DISSOLUTION:

A. DISSOLUTION TESTING METHOD DEVELOPMENT:

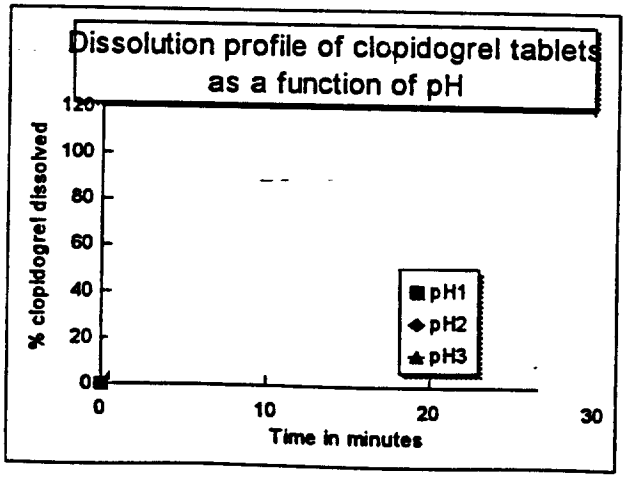
The sponsor provided solubility data for the drug substance at various pH values (details shown in the following table). The solubility of clopidogrel bisulfate significantly decreases above pH

pH	Medium	Solubility (expressed as mg base per mL)
1		
2		
3		
2.6		
3		
4		
6		
8		

* determined at 37°C, while at other pH values, solubility was determined at 25±1°C.

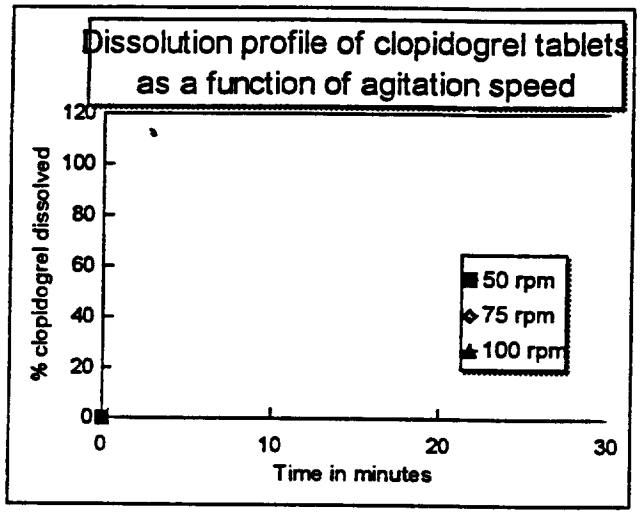
During the development of dissolution method for clopidogrel tablets, the sponsor investigated the effect of dissolution medium and agitation speed on dissolution rate of clopidogrel. USP apparatus II (paddle) was used at agitation speeds of 50, 75 and 100 rpm. Dissolution media with pH values of 1, 2 and 3 were also tested. Using the above conditions, dissolution samples were collected at different times up to 1 hour and analyzed. The dissolution samples were assayed for clopidogrel by

The graph provided below shows the dissolution profiles (tested using apparatus II at rpm) for clopidogrel tablets as a function of pH (1 to 3).

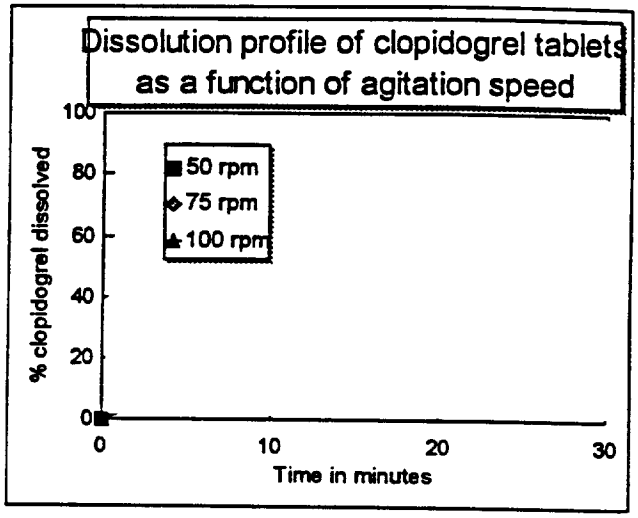


Figures below show the dissolution profiles of clopidogrel tablets using USP apparatus II (paddle) at different agitation speeds.

At Release



After 9 months of storage at 40°C/75% RH



CONCLUSION: Results indicate that the solubility of clopidogrel sharply declines above pH resulting in non-sink conditions for dissolution if a dissolution medium of pH greater thar was selected. Dissolution of clopidogrel tablets increases with agitation speed. **The final testing conditions selected by the sponsor are: USP paddle apparatus, 75 rpm, pH 2 dissolution medium at 37°C.** This method appears to be satisfactory. However, the agitation speed should be reduced to 50 rpm, since this condition will be more discriminatory and can potentially detect batches that may not have optimal performance.

FDA therefore recommends the following dissolution testing conditions for clopidogrel 75 mg tablets: **USP paddle apparatus, 50 rpm, pH 2 dissolution medium (containing KCl and 0.1N HCL) at 37°C.**

B. WAIVER FOR A BIOEQUIVALENCE STUDY BETWEEN THE 2Q2 TABLET (CLINICAL FORMULATION) AND THE 2B7 (TO-BE MARKETED FORMULATION):

2Q2 tablet formulation was used in the pivotal CAPRIE clinical trial. The to-be marketed formulation is 2AB7 tablet which only differs slightly from the 2Q2 formulation. The 2AB7 tablet has not been tested in humans. The differences in formulation between the 2Q2 and 2AB7 tablet are as follows: 1. 2AB7 contains lactose (for 2Q2) and includes a new colorant, Ferric oxide, NF (red) which gives pink color to the tablet. The formulation otherwise remains the same as 2Q2 tablet. Film coating of 2Q2 tablet utilized and purified water as solvent for coating, which gets removed in the final processing. The only

difference in film coating is that the to-be marketed formulation eliminates the use of as solvent for coating. Since these are minor changes, the sponsor provided dissolution data as justification for biowaiver. Dissolution data was provided on 6 tablets each for 2AB7 tablet and for two batches of 2Q2 clinical formulation. This data was generated with dissolution testing using USP apparatus 2, KCl/HCl medium pH 2.0 buffer, 1000 mL at 75 rpm.

The mean (% CV) for the 3 batches are shown in the following table:

Time in minutes	Mean % (% CV) clopidogrel dissolved		
	2AB7	2Q2 (batch 1)	2Q2 (batch 2)
5	30.4 (27.8)	35.1 (22.26)	25.6 (18.1)
10	74.1 (8.4)	75.9 (7.2)	78.5 (8.8)
15	95.1 (3.6)	93.9 (1.6)	96.2 (3.3)
20	98.9 (2.2)	96.9 (1.4)	99.2 (1.1)
25	98.9 (2.1)	97.2 (1.4)	100.3 (0.8)
30	98.9 (2.2)	97.4 (1.4)	100.1 (0.8)

The f_2 values for comparison of the dissolution profiles of 2AB7 tablet to 2Q2 (batch 1) tablet and 2Q2 (batch 2) tablet are 78.896 and 76.326 respectively.

Although this appears to be acceptable, the reviewer requested the sponsor to provide comparative dissolution data generated at similar dissolution testing conditions except the agitation speed which is changed to 50 rpm since this is more discriminatory. Data was obtained for 12 units each of 2AB7 tablet and 2Q2 tablet formulation. This data is provided in the following tables and figure. The corresponding f_2 value for comparison of the 2 dissolution profiles is 93.108.

Batch 0001 Commercial Formula 2AB7
Manufacturing date: 02/97

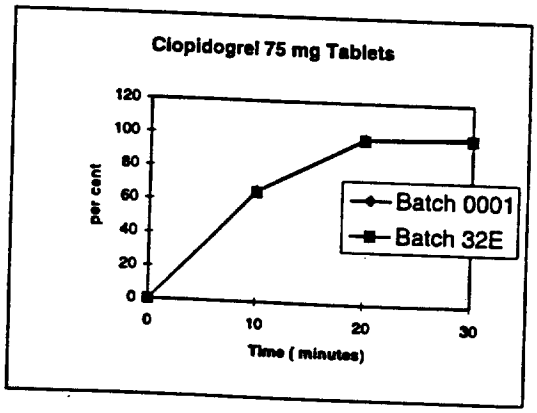
Dissolution at 50 rpm
% dissolved

Comp.	10min	20min	30min
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
Avg.	65.9	98.6	100.3

Batch 32E = Batch L146 G CAPRIE Formula 2Q2
Manufacturing date: 07/94

Dissolution at 50 rpm
% dissolved

Comp.	10min	20min	30min
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
Avg.	65.0	97.8	99.2



CONCLUSION: The dissolution profiles for the to-be marketed formulation are comparable to the clinical trial formulation. Hence, a biowaiver can be granted.

C. DISSOLUTION SPECIFICATIONS:

SPONSOR SELECTED DISSOLUTION SPECIFICATIONS:
The sponsor selected dissolution conditions and specification for clopidogrel 75 mg tablets are as follows:

USP APPARATUS 2 (PADDLE), 75 RPM, 1000 ML, 37°C, pH 2.0 BUFFER (KCl/HCl)
SPECIFICATION: Q = 'N 30 MINUTES

Using these conditions, dissolution data on 6 units from two pivotal clinical trial batches (2Q2), 6 units from one to-be marketed tablet batch and 3 to-be marketed production batches (2AB7) were generated and have been summarized in the following tables:

Time in minutes	Mean % (% CV) clopidogrel dissolved		
	2AB7	2Q2 (batch 1)	2Q2 (batch 2)
5	30.4 (27.8)	35.1 (22.26)	25.6 (18.1)
10	74.1 (8.4)	75.9 (7.2)	78.5 (8.8)
15	95.1 (3.6)	93.9 (1.6)	96.2 (3.3)
20	98.9 (2.2)	96.9 (1.4)	99.2 (1.1)
25	98.9 (2.1)	97.2 (1.4)	100.3 (0.8)
30	98.9 (2.2)	97.4 (1.4)	100.1 (0.8)

Mean of cumulative % clopidogrel dissolved (n=12, production/validation batches)

TIME (minutes)	2AB7 (batch 1)	2AB7 (batch 2)	2AB7 (batch 3)
10	78.6	78.7	79.1
20	98.6	100.3	101.1
30	98.9	100.3	101.3

As mentioned previously, 75 rpm for paddle apparatus is faster than the normally accepted agitation speed of 50 rpm. Since the lower agitation speeds are more discriminatory, it is recommended that 50 rpm be used as the dissolution testing condition. The only data available at 50 rpm is from one batch of clinical 2Q2 formulation and one production batch of to-be marketed

(2AB7) formulation, hence only an interim dissolution specification can be set at this time. The corresponding dissolution data is provided in the following 2 tables.

Batch 0001 Commercial Formula 2AB7
Manufacturing date: 02/97

Dissolution at 50 rpm
% dissolved

Comp.	10min	20min	30min
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
Avg.	65.9	98.6	100.3

Batch 32E = Batch L146 G CAPRIE Formula 2Q2
Manufacturing date: 07/94

Dissolution at 50 rpm
% dissolved

Comp.	10min	20min	30min
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
Avg.	65.0	97.8	99.2

CONCLUSION:
FDA SELECTED DISSOLUTION CONDITIONS AND INTERIM DISSOLUTION SPECIFICATIONS:

USP APPARATUS 2 (PADDLE), 50 RPM, 1000 ML, 37°C, pH 2.0 BUFFER (KCl/HCl) INTERIM SPECIFICATION: Q = IN 20 MINUTES. The sponsor should provide dissolution data using these conditions on 3 production batches.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY

PROTEIN BINDING:

LPR301 - INTERACTIONS TO PLASMA PROTEIN BINDING SITES OF SR25990C - "IN-VITRO" STUDY

Study ID: LPR301
Volume: 1.60
Objective:
1. To determine possible protein binding interactions between SR25990C (parent clopidogrel) and its main metabolite (SR26334) and other drugs, nifedipine, atenolol, digoxin and ranitidine (effect of these drugs on protein binding of clopidogrel).
2. To study the effect of bilirubin and palmitic acid as endogenous compounds on the binding of SR25990C.

Concentrations of clopidogrel selected for this study were 0.15 and 25 μg/mL. The C_{max} for clopidogrel obtained in normal volunteers (2 ng/mL) is much lower than the concentrations studied here.

Study Design:

Human plasma was obtained. An aliquot of radiolabeled drug (^{14}C) stock solutions was added to normal saline to obtain final concentration as shown in the table below.

Protein binding was determined by equilibrium dialysis conducted at 37°C. SR25990C in normal saline at concentrations of 0.15 and 25 μg/mL with appropriate amounts of radioactivity were dialyzed against plasma samples spiked with xenobiotics at their usual therapeutic concentrations or endogenous compounds at 3-fold their usual physiological concentrations. At the end of dialysis, the radioactivity and protein concentrations were measured in each compartment of the dialysis cells by . Percent binding was then determined from these results. The dpm in the buffer compartment represents free drug concentration. Dpm in protein compartment represents free + bound drug concentrations. The difference in dpm between the protein cell and buffer cell represent the bound drug concentrations. The percent bound is determined by dividing the dpm bound by the dpm in the protein cell multiplied by 100.

Results:

Addition of SR26334, nifedipine, atenolol, digoxin, ranitidine, bilirubin and palmitic acid to SR25990C did not have any effect on protein binding of SR25990C (see table below).

Drug	Competitor concentrations (µg/mL)	% binding of clopidogrel at	
		0.15 µg/mL	25 µg/mL
SR25990C alone		94 ± 0.6	96 ± 0
+ SR26334	1	95 ± 1	96 ± 0
+ Nifedipine	0.2	94 ± 0	96 ± 0
+ Atenolol	0.5	94 ± 0.6	96 ± 0
+ Digoxin	0.002	94 ± 0.6	96 ± 0
+ Ranitidine	0.1	95 ± 0	96 ± 0
+ Bilirubin	20	94 ± 0.6	95 ± 1
+ Palmitic acid	100	94 ± 0	95 ± 0

Conclusion: Results of this study show that these potentially coadministered drugs do not alter SR25990C binding when present in plasma at therapeutic concentrations or at 3 fold their physiologic concentrations.

Comment: It is not clear why the sponsor has not studied the effect of warfarin on the protein binding of clopidogrel and vice versa. This could be especially important since these 2 drugs are very likely to be co-administered.

APPEARS THIS WAY
ON ORIGINAL

LPR303 - SR26334A (METABOLITE OF SR25990C) AND INTERACTIONS ON SERUM BINDING SITES - "IN VITRO" STUDY

Study ID: LPR303
Volume: 1.60
Objective:

1. To determine possible protein binding interactions between SR26334A (metabolite of clopidogrel) and its parent drug (SR25990C); other drugs, nifedipine, atenolol, digoxin and ranitidine and endogenous compounds, bilirubin and palmitic acid (effect of these drugs on protein binding of SR26334A).
2. To study the effect of SR26334A on protein binding of SR25990C, digoxin and ranitidine.

Concentrations of clopidogrel carboxy metabolite selected for this study were 0.1, 25 and 100 µg/mL. These concentrations encompass the therapeutic concentrations of SR26334 achieved.

Study Design:

Human serum sample was obtained. An aliquot of radiolabeled drug (^{14}C) stock solutions was added to Sorensen buffer to obtain final concentrations as shown in the table below.

Protein binding was determined by equilibrium dialysis conducted at 37°C. SR26334A in Sorensen buffer at concentrations of 0.1, 25 and 100 µg/mL with appropriate amounts of radioactivity were dialyzed against serum samples spiked with xenobiotics at their usual therapeutic concentrations or endogenous compounds at 10 times higher than their usual physiological concentrations. At the end of dialysis, the radioactivity and protein concentrations were measured in each compartment of the dialysis cells by
Percent binding was then determined from these results. The dpm in the buffer compartment represents free drug concentration. Dpm in protein compartment represents free + bound drug concentrations. The difference in dpm between the protein cell and buffer cell represent the bound drug concentrations. The percent bound is determined by dividing the dpm bound by the dpm in the protein cell multiplied by 100.

