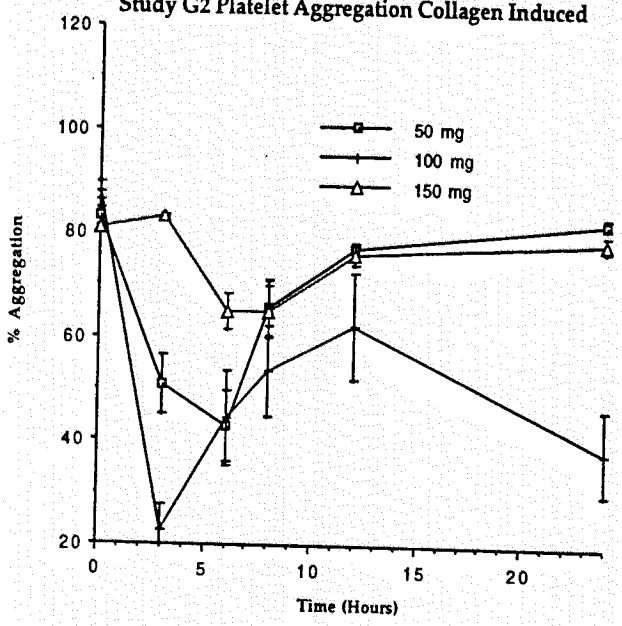
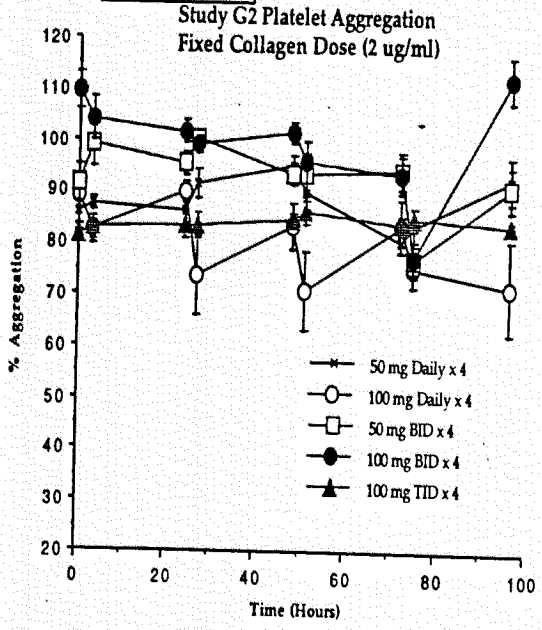


Figure 3.5



trough effects), particularly for the multiple daily dose regimen shows minimal platelet inhibition. The pattern appears to be the same for aggregation when collagen is used as the stimuli.

When epinephrine is used as the stimuli, the results during the single dose