When radiolabeled competitors were available, the reverse effect (effect of SR26334A on their protein binding) was also studied.

Results:

The % binding of SR26334A in the absence or presence of competitors are shown in the following table. Results indicate that clopidogrel and other competitors studied have no effect on binding of SR26334A.

Model	Competitor concentrations (mg/l)	% binding at 0.1 mg/l	% binding at 25 mg/l	% binding at 100 mg/l
SR 26334A alone		93 ± 0.8(21)	93 ± 0.7(21)	93 ± 1.1(19)
+ SR 25990C	25 0.1	93 ± 0.6 (3) 91 ± 0.7 (2)	92 ± 0.6 (3)	92 ± 0.7 (2)
+ NIFEDIPINE	2 0.2[1])	93 ± 0 (3) 93 ± 0 (2)	94 ± 0.6 (3)	93 ± 0 (3)
+ ATENOLOL	5 0.5[2])	92 ± 0.6 (3) 94 ± 0.7 (2)	94 ± 0 (3)	93 ± 0.6 (3)
+ DIGOXINE	0.02 0.002[3])	94 ± 0.6 (3) 95 ± 0 (2)	94 ± 0.6 (3)	93 ± 0 (3)
+ RANITIDINE	1.1 0.1[4])	94 ± 0 (3) 94 ± 0 (2)	94 ± 0 (3)	94 ± 0 (3)
+ BILIRUBINE	20[5]) 7	92 ± 0.6 (3) 94 ± 1.4 (2)	92 ± 0.6 (3)	92 ± 1.0 (3)
+ PALMITIC ACID	100[5]) 30	93 ± 0.7 (3) 93 ± 0.7 (2)	93 ± 0 (3)	92 ± 0.6 (3)

(n)

The effect of SR26334A on the % binding of SR25990C, digoxin and ranitidine are shown in the following table. Results indicate that SR26334A has no effect on clopidogrel binding. A small effect is seen on binding of digoxin and ranitidine (2 - 5%).

DRUGS	MODEL	% binding at drug concentrations	
SR 25990C		0.06 mg/l	25 mg/l
% binding	alone	89 ± 0.6 (3)	92 ± 0 (3)
% binding	+ SR 26334A (0.1 mg/l)	89 ± 0.6 (3)	92 ± 0 (3)
% binding	+ SR 26334A (100 mg/l)	89 ± 0 (3)	92 ± 0 (3)
DIGOXINE		0.002 mg/l	0.02 mg/l
% binding	alone	51 ± 1.5 (3)	46 ± 4.6 (3)
% binding	+ SR 26334A (0.1 mg/l)	48 ± 1.4 (2)	47 ± 1.5 (3)
% binding	+ SR 26334A (100 mg/l)	49 ± 1.5 (3)	44 ± 3.5 (2)
RANITIDINE		0.1 mg/l	1 mg/l
% binding	alone	16 ± 1.5 (3)	15 ± 1.5 (3)
% binding	+ SR 26334A (0.1 mg/l)	15 ± 2 (3)	19 ± 1 (3)
% binding	+ SR 26334A (100 mg/l)	18 ± 2 (3)	10 ± 2.5 (3)

(n)

Conclusion: Results of this study show that these potentially coadministered drugs do not alter SR26334A binding when present in plasma at therapeutic concentrations or at 10 fold their physiologic concentrations. SR26334A has no effect of SR25990C binding and has minimal effect on digoxin and ranitidine binding.

Comment: It is not clear why the sponsor has not studied the effect of warfarin on the protein binding of clopidogrel carboxy metabolite and vice versa. This could be especially important since warfarin and clopidogrel are very likely to be co-administered.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

LPR201 - HUMAN SERUM PROTEIN BINDING OF CARBOXYLIC ACID DERIVATIVE OF CLOPIDOGREL (SR26334) - IN VITRO STUDY

Study ID: LPR201
Volume: 1.60
Objective:

To determine the in vitro protein binding of SR26334 (99% pure S-enantiomer) in human serum.

Concentration range of drug selected for this study was 0.04-2500 µg/mL, so as to accommodate the anticipated therapeutic plasma levels.

Study Design:

Human serum sample was obtained. An aliquot of radiolabeled drug (^{14}C) stock solutions was added to Sorensen buffer to obtain final concentrations as shown in the table below.

Protein binding was determined by equilibrium dialysis conducted at 37°C. SR26334A in Sorensen buffer at concentrations of 0.04 to 2500 µg/mL with appropriate amounts of radioactivity was dialyzed against serum. At the end of dialysis (after 2 hours of incubation), the radioactivity and protein concentrations were measured in each compartment of the dialysis cells b) Percent binding was then determined from these results. The dpm in the buffer compartment represents free drug concentration. Dpm in protein compartment represents free + bound drug concentrations. The difference in dpm between the protein cell and buffer cell represent the bound drug concentrations. The percent bound is determined by dividing the dpm bound by the dpm in the protein cell multiplied by 100.

Results: At concentrations ranging from 0.04-100 µg/mL, the protein binding of SR26334-^{14}C averaged 94-95% in human serum. Protein binding appears to be saturable at higher concentrations of drug.

% protein binding of SR26334A in human serum

Concentrations, µg/mL	0.04	0.08	1.0	5.1	10	25	50	100
% binding	94±1.5	95±2.8	94±1.5	95±2.1	95±1.7	94±1.7	94±1.5	94±1.7
Concentrations, µg/mL	250	500	750	1000	1500	2000	2500	
% binding	91±1.1	90±2.1	86±1.5	83±3.2	76±2.3	72±3.2	67±3.7	

Conclusion: The binding of SR26334A to human serum protein appears to be saturable at concentrations greater than 100 µg/mL. At concentrations that are equal to or higher than (up to 30 fold) the therapeutically achievable concentrations of SR26334A, the binding was found to be linear and high (94 - 95%).

IN VITRO BINDING OF SR25990C AND SR26334A TO PLASMA PROTEINS AND TO ERYTHROCYTES IN MALE CAUCASIAN HUMANS

Study ID: RA850890906/ML1
Volume: 1.60
Objective:
1. To determine the in vitro red blood cell binding of clopidogrel-^{14}C and its carboxy metabolite in human whole blood.
2. To determine the protein binding of clopidogrel-^{14}C and its carboxy metabolite in human plasma.

Study Design:

Blood obtained from healthy male caucasian humans (n = 6), of age range 31 to 39 years, was used for this study. For protein binding experiments, blood samples were centrifuged to separate erythrocytes from plasma. For erythrocyte binding experiments, hematocrit was measured in these blood samples. Appropriate aliquots of stock solutions were added to whole blood aliquots to give various concentrations of ^{14}C-clopidogrel or ^{14}C-SR26334. Experiments were performed at 0.025, 0.05, 0.10, 0.25, 0.50, 1, 5, 10, 25, 50 and 100 µg/mL for SR25990C and at 0.10, 0.50, 1, 5, 10, 25, 50, 100, 250, 500 and 1000 µg/mL for SR26334A. These concentrations were selected to encompass the levels that may occur in human clinical studies.

PLASMA PROTEIN BINDING: Protein binding was determined by . Plasma containing increasing drug concentration with appropriate amounts of radioactivity was dialyzed against normal saline solution. At the end of dialysis (after 2 hours of incubation), the radioactivity was measured in each compartment of the dialysis cells by and drug concentrations calculated. Percent binding was then determined from these results. The dpm in the buffer compartment represents free drug concentration. Dpm in protein compartment represents free + bound drug concentrations. The difference in dpm between the protein cell and buffer cell represent the bound drug concentrations. The percent bound is determined by dividing the dpm bound by the dpm in the protein cell multiplied by 100.

COVALENT BINDING OF SR25990C AND SR26334A: Plasma covalent binding was determined at various concentrations of clopidogrel and its metabolite after incubation with plasma and precipitation with trichloroacetic acid/methanol mixture. The precipitated proteins are washed to remove unbound drug. Then concentrations of drug covalently bound to proteins is determined by liquid scintillation counting of the sample containing precipitated proteins. Plasma covalent binding is obtained by dividing the radiolabel recovered in protein pellet after extensive washing by the total initial radiolabel.

BINDING TO ERYTHROCYTES: An appropriate volume of drug was dried and suitable volume of blood was added. After incubation, an aliquot was weighed, dried and mineralized and then analyzed by . Another aliquot was centrifuged to separate the

erythrocytes from plasma. Radioactivity was determined in plasma by
The % binding to erythrocytes is then determined as follows:

E% = 1 - [(1-H)*S/T] where E = radiolabel bound to erythrocytes; H = hematocrit; S = radioactivity in plasma and T = total drug.

Results: The results of % binding to plasma proteins are shown in the following tables:

SR 25990C concentration (mg/l)	(μM)	Plasma Protein Binding (%) (mean ± SD)
0.025	0.059	-
0.050	0.119	-
0.10	0.238	96.08 ± 3.41
0.25	0.595	98.50 ± 0.20
0.50	1.19	97.68 ± 0.39
1.0	2.38	98.58 ± 0.44
5.0	11.91	98.79 ± 0.0
10.0	23.81	98.73 ± 0.11
25.0	59.53	98.54 ± 0.14
50.0	119.07	98.32 ± 0.07

SR 26334A concentration (mg/l)	(μM)	Plasma Protein Binding (%) (mean ± SD)
0.10	0.29	92.46 ± 0.95
0.50	1.45	94.28 ± 0.94
1.0	2.90	94.48 ± 0.62
5.0	14.50	94.51 ± 0.79
10.0	29.00	94.58 ± 0.48
25.0	72.60	95.03 ± 0.13
50.0	145.20	93.28 ± 1.65
100.0	290.5	93.82 ± 1.09
250.0	726.3	90.86 ± 3.80
500.0	1452.6	88.34 ± 2.28
1000.0	2905.1	80.79 ± 5.05

The results of plasma covalent binding of both clopidogrel and its metabolite are presented below:

SR 25990C concentration (mg/l)	(μM)	Plasma covalent binding (%) (mean ± S.D.)
0.025	0.059	1.45 ± 1.03
0.25	0.595	1.41 ± 0.16
1.0	2.38	1.31 ± 0.10
10.0	23.81	1.23 ± 0.17
50.0	119.07	1.17 ± 0.27

SR 26334A concentration (mg/l)	(μM)	Plasma covalent binding (%) (mean ± S.D.)
0.1	0.29	1.73 ± 0.27
1.0	2.90	1.35 ± 0.26
10.0	29.05	1.30 ± 0.08
100.0	290.46	1.42 ± 0.32
500.0	1452.30	1.32 ± 0.22

BEST POSSIBLE COPY

% binding of clopidogrel and SR26334 to erythrocytes are summarized in the following tables:

SR 25990C concentration (mg/l)	(μM)	Erythrocyte binding (%)
0.025	0.059	-a
0.050	0.119	-
0.10	0.238	5.38 ± 4.98
0.25	0.595	4.82 ± 2.29
0.50	1.19	6.86 ± 7.14
1.0	2.38	4.61 ± 4.00
5.0	11.91	5.31 ± 5.20
10.0	23.81	13.52 ± 21.80
25.0	59.53	20.73 ± 17.14
50.0	119.07	10.34 ± 10.32
Mean S.D.		8.94 ± 5.34

SR 26334A concentration (mg/l)	(μM)	Erythrocytes binding (%)
0.10	0.29	11.55 ± 2.51
0.50	1.45	13.22 ± 4.16
1.0	2.90	9.89 ± 6.16
5.0	14.50	9.28 ± 7.33
10.0	29.00	9.71 ± 8.83
25.0	72.60	8.38 ± 7.59
50.0	145.20	8.14 ± 5.80
100.0	290.5	8.76 ± 6.18
250.0	726.3	13.75 ± 8.85
500.0	1452.6	15.50 ± 3.93
Mean S.D.		10.81 ± 2.42

Conclusion:

1. % binding of SR25990C and SR26334 to plasma proteins is 98 and 94% respectively and is not saturable up to 100 μg/mL concentrations. Binding is saturable at higher concentrations.
2. About 2% of radiolabel for clopidogrel and its carboxy metabolite is covalently bound to plasma proteins.
3. % binding to erythrocytes was about 10% for both clopidogrel and its metabolite.

STUDY LPR0602: BINDING OF SR25990C TO ISOLATED HUMAN SERUM PROTEINS: This study has already been reviewed by Dr. Phil Colangelo (see review dated August 31, 1995). It was concluded that binding of clopidogrel was extensive (80 - 90%) to physiological concentrations of human serum albumin and low density lipoproteins, and moderate (30 - 60%) to α_1-acid glycoprotein and high density lipoproteins. This binding to HSA, LDL and HDL was not saturable in the concentrations studied, but binding to AAG was saturable. The extensive binding to albumin suggests potential binding displacement interactions may occur with other drugs. However, since therapeutic concentrations of clopidogrel are extremely low, this protein binding may be of limited clinical importance.

STUDY LPR0603: BINDING OF SR26334A TO ISOLATED HUMAN SERUM PROTEINS: This study has already been reviewed by Dr. Phil Colangelo (see review dated August 31, 1995). It was concluded that binding of SR26334A was primarily to human serum albumin and was extensive (80 - 90%). This binding was saturable at high concentrations of SR26334A (893 - 2580 mg/l). Binding to other proteins, including AAG was low (<10%) to moderate (20 - 50%). The extensive binding to albumin may potentially result in protein binding displacement interactions with other highly bound drugs.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

IN VITRO METABOLISM:

MIH0012 - DETERMINATION OF THE CYTOCHROME P450 (CYP) ISOFORMS INVOLVED IN THE OXIDATIVE METABOLISM OF SR25990C AND SR26334A IN HUMAN LIVER MICROSOMES IN VITRO

Study ID: MIH0012
Volume: 1.60
Objective:

To identify the hepatic Cytochrome P-450 (CYP) isoforms involved in the oxidative metabolism of clopidogrel and its carboxylic acid metabolite by human liver microsomes.

IN VITRO METABOLISM OF CLOPIDOGREL : Clopidogrel concentrations in the range of 0.5 and 10 µM were incubated with human liver microsomes (0.1 mg/mL microsomal protein), pooled microsomes from 3 human livers (9 livers for CYP isozyme determination), at 37°C (optimal reaction conditions were used to produce linear rates of loss). This mixture also contained potassium phosphate buffer, magnesium chloride, ß-$NADP^+$, glucose 6-phosphate, and glucose 6-phosphate dehydrogenase. The reactions were allowed to proceed for about 15 minutes. The samples were centrifuged and analyzed by to monitor the disappearance of clopidogrel. K_m and V_{max} parameters for metabolism of clopidogrel were then determined from this data.

Specific cytochrome P450 enzymes responsible for metabolism of clopidogrel and its metabolite were identified by chemical inhibition studies, CYP-selective inhibitory antibodies and by incubation with purified CYP forms [individual CYP isoforms in microsomes from transfected β-lymphoblastoid cells (cDNA-expressed CYP systems)]. The isoforms investigated were CYP1A2, CYP2B6, CYP2A6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4.

Human liver microsomes and clopidogrel were incubated with an NADPH generating system, with and without competitive substrates, chemical inhibitors or antibodies, in potassium phosphate buffer (pH 7.4) at 37°C for 15 minutes. Rates of clopidogrel disappearance were compared to determine inhibition.

CYTOCHROME P-450 SELECTIVE INHIBITORS: A series of inhibitors were incubated with 0.5 and 5 µM clopidogrel to determine which compounds could inhibit the metabolism of this drug. The inhibitors (and competitive substrates) selected were 7,8-benzoflavone (0.1 - 1 µM) and furafylline (0.1 - 1 µM) for CYP1A2; pilocarpine (1 - 10 µM) for CYP2A6; orphenadrine (2 - 20 µM) and cyclophosphamide (2 - 20 µM) for CYP2B6; sulfaphenazole (1 - 10 µM) for CYP2C9; S-mephenytoin (10 - 100 µM) and tranylcypromine (2 - 20 µM) for CYP2C19; quinidine (0.1 - 5 µM) for CYP2D6; diethyldithiocarbamate (2 - 20 µM) and chlorzoxazone (10 - 100 µM) for CYP2E1; ketoconazole (0.05 - 0.5 µM) and troleandomycin (10 - 100 µM) for CYP3A4.

PURIFIED CYP FORMS AND cDNA-EXPRESSED CYP ACTIVITIES: Clopidogrel (at 0.5 and 5 µM concentration) in presence of an NADPH-generating system at 37°C was incubated with human ß-lymphoblastoid cells containing cDNA-expressed specific CYP-isoforms, CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4 for 15 minutes to investigate clopidogrel disappearance.

ISOFORM-SELECTIVE INHIBITORY ANTIBODIES: Clopidogrel (at 0.5 and 5 µM concentration) in presence of an NADPH-generating system at 37°C was incubated with isoform-selective inhibitory antibodies for 15 minutes to investigate clopidogrel disappearance.

Results:

All samples were analyzed by to monitor the oxidative metabolism of clopidogrel. LOQ of the assay was 0.1 µM. Within run and between run CV was less than 10.4%. Within run and between run accuracy ranged from -4 to 7%.

Mean apparent Km value for clopidogrel metabolism was found to be 6.6 ± 2.1 µM and Vmax = 2637 pmol/min/mg.

Results from chemical inhibition studies are shown in the following table. Inhibition was not extensive at clopidogrel concentrations of 0.5 µM.

Competitive Substrate/Inhibitor	Percent of Control Activity		
	0.5 µM	5 µM	Isoform-Selective Substrate
Furafylline (CYP1A2):			
0.1 µM	98	105	88
1 µM	91	86	38
7,8-benzoflavone (CYP1A2):			
0.1 µM	103	55	ND
1 µM	98	43	ND
Pilocarpine (CYP2A6):			
1 µM	104	88	77
10 µM	96	79	25
Orphenadrine (CYP2B6):			
2 µM	95	95	ND
20 µM	86	62	ND
Cyclophosphamide (CYP2B6):			
2 µM	102	46	ND
20 µM	102	41	ND
Sulfaphenazole (CYP2C9):			
1 µM	94	68	69
10 µM	83	47	24
S-Mephenytoin (CYP2C19):			
10 µM	103	85	ND
100 µM	91	55	ND
Tranylcypromine (CYP2C19):			
2 µM	94	93	86
20 µM	81	89	49
Quinidine (CYP2D6):	99 (0.1 µM) 101 (1 µM)	87 (0.5 µM)* 100 (5 µM)*	79 (0.1 µM) 35 (1 µM)
Diethyldithiocarbamate (CYP2E1):			
2 µM	93	103	92
20 µM	92	73	81
Chlorzoxazone (CYP2E1):			
10 µM	97	118	ND
100 µM	87	93	ND
Ketoconazole (CYP3A4):			
0.05 µM	88	71	60
0.5 µM	84	35	13
Troleandomycin (CYP3A4):			
10 µM	84	58	ND
100 µM	82	55	ND

Results from cDNA-expressed purified isozymes are shown in the following table:

Clopidogrel metabolism in microsomes containing heterologously expressed human CYP isoforms

Microsome preparation	Clopidogrel Metabolism (Percent of control activity)		Isoform-Selective Substrates
	0.5 μM*	5 μM*	Oxidation Rate
Vector alone	ND	103	–
CYP1A2 (17 pmol CYP)	44	78	263
CYP2A6 (14 pmol CYP)	106	102	8625
CYP2B6 (18 pmol CYP)	0	4	ND
CYP2C9 (5 pmol CYP)	99	94	13
CYP2C19 (3 pmol CYP)	14	80	81
CYP2D6 (5 pmol CYP)	106	113	355
CYP2E1 (25 pmol CYP)	107	98	175
CYP3A4 (9 pmol CYP)	30	36	1402

* Clopidogrel concentration

Results from antibody inhibition studies are shown in the following table:

Antibody preparation	Percent of Control Activity		
	0.5 μM*	5 μM*	Isoform-Selective Substrates
CYP1A2			
low	102	126	
high	101	100	64
CYP2A6			
low	110	103	
high	110	80	52
CYP2B6			
low	89	43	
high	89	29	ND
CYP2C9			
low	92	119	
high	94	57	86
CYP2C19			
low	92	119	
high	94	57	ND
CYP2D6			
low	104	74	
high	99	68	29
CYP2E1			
low	101	97	
high	89	67	80
CYP3A4			
low	92	72	
high	77	47	21

*Clopidogrel concentration

The following summary table shows the CYP isoenzymes involved in the metabolism of clopidogrel:

	Type of Experiment			Conclusion*
CYP Isoform	Substrates/ Inhibitors	Inhibitory Antibodies	Expressed Isoforms	Involved in clopidogrel metabolism
CYP1A2	++	–	++	Possible
CYP2A6	–	–	–	No
CYP2B6	+	++	++	Yes
CYP2C9	++	+	–	Possible
CYP2C19	++	+	++	Yes
CYP2D6	–	+	–	No
CYP2E1	+	+	–	Possible
CYP3A4	++	++	++	Yes

++ Active
+ Possibly active
– Not active
* 0 - 1 +. Isoform not involved in clopidogrel metabolism
2 - 4 +. Isoform possibly involved in clopidogrel metabolism
5 - 6 +. Isoform involved in clopidogrel metabolism

Conclusion: Results from this study indicate that CYP2B6, CYP2C19 and CYP3A4 are involved clopidogrel metabolism. CYP1A2, CYP2C9 and CYP2E1 may also possibly be involved. The bulk of metabolic clearance, however, is not due to cytochrome P450 mediated metabolism. This involves de-esterification, to form the carboxy metabolite (SR26334). The primary clearance pathway for the carboxy metabolite is glucuronidation. Attempts to identify the cytochrome P450 isozymes involved in its metabolism were inconclusive due to undetectable rates of metabolism in human liver microsomes.

Comments: Since this drug is metabolized by CYP1A2, 2C9, 2B6, 2C19 and 3A4, it may potentially interact with drugs like theophylline, warfarin, orphenadrine, phenytoin and ketoconazole.

STUDY MIH0011 (IN VITRO ENZYME INHIBITION STUDY):

INVESTIGATING THE POTENTIAL FOR SR25990C AND SR26334A TO INHIBIT CYTOCHROME P450 (CYP) ENZYMES USING HUMAN LIVER MICROSOMES IN VITRO

Reference: Volume 60
Investigator: Jennifer Brandl
Study Location: Sanofi Research Division, Malvern, PA
Objective:

To investigate the effects of clopidogrel and its carboxy metabolite on human cytochrome P450 activities (CYP1A2, CYP3A4, CYP2A6, CYP2C9, CYP2C19, CYP2D6 and CYP2E1) in vitro and to determine its potential to inhibit the metabolism of other drugs in man.

Study design:

Human liver microsomes obtained from 3 donor livers were incubated with various substrates (nifedipine: 40 μM; tolbutamide: 500 μM; phenacetin: 10 μM; coumarin 1 μM (for CYP2A6), bufuralol 25 μM, chlorzoxazone 100 μM and mephenytoin 100 μM), reaction cofactors and clopidogrel 0.4 μM and carboxy metabolite 200 μM (concentrations approximately 20 fold higher than expected plasma concentrations after a daily dose of 75 mg). Sodium fluoride was added to the reaction mixtures containing clopidogrel to inhibit de-esterification of the compound during incubation. Quantitation of the specific metabolites formed by various isozymes (1A2, 2C9, 2D6, 2C19, 2E1 and 3A4) of cytochrome P450 were performed using methods. .

Where inhibition was found, K_is (inhibition constants) for clopidogrel and its metabolite were determined for different isozymes using non-linear regression. % inhibition expected clinically was estimated using the following equation for competitive enzyme inhibitors:

$i = \{I/[I+Ki(1+S/Km)]\}*100$ where i = % inhibition; I = expected plasma concentration of inhibitor (10 μM for SR26334A based on C_{max} value after 75 mg dose); S = expected plasma concentration of substrate; Ki = apparent Ki value for inhibitor against drug metabolism; and Km = apparent Km value determined for substrate metabolite production.

Results:

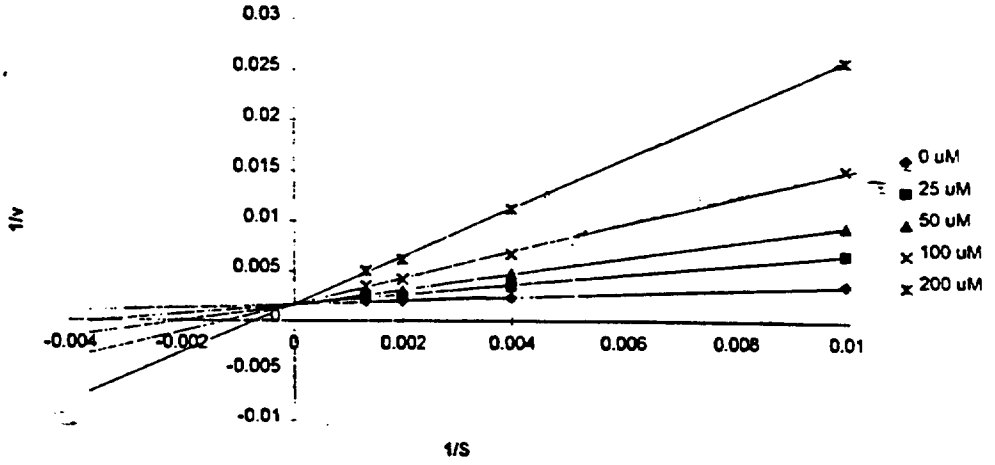
Clopidogrel did not significantly inhibit (defined as >30% inhibition relative to control values) any of the CYP isoform activities. Addition of isoform-selective inhibitors (CYP1A2, furafylline; CYP2D6, quinidine; CYP2E1, diethyldithiocarbamate; CYP2C9, sulfaphenazole; CYP2A6, pilocarpine; CYP2C19, tranylcypromine; and CYP3A4, ketoconazole), to reaction mixtures, however, resulted in significant inhibition relative to control reaction mixtures confirming the ability of these assays to show CYP inhibition.

SR26334A did not significantly inhibit phenacetin O-deethylation (CYP1A2), bufuralol 1'-hydroxylation (CYP2D6), coumarin 7-hydroxylation (CYP2A6), mephenytoin 4-hydroxylation (CYP2C19), nifedipine oxidation (CYP3A4), or chlorzoxazone 6-hydroxylation (CYP2E1).

SR26334A, however, decreased tolbutamide hydroxylation (CYP2C9) to approximately 45% of control values in all three microsomal preparations. In comparison to this, 50 μM sulfaphenazole, a selective 2C9 inhibitor, decreased tolbutamide hydroxylation by 90%.

Apparent Ki values for SR26334A inhibition (competitive inhibitor) of CYP2C9, were 25, 27 and 32 μM in the tested microsomes (see figure). Based on an expected SR26334A C_{max} of 10 μM and a mean apparent Ki value of 28 μM, the calculated % inhibition for CYP2C9 was 26.3%.

Lineweaver Burk representation of the effect of SR 26334A on tolbutamide hydroxylation in microsomes from human liver



Conclusions:

Clopidogrel did not inhibit cytochrome P450 mediated metabolism. SR26334A inhibited CYP2C9 (tolbutamide hydroxylation) in vitro. This suggests that inhibition of CYP2C9-mediated metabolism of drugs is possible upon concomitant administration with clopidogrel.

Comment:

Based on the above results, one can expect drug interactions with phenytoin, S-warfarin, tolbutamide and may be even torsemide. S-warfarin interaction is especially important since warfarin has a high potential to be used in patients taking clopidogrel due to the nature of the disease states in which both these drugs are used.

STUDY MIH0009 (IN VITRO ENZYME INHIBITION STUDY):

INVESTIGATING THE POTENTIAL OF SR25990C AND SR26334A TO INHIBIT THE OXIDATIVE METABOLISM OF GLYBENCLAMIDE (GLYBURIDE) IN HUMAN LIVER MICROSOMES

Reference: Volume 60
Investigator: Robert Van Horn
Study Location: Sanofi Research Division, Malvern, PA
Objective:

To investigate the potential of clopidogrel and its carboxy metabolite to inhibit the metabolism of glyburide in human liver microsomes.

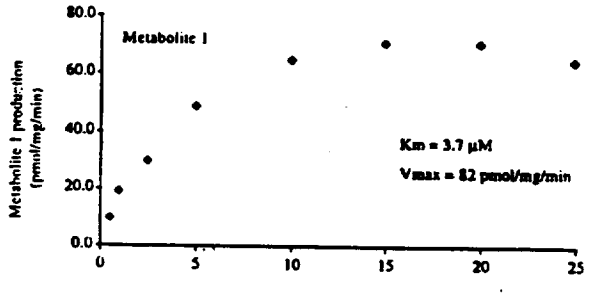
Study design:

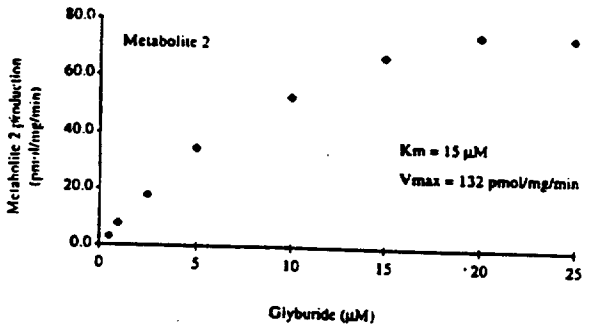
Human liver microsomes obtained from 3 donor livers were incubated (for 30 minutes using 0.5 mg/mL microsomal protein to be in the linear range) with various substrates (glyburide 1, 2.5, 5 and 10 µM), reaction cofactors and a range of clopidogrel (400 nM) and carboxy metabolite concentrations (0, 50, 100, 200, 400 and 600 µM). Quantitation of the metabolites 1 and 2 of glyburide were performed by Apparent Km and Vmax values for metabolites 1 and 2 and apparent Ki value for SR26334A with metabolite 1 were determined by non-linear regression. % inhibition expected clinically was estimated using the following equation for competitive enzyme inhibitors:

$i = \{I/[I+Ki(1+S/Km)]\}*100$ where i = % inhibition; I = expected plasma concentration of inhibitor (10 µM for SR26334A based on C_{max} value after 75 mg dose); S = expected plasma concentration of glyburide (1 µM based on a daily dose of 5 mg); Ki = apparent Ki value for inhibitor against glyburide oxidation; and Km = apparent Km value determined for glyburide metabolite production (metabolite 1 or 2).

Results:

Km and Vmax values for formation of glyburide metabolites are shown in the following figures:





Clopidogrel did not affect the oxidative metabolism of glyburide when incubated with human liver microsomes. SR26334A appeared to be a competitive inhibitor of metabolite 1 production (see tables below), with a mean apparent Ki of 111 μM, with a range of 68 to 140 μM (see figure below). However, SR26334A had no effect on metabolite 2 formation. Based on expected plasma concentrations and apparent Km and Ki values, the predicted inhibition of the metabolite 1 pathway is approximately 6.5%.

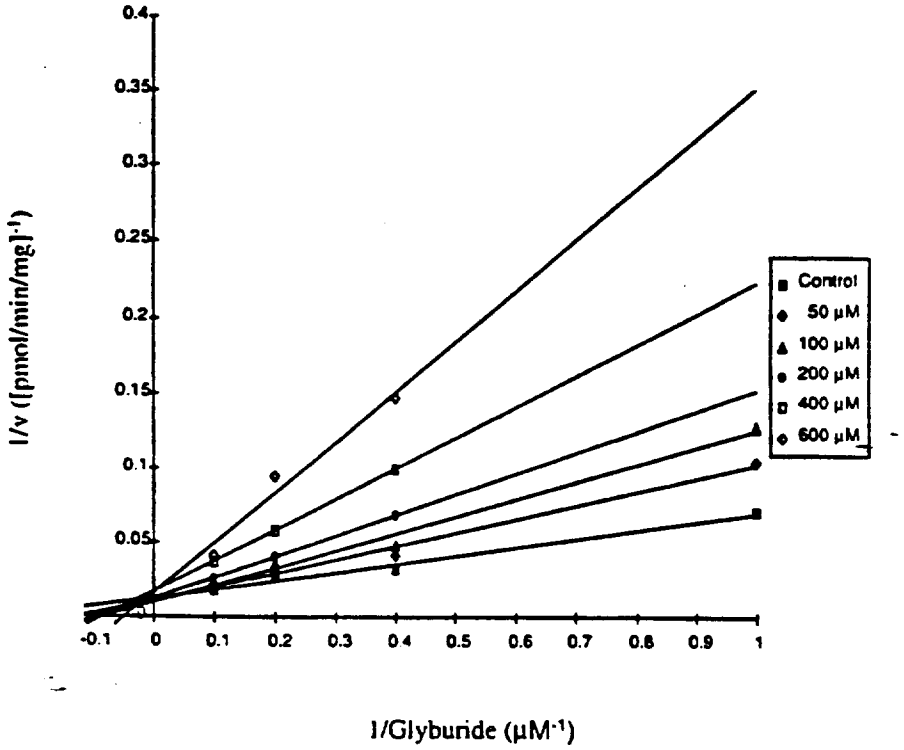
	Metabolite 1*	Inhibition (% of Control)	Metabolite 2*	Inhibition (% of Control)
HL 23-May-94				
Control†	0.25	100	0.24	100
Clopidogrel	0.25	100	0.24	100
HL 4-Sep-92				
Control†	0.29	100	0.23	100
Clopidogrel	0.28	96	0.23	100
HL 21-Jan-94				
Control†	0.19	100	0.06	100
Clopidogrel	0.19	100	0.06	100

* Values are expressed as μM amounts of metabolite produced
† Control reaction mixtures with clopidogrel contained 1 mM NaF

	Metabolite 1*	Inhibition (% of Control)	Metabolite 2*	Inhibition (% of Control)
HL 23-May-94				
Control	0.27	100	0.24	100
SR 26334A	0.18	67	0.23	96
HL 4-Sep-92				
Control	0.29	100	0.22	100
SR 26334A	0.19	66	0.21	95
HL 21-Jan-94				
Control	0.20	100	0.06	100
SR 26334A	0.12	60	0.06	100

* Values are expressed as μM amounts of metabolite produced

Lineweaver-Burk representation of the effect of SR 26334A on the production of metabolite 1 in microsome preparation HL



Conclusions:

Clopidogrel did not inhibit glyburide metabolism. However, the carboxy metabolite of clopidogrel inhibited the formation of glyburide metabolite 1. Therefore an interaction between SR26334A and glyburide is possible. However, this may not be clinically significant since all the metabolic pathways are not inhibited and glyburide when concomitantly administered with clopidogrel could possibly be cleared via the other pathways.

APPEARS THIS WAY
ON ORIGINAL

STUDY P1549: (HEPATIC ENZYME INDUCTION/INHIBITION: EFFECT OF CLOPIDOGREL ON ANTIPYRINE METABOLISM)

A STUDY OF THE INDUCTION OF HEPATIC ENZYME SYSTEMS BY CLOPIDOGREL (SR25990C) IN HEALTHY MALE VOLUNTEERS

Reference: Volumes 15, 16, 17, 18 and 19
Investigator:
Study Location:
Objective:

To determine whether clopidogrel and/or its metabolites are capable of causing induction or inhibition of hepatic enzymes using antipyrine kinetics as a marker of both the inhibition and induction of hepatic oxidative activity.

Study design:

This is a randomized double-blind, parallel-group, placebo-controlled trial in 20 healthy young male volunteers (age 18 - 35 years) involving multiple oral doses of clopidogrel (75 mg qd for 10 days) or placebo and single oral doses of antipyrine (10 mg/kg pre-clopidogrel dose and on day 10).

Clopidogrel was administered as 75 mg dose (3 x 25 mg tablets) once a day for 10 days. Antipyrine was administered at a dose of 10 mg/kg before and after the 10 day treatment with clopidogrel. Antipyrine powder was reconstituted with 100 ml of bottled water prior to dosing. No food was permitted 12 hours prior to and 4 hours following antipyrine administration.

Blood samples were drawn for analysis of antipyrine at 0, 0.5, 1, 2, 3, 4, 5, 8, 12, 16, 24 and 36 hours on after antipyrine administration on days 2 (pre-clopidogrel dosing) and 10. Urine samples were collected at 12 hour intervals over a 48 hour period for determination of antipyrine and its metabolite levels (norantipyrine and 3-OH methylantipyrine) on days 2 and 10. Urine was also assayed for 6 β OH-cortisol. Plasma samples were collected on day -4, -3 and 9 for cortisol plasma concentration.

The primary analysis parameters were plasma antipyrine clearance and cumulative urinary recoveries of antipyrine and its metabolites. ANOVA was performed on log-transformed plasma antipyrine clearance and on the cumulative urinary recoveries of antipyrine and its metabolites to compare clopidogrel and placebo. To evaluate combined inductive and inhibitory effect, parameters obtained after coadministration of antipyrine with clopidogrel (day D10) were compared with antipyrine alone (D2).

Comparisons between clopidogrel and placebo groups were made for safety in terms of laboratory tests, bleeding time and vital signs (changes from baseline were compared between 2 groups).

Redacted 1

page(s) of trade secret and/or confidential commercial information

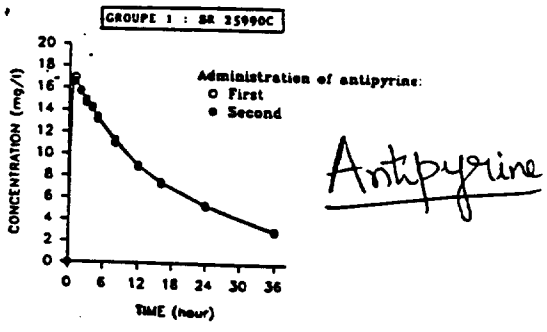
Assays were found to be acceptable.

PHARMACOKINETIC RESULTS:

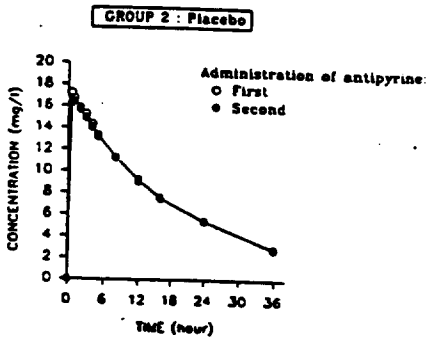
Mean PK parameters of antipyrine (before and after clopidogrel and placebo groups) is shown in the table and figure below:

	C max (mg/l)		T max (h)		AUC0-36h (mg-h/l)		T ½ (h)		Cl/F (l/h)	
	D-2*	D10*	D-2	D10	D-2	D10	D-2	D10	D-2	D10
SR										
Mean	17.5	17.7	0.7	1.0	280.9	285.3	14.7	14.9	2.3	2.2
SEM	0.5	0.8	0.1	0.2	20.2	18.7	1.9	1.8	0.3	0.2
Min										
Max										
Placebo										
Mean	17.9	17.1	0.8	0.9	285.3	284.6	14.0	14.4	2.3	2.2
SEM	0.4	0.3	0.2	0.1	10.8	11.2	0.6	0.7	0.1	0.1
Min										
Max										

* Day-2 administration of antipyrine before treatment
Day10 administration of antipyrine after 10 days of treatment



Antipyrine



Statistical analysis of $t_{1/2}$ and Cl/F is shown in the following table:

		Parameters			
		T ½		Cl/F	
Factors	**d.f.**	**F**	**p**	**F**	**p**
Treatment on Day-2	1,18	0.14	0.715	0.01	0.927
Treatment on D10	1,18	0.07	0.801	0.02	0.895
Visit	1,18	1.61	0.221	3.92	0.063
Treatment x visit	1,18	0.47	0.500	0.64	0.433

No significant differences in antipyrine plasma PK parameters were found.

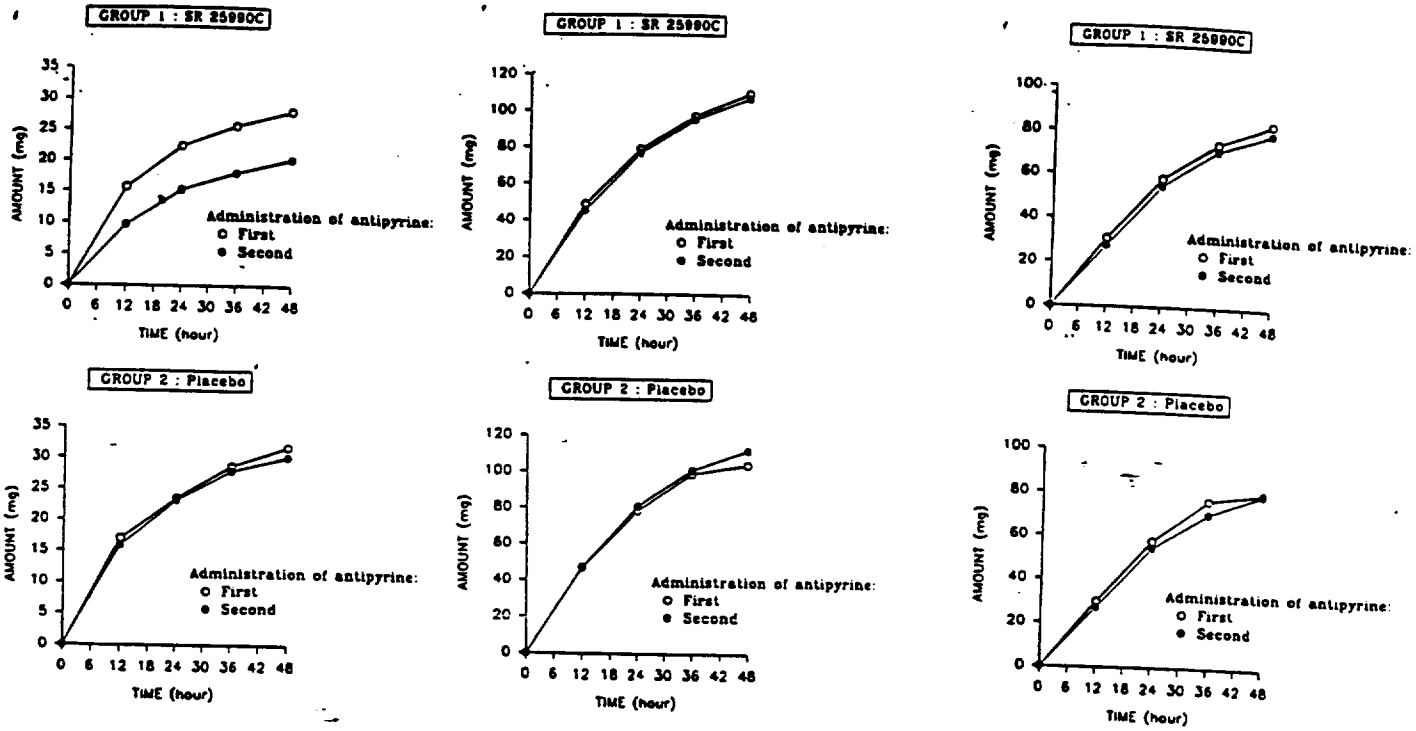
Mean urinary recovery of antipyrine, norantipyrine and 3-OH methylantipyrine (before and after clopidogrel and placebo groups) is shown in the table and the 3 figures below:

	Ae-Antipyrine (mg)		Ae Norant (mg)		Ae 3 HMA (mg)	
	D-2	D10	D-2	D10	D-2	D10
SR						
Mean	28.0	20.0	110.0	107.3	84.2	79.9
SEM	2.2	1.5	12.0	11.4	7.2	6.4
Min						
Max						
Placebo						
Mean	30.6	30.2	108.2	112.3	84.0	79.8
SEM	3.4	4.3	7.0	8.6	5.6	4.6
Min						
Max						

* Ae 0-36h

URINARY PHARMACOKINETICS

ANTIPYRINE NORANTIPYRINE 3-HMA



Statistical analysis of A_e for antipyrine and its metabolites is shown in the following table:

		Ae parameters					
		Antipyrine		Norant.		3 - HMA	
Factors	d.f.	F	p	F	p	F	p
Treatment Day-2	1,18	0.03	0.856	0.17	0.689	0.20	0.659
Treatment D10	1,18	5.09	0.037	0.28	0.603	0.02	0.902
Visit	1,18	3.53	0.076	0.78	0.388	0.30	0.594
Treatment x visit	1,18	4.60	0.046	1.12	0.305	0.14	0.708

Conclusions:

There was no significant difference in PK parameters of antipyrine (clearance and half-life) and in the urine excretion of antipyrine and the 2 metabolites obtained before and after clopidogrel treatment. There was no effect on γGT or on cortisol blood levels or urinary excretion of 6 β OH-cortisol. This indicates that clopidogrel does not significantly induce or inhibit hepatic enzyme activity.

CONCLUSION: This method is precise and accurate.

Comments: 1. The data for stability of clopidogrel and its metabolite in plasma samples upon storage and during freeze-thaw cycles has not been provided.
2. The calibration curve plot provided contains concentration (a truly independent variable) on Y-axis instead of X-axis. The sponsor, in future should plot concentration which is an independent variable on X-axis for calibration curves.

CONCLUSION: This method is precise and accurate.

Comments: 1. The data for stability of clopidogrel and its metabolite in plasma samples has not been provided.

Redacted 2

pages of trade

secret and/or

confidential

commercial

information

CONCLUSION: This method is precise and accurate and validation results are found to be acceptable.

CONCLUSION: This method is precise and accurate and validation results are found to be acceptable.

ASSAY PERFORMANCE: Conducted at Sanofi Research, Montpellier, France

CONCLUSION: This method is precise and accurate to determine the R and S enantiomers of clopidogrel carboxy metabolite and validation results are found to be acceptable. However, the method is not very sensitive.

STUDY P1717 (volumes 51, 52 and 53): RELATIVE BIOAVAILABILITY OF CLOPIDOGREL IN ELDERLY HEALTHY VOLUNTEERS IN THE FED AND FASTING STATES: This study has already been reviewed by Dr. Phil Colangelo (see review dated August 31, 1995). It was concluded that food did not alter the extent of SR26334 absorption/formation, as measured by AUC of SR26334 following single dose administration of clopidogrel (75 mg 2Q2 - clinical tablet). Although the rate of metabolite formation was, on average, rapid under both conditions, it may be slightly slower in the presence of food resulting in lower C_{max} (ratio of mean C_{max} fed/fasting = 0.79 and 90% C.I. 0.57 - 0.97). However, the C_{max} and T_{max} values were not overwhelmingly convincing to the reviewer, due to the truncation of the data. It was concluded that in general, food had a minimal effect on the pharmacokinetics of the primary metabolite of clopidogrel.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

STUDY P1298: (FOOD EFFECT BIOAVAILABILITY STUDY)

INFLUENCE OF FOOD INTAKE ON PHARMACOKINETIC PROFILE AND ANTIAGGREGATING ACTIVITY OF SR 25990C (CLOPIDOGREL)ADMINISTERED AS A SINGLE DOSE (400 MG) IN THE FORM OF TABLETS (8 x 50 MG) IN HEALTHY VOLUNTEERS

Reference: Volume 61
Investigators:
Study Location:
Objective:
1.To compare the pharmacokinetic parameters after administration under fasting or non-fasting conditions.
2.To compare efficacy of the compound in fasting to non-fasting conditions.
Study design:
This is a randomized open-label two-way crossover study in 12 healthy male volunteers (caucasians) of age 18-32 years. In the first arm of the study, a single 400 mg dose of clopidogrel was administered as eight 50 mg tablets taken with a glass of water under fasting conditions (and remaining so for another 4 hours), while the second arm consisted of the same administration at the end of a meal containing 50g bread, 100g potatoes, 1 egg, 50g York ham, 1 yoghurt, 1 fruit (approx. 700 calories with 30g protein, 47g fats, 60g carbohydrate). The interval between two treatment periods was 14 days.

Batch #s: Clopidogrel 50 mg tablet: 1A1 RFF16

Blood samples were drawn for the determination of plasma concentration of SR25990 and SR26334 (carboxy metabolite of clopidogrel) at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours after dosing. ADP-induced platelet aggregation was measured at 0, 2, 4, 24, 48, 72 and 192 (upto 72 hours for second administration) hours after dosing. Bleeding time was determined at 5 and 24 hours after dosing. Pharmacokinetic parameters that were compared using ANOVA were AUC, Cmax, Tmax, t1/2 and MRT. Student's paired t-test was used to detect differences between plasma concentrations for the two treatments at each time point. Wilcoxon's non-parametric test was used to compare Tmax values. Comparison of aggregation and bleeding times were also undertaken using ANOVA.

Results:
ASSAY PERFORMANCE:

Mean (stdev) PK parameters for SR26334 are provided in the following table:

Parameter	Fasting	Non-Fasting	Statistical Significance
AUC_{0-} (mg.hr/l)	47.88 (11.52)	50.82 (13.63)	ns
C_{max} (mg/l)	12.34 (2.94)	10.56 (2.18)	ns
Tmax (hr)	1.04 (0.35)	1.50 (0.71)	ns
MRT (hr)	7.23 (1.39)	7.75 (2.04)	ns
T1/2 (hr)	7.71 (1.96)	7.38 (2.19)	ns

Mean plasma concentration profiles are shown in the figures below:

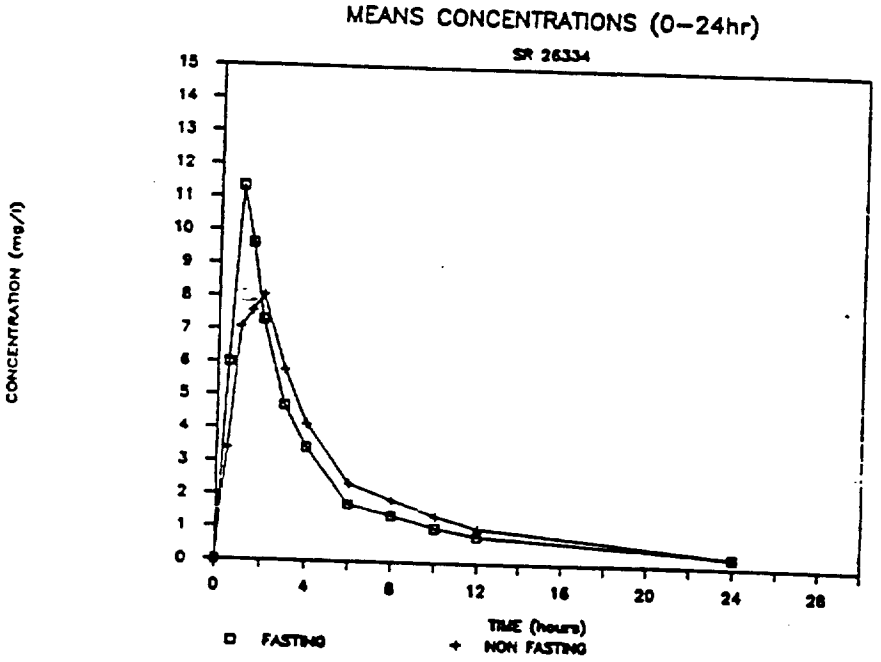


Figure (14.5)1

Mean plasma levels of SR 26334 after SR 25990C administration in fasting and non fasting conditions.

Percentage inhibition (based on time zero) of 5 µmol/l ADP-induced platelet aggregation following administration of clopidogrel under fed and fasted conditions are shown in the following table:

Treatment	2 hours	4 hours	24 hours	48 hours	72 hours	192 hours
Fasting	43	69	63	53	53	3
Non-Fasting	40	68	54	56	40	9

There was no significant difference in aggregation inhibition after administration of clopidogrel either under fasted or non-fasted conditions.

Bleeding time (seconds) following clopidogrel administration under fasted or non-fasted conditions is shown below:

Time of Measurement	Fasted	Multiplication factor	Non-Fasted	Multiplication factor
Before treatment	478		414	
5 hours after administration	1380	3	1200	3
24hours after administration	1080	2.4	900	2.2

No significant difference was noted in bleeding time after administration of clopidogrel either under fasted or non-fasted conditions.

Conclusions:
Co-administration with meals reduced the Cmax for SR26334 marginally, by about 14% and increased the Tmax by about 0.5 hours (from 1 to 1.5 hours). This is likely to be due to delayed gastric emptying time for the drug when it is administered with meals. AUC remained unchanged. Based on % inhibition of ADP induced platelet aggregation and bleeding times, administration under fasted or fed conditions showed similar results. In agreement with other study results, significant inhibition of platelet aggregation persisted upto about 72 hours (3 days) after dosing. Baseline values were regained by 8th day after drug administration.

Comments:
1. Although the meal used by the sponsor is not the typical meal generally recommended by the FDA for such studies, it appears to be a high calorie breakfast with adequate proportions of carbohydrate, fat and protein.

2. The sponsor should provide data on QC samples that are generally run with the plasma samples.

STUDY P1331: (PHARMACOKINETIC STUDY IN YOUNG, ELDERLY (WITHOUT ARTERIOPATHY), AND ELDERLY (WITH ARTERIOPATHY) SUBJECTS

COMPARISON OF PHARMACOKINETICS AND ANTIPLATELET ACTIVITY OF SR 25990 (CLOPIDOGREL) ADMINISTERED AS 75 MG ONCE DAILY FOR 10 DAYS (50MG + 25MG TABLETS), IN THREE POPULATION GROUPS

Reference: Volume 20
Investigator:
Study Location:

Objective: To assess in three different populations,
1. Platelet aggregation and bleeding time
2. Clinical and biological tolerability
3. Pharmacokinetic profile at steady state

Study design:
This is a non-randomized open-label parallel group study in healthy young (N=10, age 18-35y), elderly without arteriopathy (N=10, age >65y) and elderly with arteriopathy (N=10, age >65y), male subjects. A daily dose of 75mg (50mg + 25mg tablet) was administered for 10 days , once daily before breakfast. A follow-up continued for a further 14 days after cessation of study treatment.
Batch #s: Clopidogrel-50 mg tablet: RFN25, RGE17, RGN23; REF 1A1
Clopidogrel 25 mg tablet: RFN15, RGE18, RGN10; REF 1A1

Assessments for platelet aggregation (ADP conc. 1,2, and 5 μM, collagen 10, 20 μg/ml) were made pre-dose and then daily during the treatment period (days 1-9: 2 hours after the dose, day 10: 2, 5, 24 hours after dose) and on alternate days during follow-up, except weekends. Bleeding time assessments were made pre-dose and on days 10 (5 hours after dosing) and 18 (at 10 am). Plasma concentration of clopidogrel (not detectable in majority of the subjects) and SR26334 (carboxy metabolite of clopidogrel) were determined daily before dosing (except weekends) and on day 10 at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 24, 36, 48 and 72 hours post-dose. Pharmacokinetic parameters determined were Cssmin, Cssmax, Tmax, AUC(0-24) and t1/2. Maximum % aggregation and velocity for each agonist was studied. Pharmacokinetic parameters were compared using Duncan's test (conc. and AUCs), Kruskal Wallis test (Tmax) and Spearman correlation coefficient (AUC vs. Clcr, Cmax and AUC vs maximum aggregation).

Demographic characteristics for subjects are as follows:

Characteristic	Healthy volunteers	Elderly subjects	Arteriosclerotics
n*	12	10	10
Age (years)	24.21 ± 0.86**	75.57 ± 1.92	75.69 ± 1.01
Weight (kg)	68.42 ± 1.47	61.50 ± 1.56	66.70 ± 2.19
Height (cm)	176.42 ± 2.15	164.60 ± 1.42	168 ± 1.79

mean ± SEM

At inclusion, ADP aggregation was significantly lower for young subjects than in the elderly population (Duncan's Test); the velocity of aggregation in response to increasing agonist concentrations was higher in the elderly, as well:

Result	Healthy volunteers	Elderly subjects	Arteriosclerotics	p[b]
ADP 1µM				
n	10	10	10	
% Max	10.00±1.83[a]	26.80±7.12	20.70±5.64	0.0984
velocity	9.50±2.20	19.60±3.92	13.70±3.18	0.0970
ADP 2µM				
n	10	10	10	
% Max	20.80±3.76	52.90±8.03	34.10±4.49	0.0020**
velocity	18.80±2.99	31.30±3.86	22.50±2.98	0.0358*
ADP 5µM				
n	10	10	10	
% Max	43.00±4.12	70.60±4.88	67.10±7.13	0.0027**
velocity	28.30±3.30	38.30±3.35	35.70±2.91	0.0894
Collagen 10µg/ml				
n	10	10	10	
% Max	44.10±10.94	55.50±8.37	24.40±6.86	0.0596
velocity	20.10±5.37	21.80±3.93	9.10±2.48	0.0767
Collagen 20µg/ml				
n	10	10	10	
% Max	44.80±9.12	69.20±5.65	60.20±7.11	0.0813
velocity	17.40±4.05	28.20±3.59	19.80±3.39	0.1129 NS

At steady state on drug, the data revealed a significant reduction (from baseline) of the maximum % aggregation (t-test for paired data). The decrease appears less marked in the young subjects as compared to the elderly:

Percentage inhibition of aggregation (%)	Healthy volunteers	Elderly subjects	Arteriosclerotics	p*
n	10	10	10	
Mean	- 25.8	- 41.3	- 39.5	0.1087 NS
SEM	3.9	5.2	6.9	
Minimum	- 44.0	- 58.3	- 69.3	
Maximum	- 7.7	- 16.3	- 6.0	

* ANOVA
NS : not significant

Although not statistically significant, a similar trend is observed for velocity of aggregation:

CHANGE IN VELOCITY AT STEADY STATE (ADP 5 µM)

Differences/ inclusion	Healthy volunteers	Elderly subjects	Arteriosclerotics	p* (ANOVA)
n	10	10	10	0.3563 NS
Mean	-11.0	-17.1	-15.8	
SEM	3.03	3.30	2.95	
Minimum	-29.3	-41.5	-36.7	
Maximum	-1.0	-6.3	-7.0	

The mean bleeding time between groups was not significantly different at pre-treatment. At steady state on drug, there was a significant lengthening of bleeding time in all groups and was similar across groups.

GROUP	INCLUSION	DAY 10	PROLONGATION FACTOR
Healthy volunteers	7:54±0:18* (n=10)	11:54±0:22 (n=10)	1:52
Elderly subjects	8:09±0:17 (n=10)	12:51±0:21 (n=10)	1:59
Arteriosclerotics	8:42±0:15 (n=10)	12:42±0:20 (n=10)	1:47

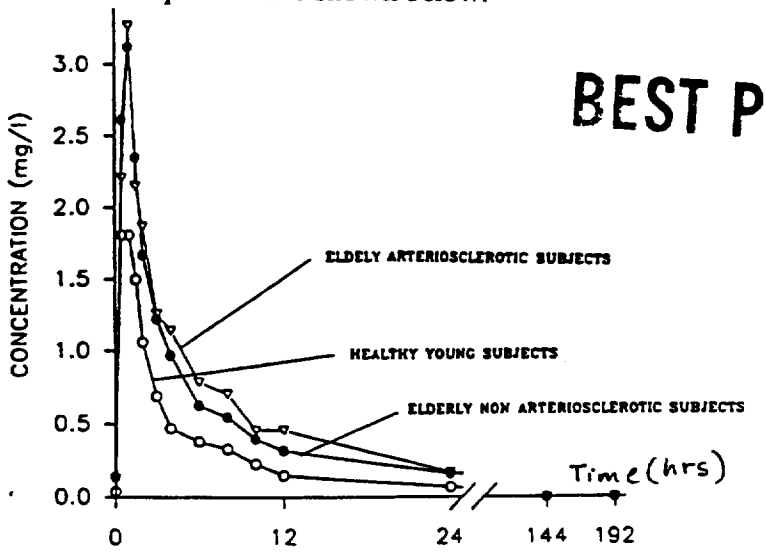
* mean ± SEM

Mean (stdev) PK parameters[a] of SR 26334 for day 10 are provided in the following table:

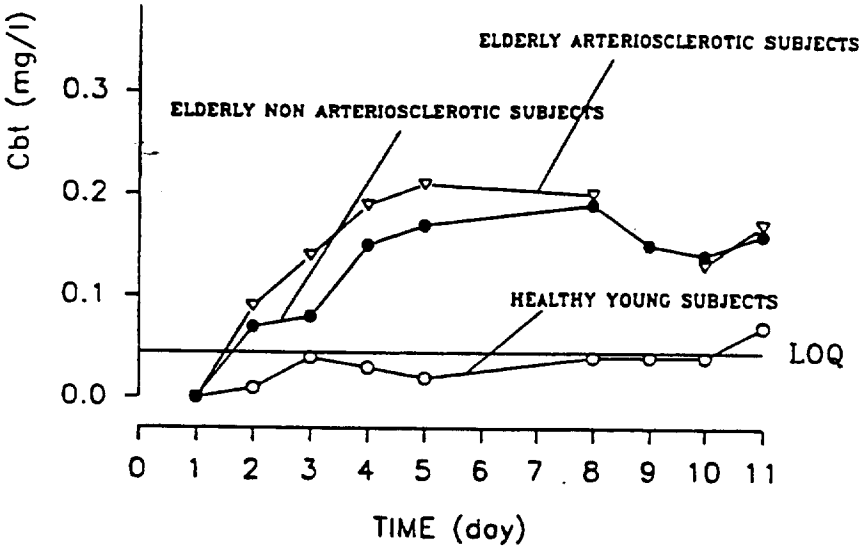
Parameter	Young	Elderly	Elderly arteriosclerotic
AUC_{0-24} (mg.hr/l)	8.33 (1.94)	14.45 (4.86)	16.97 (3.16)
C_{max} (mg/l)	2.65 (1.02)	3.39 (0.71)	3.47 (0.55)
Cmin (mg/l)	0.04 (0.05)	0.13 (0.09)	0.12 (0.08)
Tmax, hours	1 (0.5-1)	1 (0.5-1.5)	1 (0.5-1.5)

[a] Tmax is presented as mean (range)

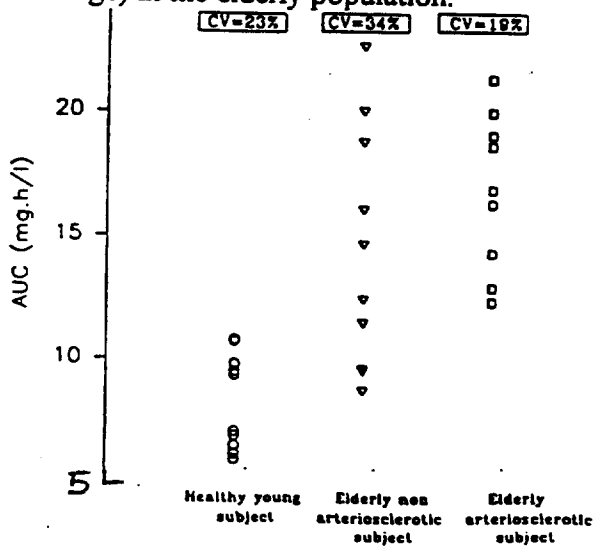
Mean plasma concentration profiles are shown below:

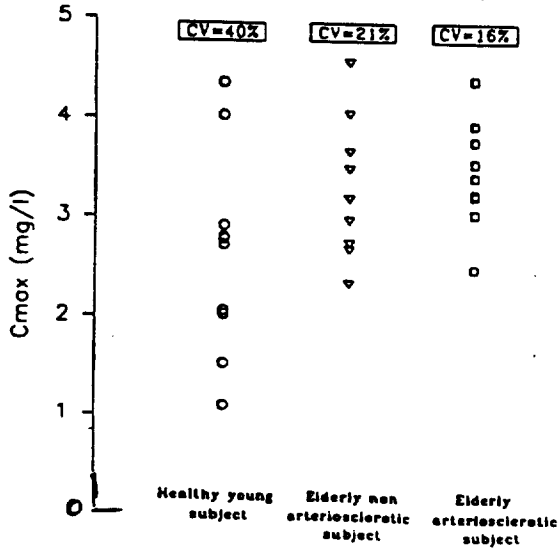


Based on concentration measurements before drug administration (Cmin), illustrated in the following figure, there appears to be a significant accumulation of drug in the elderly population, as compared to the young. This is an expected outcome of longer t1/2 in the elderly population.

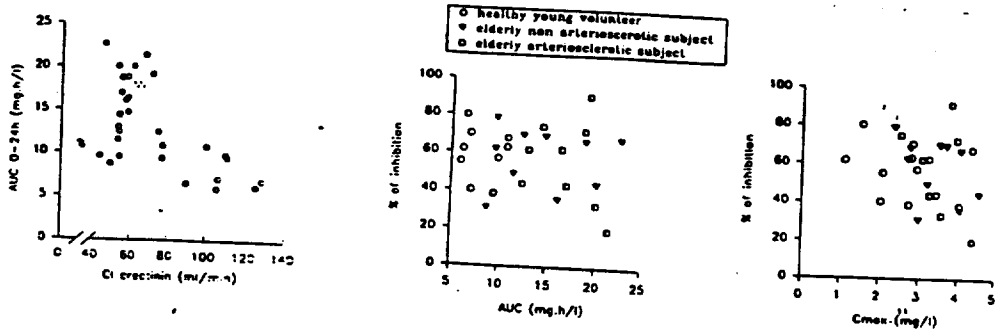


Cmax and AUC values were also higher (30% higher for Cmax and 75-100% higher for AUC, on average) in the elderly population.





Creatinine clearance, calculated as {(140-age) * wt/(72 * blood creatinine)}, correlated with age, however, no correlation was detected between the pharmacokinetic parameters and % inhibition of maximum amplitude of aggregation with ADP:



Conclusions:

Based on pre-treatment data, the elderly subjects appear to be more susceptible to ADP and collagen induced platelet aggregation. At steady state on clopidogrel, the decrease in maximal aggregation compared to pre-treatment was also higher in the elderly population. A similar trend (higher response in elderly) was also perceived for velocity of aggregation . This may lead one to conclude that the elderly population is more susceptible to the platelet aggregation and also more sensitive to the treatment. Dosage adjustment or caution should be considered. There appeared to be accumulation of SR 26334 in the elderly population but not in young subjects. An inverse correlation existed between creatinine clearance and AUCs however, in the concentration ranges available, AUCs and Cmax values did not correlate with the inhibition of aggregation.

Comments:

1. While the study ended in 6/1990, some lots used in the study had expiration dates of 5/1990.
2. Note that the alcohol consumption was more prevalent in the elderly groups (4/10 eldery, 9/10 elderly with arteriopathy) as compared to young (1/12) subjects. Five subjects (3 elderly and 2 with arteriopathy) were on co-medications (lorazepam, nitrazepam, flunitrazepam).
3. "velocity of aggregation" is not defined.
4. The sponsor determined terminal t1/2 for all subjects however this parameter was not reported. Examination of these numbers indicate a higher average terminal t1/2 in the elderly as compared to young subjects (15 and 9.7 hours resp.). Although variability was high (cv 50%), this seems to be the likely cause of higher accumulation in the elderly population. Elderly patients had an overall average t1/2 of 22 hours however, 106 hours t1/2 was calculated in one of the subjects. Excluding this value, average t1/2 was 11 hours in this group.

STUDY IRN2194: (PHARMACOKINETIC STUDY IN PATIENTS WITH CHRONIC RENAL FAILURE)

COMPARISON OF PHARMACOKINETICS, TOLERABILITY AND ANTIPLATELET ACTIVITY OF SR 25990 (CLOPIDOGREL) ADMINISTERED AS 75 MG TABLET ONCE DAILY FOR 8 DAYS IN CHRONIC RENALLY IMPAIRED PATIENTS

Reference: Volume 54
Investigator:
Study Location:

Objective: To assess in chronic renal failure patients,
1. Tolerability and anti-aggregation effect
2. Pharmacokinetic profile of metabolite at steady state

Study design:
This is a non-randomized study. Two groups, with 8 patients in each group (total 16), were included in the study. Group 1 was patients with Clcr of 5-15 ml/min (severe) and Group 2 was patients with Clcr of 30-60 ml/min (moderate), (Cockroft Gault). Patients went through 15 days of run-in phase, 8 days of treatment period, where a dose of 75mg of clopidogrel was administered for 8 days, once daily before breakfast. A follow-up continued for a further 14 days after cessation of study treatment.

Batch #s: Clopidogrel 75 mg bi-convex film coated tablet: 2Q2, batch J789F

Pharmacokinetics were evaluated for SR26334 from blood and urine. Blood samples were collected pre-dose on days 1, 3, 5 and 8 and at 0.5, 1, 1.5, 2, 4, 8, 12, 24, 48 and 96 hours after the last administration on day 8. Urine was collected before treatment and on day 8 (0-8 hours and 8-24 hours post-dose). Pharmacokinetic parameters determined were Cbt (pre-dose levels on dosing days), Cmin (day 8), Cmax (day 8), Tmax, AUC(0-24), t1/2, Ae(0-24) and Clr (renal clearance). Assessments for inhibition of platelet aggregation (5 μM ADP induced) and bleeding time were made at pre-dose and at 2 hours following blood sampling on days 1, 3, 5, 8, 9, 12, 15, 20. Clinical tolerability was assessed by occurrences of adverse events, vital signs, physical exam, ECG, bleeding time, hemogram and urinalysis. Only Cmax and Tmax were reported for SR25990C.

Changes in aggregation were reported as % inhibition, bleeding time and pharmacokinetic parameters were compared using t-test, Kruskal Wallis test (Tmax) and Spearman correlation coefficient (AUC, Cmax and Clr vs. Clcr).

Demographic characteristics for subjects are as follows:

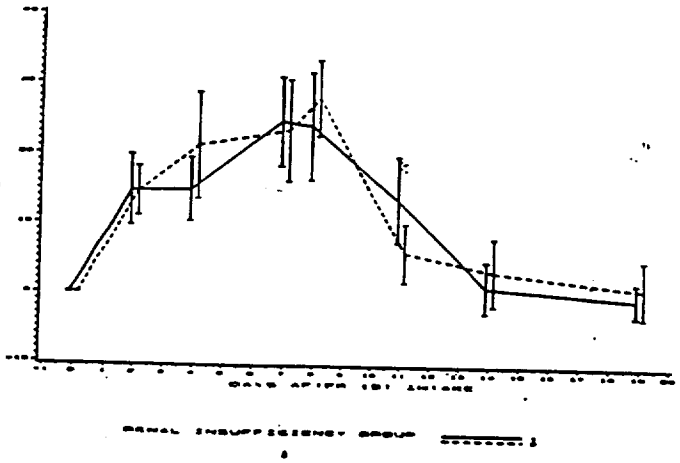
GROUP	CENTRE PATIENT	DATE OF BIRTH (dd/mm/yy)	SEX	AGE (years)	RACE	HEIGHT (cm)	WEIGHT (kg)	Clcr
1	10001	25/11/39	Female	53	Black	166.00	67.40	
	10002	04/06/23	Male	70	Caucasian	165.00	74.00	
	10005	11/03/21	Male	72	Caucasian	168.00	73.00	
	10011	13/05/35	Female	58	Caucasian	156.00	84.00	
	10012	06/06/54	Male	39	Black	179.00	87.00	5-15 ml/min
	10014	02/02/62	Female	32	Black	168.00	76.00	
	10015	28/08/35	Male	58	Caucasian	170.00	56.00	
	10016	16/12/37	Female	56	Caucasian	146.00	70.00	
2	10003	25/07/20	Male	73	Caucasian	163.00	73.00	
	10004	14/05/27	Female	66	Caucasian	163.00	52.00	
	10006	03/06/58	Male	35	Black	168.00	70.00	
	10007	12/12/47	Male	45	Caucasian	177.00	59.00	
	10008	18/07/47	Male	46	Caucasian	168.00	72.00	
	10009	03/06/31	Male	62	Caucasian	167.00	70.00	30-60 ml/min
	10010	01/01/32	Male	62	Caucasian	161.00	63.00	
	10013	14/09/45	Female	48	Caucasian	168.00	58.00	

Following are the ADP induced aggregation (pre-treatment) and its inhibition for the 2 chronic renally insufficient groups, mean (SEM) :

Group	baseline	Day 3	Day 5	Day 8	Day 9	Day 12	Day 15	Day 20
1[a]	77.3(2.7)	66.1(5.2)	66.4(5.2)	58.9(6.2)	59.4(7.1)	67.5(6.3)	76.1(2.7)	77.4(1.5)
2[a]	77.1(2.7)	65.6(3.1)	59.6(4.6)	58(5.7)	55.3(4.2)	72.1(2.6)	74(2.2)	76(2.2)
1[b]	-	14.8(5.1)	15(4.6)	24.7(6.3)	24(7.6)	13.5(6.2)	1(3.6)	-0.7(2.3)
2[b]	-	14.7(3.6)	21.3(7.6)	23.5(7.2)	28(5.4)	5.8(4.2)	3.1(4.8)	0.8(4.1)

a: maximum % induction of aggregation, b: inhibition of maximum % of induced aggregation

Although there were some differences between groups on specific days, the variability is high. Overall, the inhibition of platelet aggregation between the 2 groups is similar. Baseline values are approached by day 15 as illustrated below:

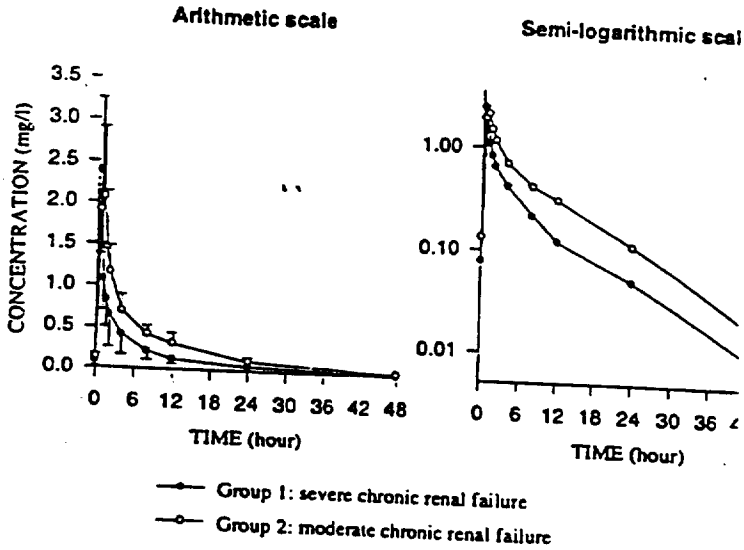


Mean plasma concentrations for the 2 groups before drug administration over the study period are reported in the table and figure below. Although the average values appear higher for the moderate patients, this was not statistically significant.

Mean and standard deviations (SD) of Cbt are presented below (n=8 per group):

	Cbt (mg/l)			
	Day 3	*Day 5*	*Day 8*	*Day 9*
Group 1	0.080 (0.110)	0.074 (0.058)	0.077 (0.056)	0.051 (0.032)
Group 2	0.111 (0.055)	0.119[a] (0.043)	0.131 (0.044)	0.115 (0.048)

a: n=7 (technical problem during the assay for Patient 10008)



Mean (SD) PK parameters of SR 26334 and mean Cmax and Tmax for SR25990 for day 8 are provided in the following tables:

SR 26334

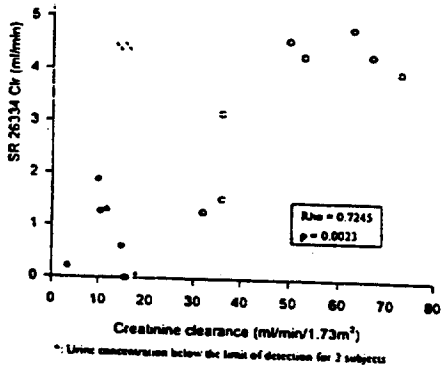
Patient (no.)	Cmax (mg/l)	Tmax (h)	Cmin (mg/l)	AUC 0-24h (mg.h/l)	T1/2 (h)	Ae 0-24h (mg)	CLR 0-24h (ml/min)
Group 1							
1							
2							
5							
11							
12							
14							
15							
16							
Mean	2.207	0.56	0.049	6.192	9.41	0.364	0.75
SD	1.038	0.18	0.033	2.368	5.65	0.419	0.74
Median	2.238	0.50	0.047	6.133	7.23	0.276	0.60
CV (%)	47	NR	67	38	60	115	99
Group 2							
3							
4							
6							
7							
8							
9							
10							
13							
Mean	2.591	0.94	0.108	11.027	8.25	2.288	3.49
SD	0.951	0.56	0.048	2.857	1.31	1.163	1.38
Median	2.677	0.75	0.106	11.180	7.82	2.237	4.13
CV (%)	37	NR	44	26	16	51	40

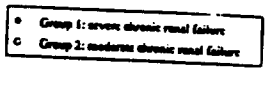
Group 1 : Severe chronic renal failure: 5 < creatinine clearance < 15 ml/min/1.73m²
Group 2 :Moderate chronic renal failure: 30 < creatinine clearance < 60 ml/min/1.73m²
: not determined (les than 3 points lined up on the regression curve)

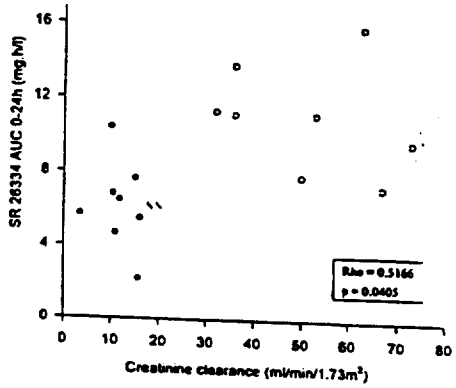
Patient (no.)	Age (yr)	Cmax (ng/ml)	Tmax (h)
Group 1			
1	54	9.640	0.52
2	70	2.670	0.50
5	73	4.150	0.50
11	38	3.840	0.50
12	34	1.010	0.50
14	32	3.790	0.50
15	58	7.910	0.50
16	57	0.000	-
Mean		4.126	0.50
SD		3.246	0.01
Median		3.815	0.50
CV (%)		79	NR
Group 2			
3	73	3.750	1.00
4	66	5.850	0.50
6	35	2.420	1.00
7	46	0.000	-
8	46	1.600	2.00
9	62	3.550	0.50
10	62	2.330	1.50
13	49	2.130	0.50
Mean		2.704	1.00
SD		1.723	0.58
Median		2.375	1.00
CV (%)		64	NR

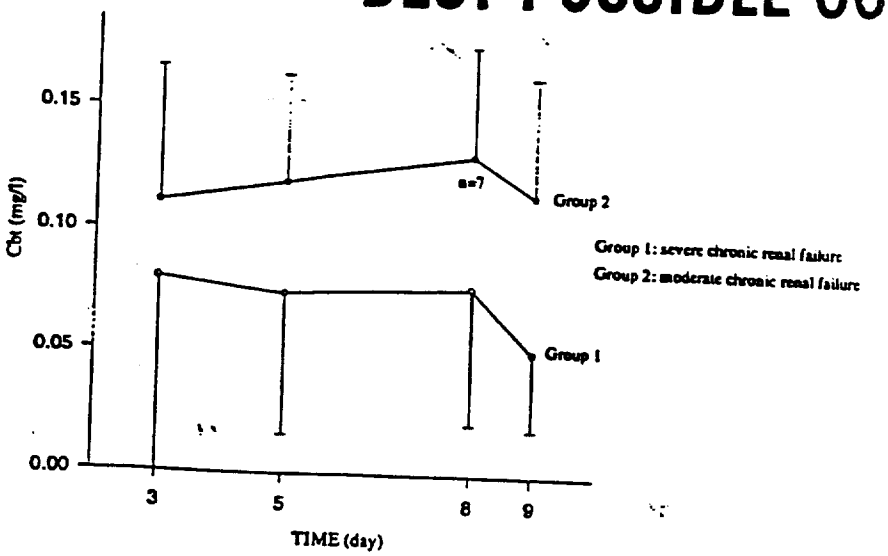
SR 25990

AUC values appear higher in moderate patients. Protein binding data is not available in the patients. Amount excreted in urine and renal clearance are lower for the severe patients. Renal clearance of the metabolite is proportional to creatinine clearance, and therefore renal function status of patients. Creatinine clearance did not correlate to Cmax values but, interestingly, unlike the relationship shown in the study with elderly subjects, there appears to be a positive correlation of AUC with creatinine clearance. This is contrary to what is expected.









Conclusions: As observed before, creatinine clearance correlated with the renal clearance of the drug. Contrary to the expectation however, an inverse relation was not observed for creatinine clearance and AUC. Cbt values were also higher in the milder group. A contributing factor may be high variability. There was no significant difference in the pharmacodynamic measurements for the 2 groups.

Comments:

1. It has been established that there is bleeding time prolongation in uremia. This is the purpose of the trial. Note however, that a control group is not included in the study.

2. Aspirin, anti-aggregating agents, NSAIDS, heparin, EPO and oral anticoagulants were prohibited during the 15 days preceding the screening and during the entire trial.

3. Patients in group 1: subject 10002 recorded a subconjunctival hemorrhage on day 2, subject 10005 had a vasovagal attack (sweating, stomach cramp, hypoglycemia) on day 3.

4. Cmax and Tmax values were reported for the parent drug. Although the overall average Cmax appeared higher for severe patients (4.13 vs 2.7 ng/ml), this seems to be due to subjects 1 and 15 who had Cmax of 9.64 and 8 ng/ml resp.

5. No control group. However, comparing the data on renal patients with the normals from other 2 studies I reviewed, here is what I see:

Bleeding time: vol 1.20/p249 and vol 1.54/p39: young normals and elderly normals at baseline: about 480 seconds; renal patients at baseline: about 180-280 seconds. Maximum change with drug for normal young and eldery +240 to 280 seconds; renal patients about +300 seconds.

Platelet aggregation: vol 1.20/p202, vol 1.54/p36 : 5 uM ADP induced platelet aggregation 77% for renal patiens at baseline and for young and elderly normals is about 50% and 75% resp. On drug, (vol 1.20/p214 and vol 1.54/p36) difference from inclusion at SS, about -30% and about -48% for young and elderly resp, and about -20% for both groups in renal impairment.

STUDY P1722: (DRUG INTERACTION STUDY WITH DIGOXIN)

EVALUATION OF THE INFLUENCE OF CLOPIDOGREL ON PLASMA CONCENTRATIONS OF DIGOXIN AFTER REPEATED ADMINISTRATION

Reference: Volume 65
Investigator:
Study Location:
Objective:
1. To assess the influence of clopidogrel on steady state digoxin plasma concentrations.
Study design:

This is an open label fixed sequence multiple dose design study in 12 healthy male volunteers of age 20-36 years. The participants received 0.25 mg digoxin once daily from day 1 up to and including day 20. From day 11 up to and including day 20, 75 mg clopidogrel once a day was coadministered. Both the treatments were administered under fasting conditions with 200 ml water after a 10 hour overnight fast. Plasma and urine samples for the assay of digoxin were collected on day 10 and day 20. ADP induced thrombocyte aggregation test was performed at screening and at days 18, 19 and 20 days two hours post dose administration.
Batch #s: Clopidogrel 75 mg tablet: batch # 1A1 RHG08
Lanoxin 0.25 mg tablet: batch# 90H17.

Blood samples were drawn for determination of plasma concentration of digoxin at 0, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 24, after dosing. Urine samples were collected at 0 - 4, 4 - 8, 8 - 12, 12 - 24 hours after dosing. ADP-induced platelet aggregation was measured at 0, 2, 5, 24, 48 and 72 hours after dosing. Bleeding time was determined at 0 and 5 hours after dosing. Pharmacokinetic parameters were determined by non-compartmental methods. These parameters with and without clopidogrel were compared by the sponsor using two way ANOVA model determining the effect of day and subject as a source of variation. A 90 % CI for the ratio of the treatment mean of AUC_{0-24} on day 20 over the treatment mean of AUC_{0-24} on day 10.

Conclusions:

Coadministration of 75 mg clopidogrel with 0.25 mg digoxin at steady-state did not have any effect on the pharmacokinetics of digoxin.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

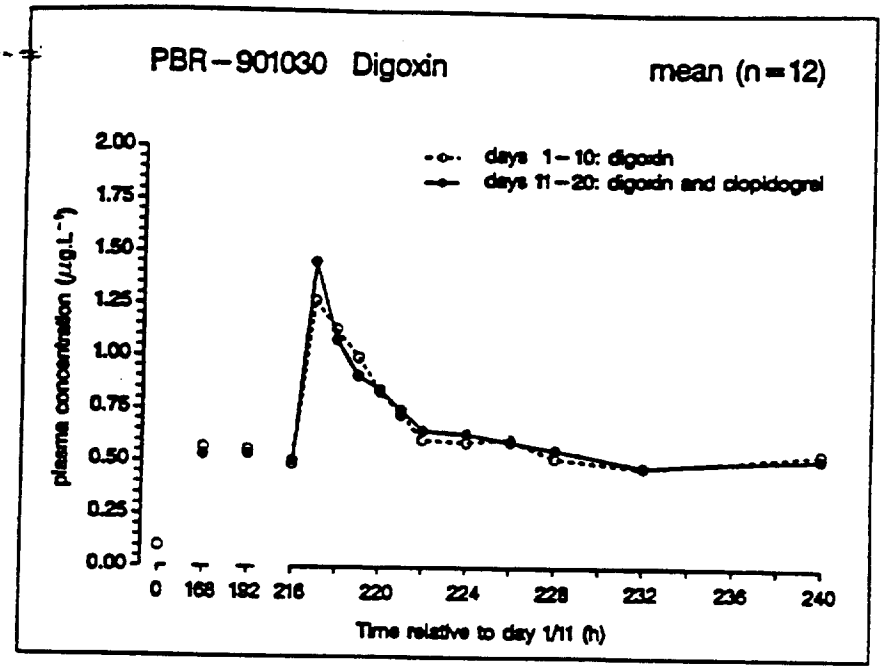


Figure 1. Mean digoxin plasma concentration-time curves during multiple oral administration of 0.25 mg digoxin daily from day 1 through day 20
Days 1-10 = without clopidogrel
Days 11-20 = during 75 mg clopidogrel daily from day 10 through day 20
Mean data, n = 12

Table 1. Mean pharmacokinetic parameters and summary statistics of digoxin as determined during multiple oral administration of 0.25 mg digoxin daily from day 1 through day 20
Days 1-10 = without clopidogrel
Days 11-20 = during 75 mg clopidogrel daily from day 10 through day 20
Mean data, n = 12

Parameter			geometric mean	Range	90%-confidence interval and point estimate of ratio (%)*	
c_{max}	($\mu g.L^{-1}$)	Day 10	1.42	1.14 - 1.92		
		Day 20	1.57	1.28 - 1.95	99.4 - 124.0	111.0
c_{min}	($\mu g.L^{-1}$)	Day 10	0.44	0.35 - 0.54		
		Day 20	0.44	0.32 - 0.58	91.9 - 107.8	99.6
AUC_{0-24}	($\mu g.L^{-1}.h$)	Day 10	15.17	12.61 - 18.54		
		Day 20	15.41	11.36 - 19.36	96.2 - 107.3	101.6
A^s_{24}	(mg)	Day 10	0.108	0.074 - 0.160		
		Day 20	0.107	0.077 - 0.174	94.0 - 103.5	98.7
t_{max}	(h)	Day 10	1.0**	0.5 - 2.0		
		Day 20	1.0**	0.5 - 2.0		

* 90%-confidence interval for ratio of means of test (day 20) and reference (day 10) (from ANOVA on log-transformed data of c_{max}, c_{min}, AUC_{0-24} and A^s_{24})

** median

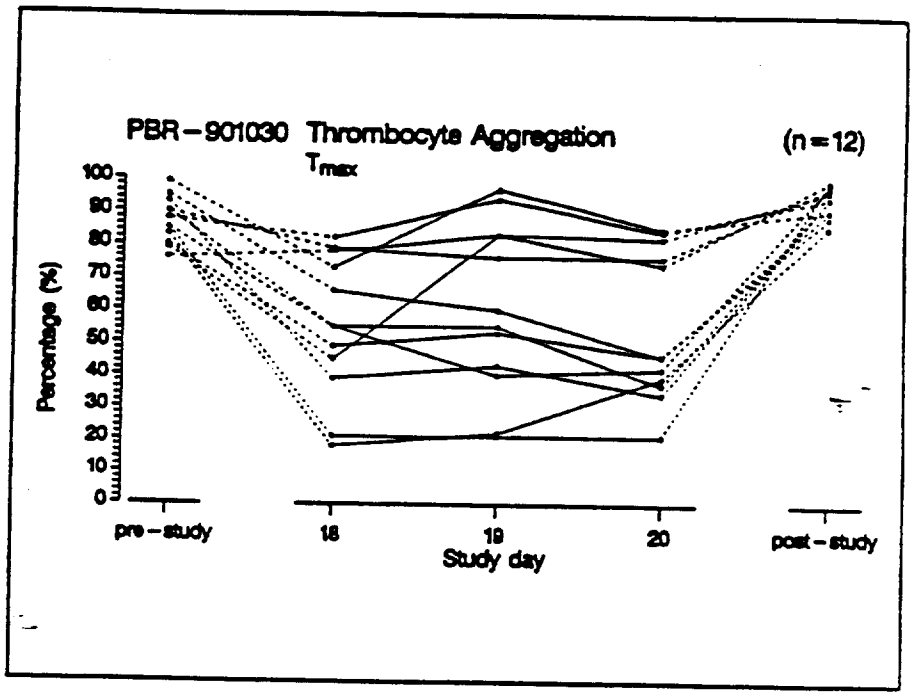


Figure 2. Individual ADP induced thrombocyte aggregation (T_{max}) versus time profiles during multiple oral administration of 0.25 mg of digoxin daily from day 1 through day 20

Days 1-10 = without clopidogrel

Days 11-20 = during 75 mg clopidogrel daily from day 10 through day 20

STUDY INT 1980: (DRUG INTERACTION STUDY WITH THEOPHYLLINE)

STUDY OF THE EFFECT OF SR 25990C AFTER A SINGLE INTAKE AND AT STEADY STATE ON THEOPHYLLINE AT THE STEADY STATE IN YOUNG HEALTHY VOLUNTEERS.

Reference: Volumes 66-67.
Investigators:

Study Location:

Objective:

1. To evaluate the effect of SR 25990C after a single intake and at steady state on theophylline blood levels in young healthy male volunteers.

Study design:

This is an open label fixed sequence multiple dose design study in 12 healthy male volunteers of age 18-35 years. The participants received 300 mg capsule of theophylline orally in the morning before breakfast and in the evening before dinner for thirteen days and on the morning of day 14. Moreover, each participants received a 75 mg clopidogrel tablet in the morning before breakfast for ten days (from day 5 to day 14). ADP induced thrombocyte aggregation test was performed at screening and at days 5, 7, 9, 11 and 14 two hours post dose administration.

Batch #s: Clopidogrel 75 mg tablet: batch # J789F
Armophylline 300 mg sustained release capsule batch # DJ 1341.

Blood samples were drawn for determination of plasma concentration of theophylline before the morning input of days 1, 6, 7, 8, 9 and 12 and on the following times on days 4, 5 and 14: 0, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hours post dose administration. Pharmacokinetic parameters were determined by non-compartmental methods. Analysis of variance on repeated measurements was carried out on ADP induced aggregation data as was a Student's t test on the percentage of inhibition of aggregation in relation to time 0 at day 1. The influence of clopidogrel on the pharmacokinetics of theophylline was studied by comparing CMAX and AUC_{0-12} on day 4 (theophylline alone) and those on day 5 and 14 (theophylline +clopidogrel) using a Student's t test. Moreover, 90 % confidence intervals were calculated on the ratios of the geometric means of CMAX, CMIN and AUC_{0-12} .

Plot of mean concentration profiles for theophylline without and with clopidogrel (single and multiple dose) are given in Figure 1. Table 1 gives a summary of the main pharmacokinetic parameters for theophylline along with the corresponding 90 % confidence intervals.

TABLE 1

	CMAX µg/ml.	CMIN µg/ml.	AUC_{0-12} µg.hr/ml.
Theophylline	10.7	6.1	102.2
Theophylline +single dose clopidogrel	10.5 (.86-1.13)[a]	6.2 (0.9-1.15)	102 (0.89-1.11)
Theophylline + multiple dose clopidogrel	10.6 (0.86-1.14)	6 (0.87-1.12)	102.6 (0.9-1.12)

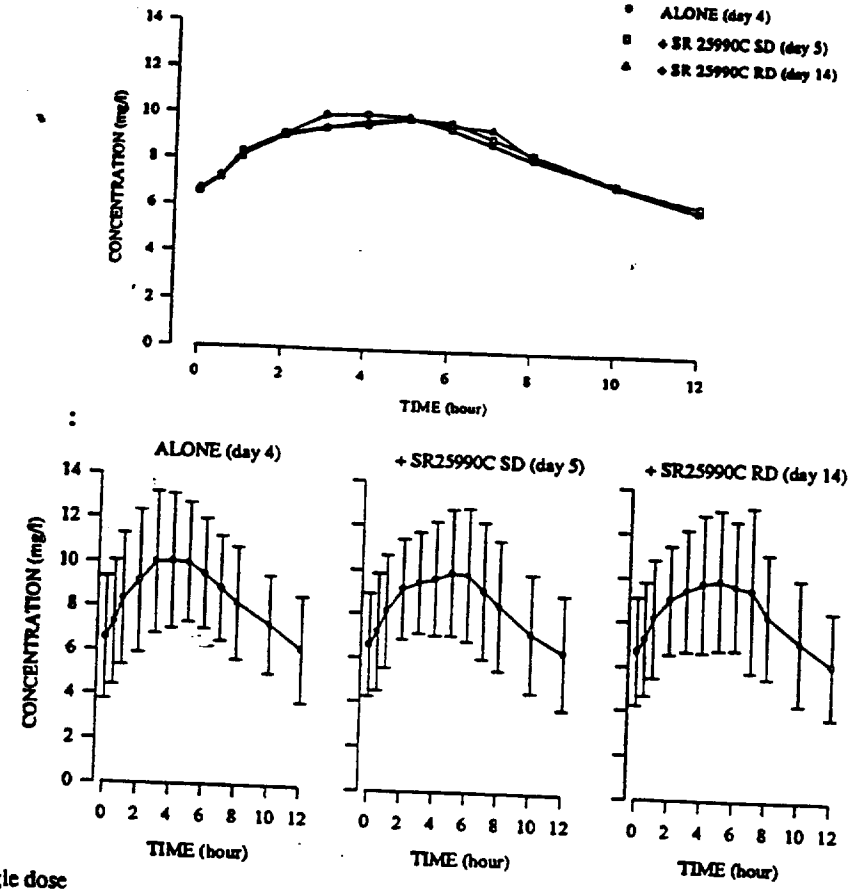
a=90 % confidence intervals of the ratios of the geometric mean of theophylline/clopidogrel.

It is to be noted that one of the subjects had to be withdrawn from the study due to bleeding times exceeding 20 minutes. This was linked to the pharmacodynamic activity of clopidogrel.

Conclusions:

Coadministration of 75 mg clopidogrel either in single or multiple dose did not have any effects on the steady state pharmacokinetics of theophylline at a dose of 300 mg twice daily.

APPEARS THIS WAY
ON ORIGINAL



SD: single dose
RD: repeated dose

Figure 1: Superimposed means of plasma concentrations of theophylline (above) and with standard deviations (below) as a function of time after repeated administration of theophylline alone (D04), with a single 75-mg dose of SR 25990C (D05), and with 75 mg/day of SR 25990C for 10 days (D14)

STUDY P1978: (DRUG INTERACTION STUDY WITH ANTACID)

STUDY OF THE INFLUENCE OF ANTACID INTAKE ON THE BIOAVAILABILITY OF A SINGLE 75 MG DOSE OF SR 25990C (CLOPIDOGREL) IN HEALTHY VOLUNTEERS.

Reference: Volume 62-64.
Investigator:
Study Location:

Objective:

1. To assess whether antacid (Maalox) one hour before administration of SR 25990C, modifies the bioavailability of SR 25990C.

Study design:

This is an open label randomized crossover study in 12 healthy male volunteers of age 18-30 years. Each participant was randomized to the following two treatments:
Treatment 1: After an overnight fast each subject received 75 mg clopidogrel with 150 ml of water.
Treatment 2: After an overnight fast each subject received 2 400 mg tablets of Maalox to be chewed then 1 hour before a 75 mg tablet of clopidogrel with 150 ml of water.
Batch #s: Clopidogrel 75 mg tablet: batch # J789F.
Maalox 400 mg tablet: batch# 0663.

Blood samples were drawn for determination of plasma concentration of SR25990 and SR 26334 on days 1 at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 10 and 12 hours post dose administration, day 2 at 16, 24 and 36 hours and day 3 48 hours post clopidogrel administration. Pharmacokinetic parameters were determined using non-compartmental methods. The influence of Maalox on the pharmacokinetics of clopidogrel and its metabolite SR26334 was studied using the Wilcoxon non parametric test. Moreover, 90 % confidence intervals were calculated on the ratios of the geometric means of the pharmacokinetic parameters of interest.

Plot of mean concentration profiles for SR26334 after clopidogrel administration with and without the coadministration of Maalox is shown in Figure 1 while the corresponding pk parameters are summarized in Table 1.

Table 1

	Clopidogrel	Clopidogrel + Maalox
CMAX (mg/l)	2.62	2.47 (0.74-1.16)
TMAX (hours)	.71	.0.67
AUC_{0-12} (mg*hr/l)	5.65	5.18 (0.86-0.96)
AUC_{obs} (mg*hr/l)	6.25	5.84 (0.89-0.97)

Conclusions:

It can be seen from the above results that coadministration of Maalox 800 mg with 75 mg clopidogrel did not have any effects on the plasma profile of metabolite SR 26334. As for the parent compound, most of the plasma samples were below the detection limit and thus calculation of the pharmacokinetic parameters of interest was not possible.

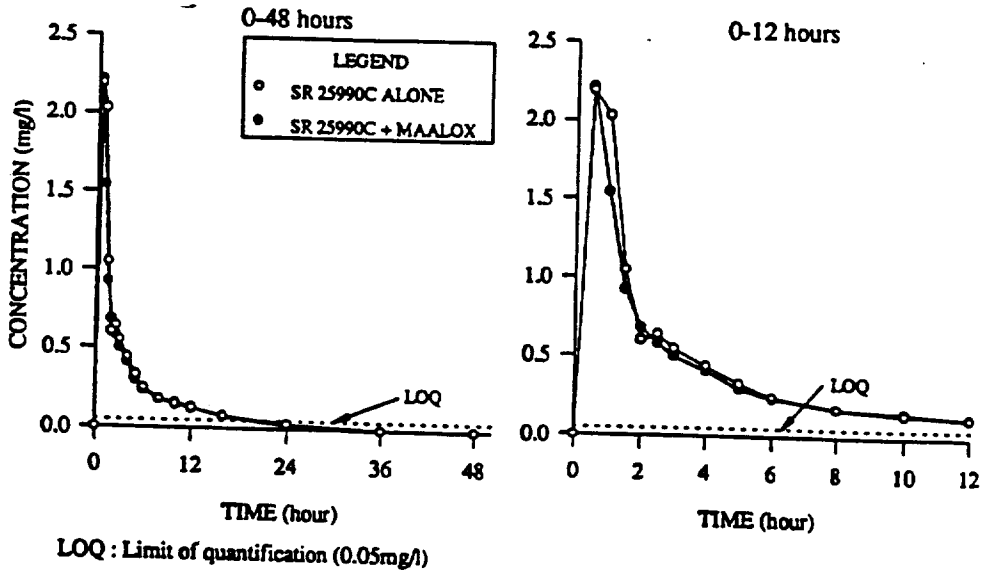


Figure 1 - Mean plasma concentrations of SR 26334 as a function of time after administration of 75 mg of SR 25990C alone and with 800 mg of Maalox® *(n=12)*
(left: 0-48h interval; right: 0-12h interval)

STUDY P1716: (DRUG INTERACTION STUDY WITH CIMETIDINE)

INTERACTION STUDY OF CIMETIDINE ON THE METABOLISM AND PHARMACODYNAMIC ACTIVITY OF SR25990C AFTER REPEATED ORAL ADMINISTRATION IN HEALTHY SUBJECTS.

Reference: Volume 68-70
Investigator:

Study Location:

Objective:

1. To determine whether cimetidine administration had an effect on platelet aggregation in subjects receiving therapeutic doses of SR 25990C.

2- To evaluate the effect of cimetidine on SR 26334 pharmacokinetic parameters in plasma. As cimetidine may inhibit SR 25990C metabolization, the influence of cimetidine on SR 25990 plasma levels was also investigated.

Study design:

This is an open label fixed sequence multiple dose design study in 18 healthy male volunteers of age 18-35 years. The participants received 75 mg clopidogrel once daily from day 1 up to and including day 28. From day 15 up to and including day 28, 400 mg cimetidine morning and evening was coadministered. Clopidogrel was administered under fasting conditions with 200 ml water after a 10 hour overnight fast. Cimetidine was administered at breakfast and during the evening meal. Plasma samples for the assay of SR 25990 were collected on day 14 and day 28. ADP induced thrombocyte aggregation test was performed before clopidogrel intake on days D1, D9, D10, D11, D14, D15, D17, D21, D23, D24, D25 and D28 and two hours after clopidogrel intake on D1, D14, D15 and D28 and also on Days 29 and 35. Aggregation test using collagen was performed on Day 1, D14 and D28 before drug intake then on days 29 and 35. Bleeding time using the modified Ivy-Nelson technique and APTT was performed two hours after clopidogrel intake on day 1, 14,28 and 35.

Batch #s: Clopidogrel 75 mg tablet: batch # 1720G expiration date June 24 1993.
Cimetidine 400 mg tablet: batch# BE 962 expiration date 30-11-1995.

Blood samples were drawn for determination of plasma concentration of SR25990 and SR 26334 on days 1, 9, 10, 11, 14, 15, 17, 21, 23, 24, 25, 28 and 29. Full plasma profiles on days 14 and 28 with samples drawn at the following times: 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 hours. On days 1 and 15 samples were collected 2 hours post clopidogrel administration. Pharmacokinetic parameters were determined using non-compartmental methods. The influence of cimetidine on the pharmacokinetics of clopidogrel and its metabolite SR26334 was studied using a Student's t test. Moreover, 90 % confidence intervals were calculated on the ratios of the geometric means of the pharmacokinetic parameters of interest.

Plasma samples:

Plot of mean concentration profiles for SR26334 after clopidogrel administration with and without the administration of cimetidine is shown in Figure 1 while the corresponding pk parameters are summarized in Table 1.

Table 1

	Clopidogrel	Clopidogrel +Cimetidine
CMAX (mg/l)	2.37	2.41 (0.81-1.25)
CMIN (mg/l)	1.06	.87
TMAX (hours)	.04	.06
AUC_{0-12} (mg*hr/l)	5.78	6.3 (1.01-1.19)
AUC_{obs} (mg*hr/l)	6.93	7.76 (1.01-1.26)

It can be seen from the above results that coadministration of cimetidine 400 mg bid with 75 mg clopidogrel did not have any effects on the plasma profile of metabolite SR 26334. As for the parent compound, most of the plasma samples were below the detection limit and thus calculation of the pharmacokinetic parameters of interest was not possible.
Table 2 shows the comparative analysis of the maximum thrombocyte aggregation % both for ADP and collagen induced while Table 3 shows the differences in mean maximum aggregation % between D35 and D1 as induced by ADP and collagen.
The above results show that there was a statistically significant increase in ADP induced maximum aggregation when cimetidine was added to clopidogrel. This increase ranged from 3.5 to 9.3 % in absolute value. However, the collagen induced values were not affected.
Table 4 shows that cimetidine did not have any effect on the prolongation of bleeding time observed with SR 25990C.

Conclusions:

Coadministration of 400 mg cimetidine bid for 14 days with 75 mg clopidogrel did not have any effects on the pharmacokinetics of SR 26334. Even though there was an increase ranging from 3.5 to 9.3 % in the ADP induced maximum % with the coadministration of cimetidine, this increase was not considered clinically significant because it was less than 10 %.

Moreover, there was no change in any of the other pharmacodynamic parameters with the coadministration of cimetidine. Therefore, no dosage adjustments are necessary for clopidogrel with the coadministration of twice a day 400 mg of cimetidine.

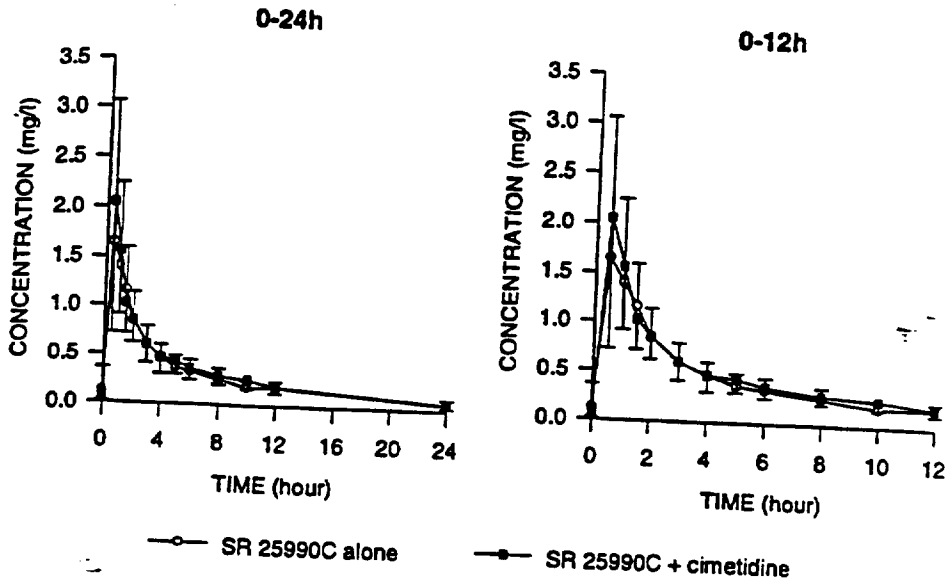


Figure 1: Mean values and standard deviations of plasma concentrations of SR 26334 obtained after administration for 14 days of 75 mg of SR 25990C o.d. alone (D14) and after co-administration for 14 days of 75 mg of SR 25990C o.d. and 400 mg of cimetidine b.i.d. (D28)
Left: 0-24h and right: 0-12h

TABLE (2) Comparative analysis of maximum aggregation percentage

ADP	n	Mean	SEM	Student t test	p	95% confidence interval
T0 value Mean with SR 25990C	18	49.7	2.79	-	-	-
T0 value Mean with SR 25990C + cimetidine	18	56.1	3.57	-	-	-
Difference at T0 D28/D14	18	6.4	1.46	4.40	<0.001 ***	[3.5 ; 9.3]
Difference on D28 (T + 2h) / D14 (T + 2h)	18	2.8	2.91	0.98	0.343	[-2.9 ; 8.5]
COLLAGEN						
T0 value Mean with SR 25990C	18	83.5	1.22	-	-	-
T0 value Mean with SR 25990C + cimetidine	18	81.8	1.72	-	-	-
Difference at T0 D28/D14	18	- 1.7	1.70	1.00	0.329	[- 5.0 ; 1.6]
Difference on D28 (T + 2h) / D14 (T + 2h)	18	- 5.1	3.71	1.38	0.187	[-12.4 ; 2.2]

Table 3 - Differences in mean maximum aggregation percentage between D35 and D01 T0 as induced by ADP and by collagen - Primary statistics

	n	Mean	SEM	Student t test	p	95% confidence interval
Percentage of aggregation induced by ADP D35 /D1 T0	18	-16.3	3.87	4.18	<0.001 ***	[-23.9 ; -8.7]
Percentage of aggregation induced by collagen D35/D1 T0	18	-13.5	4.79	2.82	0.012 *	[-22.8 ; -4.1]

TABLE (4) **Prolongation factor of bleeding time - Comparative analysis**

	Difference between means	n	SEM	95% confidence interval	Student t test	p	Ratio of geometrical means	95% confidence interval
D28 / D14	-0.087	18	0.107	[-0.313; 0.133]	0.817	0.425	0.917	[0.731 ; 1.143]
D35 / D-14	-0.086	18	0.111	[-0.320; 0.146]	0.775	0.449	0.918	[0.726 ; 1.157]

STUDY P1435: (GENDER AND DRUG INTERACTION STUDY WITH ESTROGEN)

COMPARATIVE STUDY OF THE ANTIPLATELET EFFECT OF REPEATED ADMINISTRATIONS OF SR25990C (75 MG/DAY) DURING 14 DAYS, IN POST-MENOPAUSAL WOMEN WITHOUT ESTROGEN REPLACEMENT THERAPY VERSUS MEN OF THE SAME AGE GROUP, AND IN POST MENOPAUSAL WOMEN RECEIVING ESTROGEN REPLACEMENT THERAPY.

Reference: Volume 48-50
Investigator:

Study Location:

Objective:
1. To compare postmenopausal women with no estrogen replacement therapy and men of the same age as regards to the:
-(a) effects of clopidogrel on platelet aggregation induced by ADP and collagen and on bleeding time;
-(b) SR26334 levels
-(c) the clinical and laboratory safety of SR25990C.
2-To assess the effects of clopidogrel therapy as regards to the same parameters in the same population of postmenopausal women while receiving estrogen replacement therapy.

Study design:
This is an open label non randomized parallel group trial with a washout period of one month between the two treatment periods. A screening period of 8 days was completed before enrollment into the study. Male subjects received the study drug (75 mg/day) for the first 14 day treatment period, female subjects received 75 mg/day clopidogrel for 2 14 day treatment periods separated by at least one month washout period. The treatment Period 1 population consisted of postmenopausal women who were not receiving estrogen replacement therapy and men of the same age group (between the ages of 55 and 75 years). The treatment Period 2 population consisted of the same group of postmenopausal women (from Period 1) who were given estrogen replacement therapy. An amendment to the protocol directed the conduct of an additional treatment period with three female subjects who had participated in the first two treatment periods. These women did not receive estrogen therapy in Period 3.
During treatment Period 1, all subjects received study drug (75 mg/day clopidogrel) every morning for 14 days. During treatment Period 2, the female subjects received estrogen replacement therapy (17-beta-estradiol valerate [Progynova] 2 mg/day) every morning for 29 days followed by 10 days of treatment with progesterone (Duphaston 10) in order to create an artificial menstrual cycle. They also took the study drug (75 mg/day clopidogrel) every morning for 14 days from day 16 until day 29. During treatment Period 3, the three female subjects received 75 mg/day of clopidogrel every morning for 14 days with a seven day follow up period.
The schedule of blood samples to determine platelet aggregation and plasma levels of SR26334 for the three treatment periods is summarized in Table 1. Bleeding time assessments were

measured at screening and on days 14 and 21 of treatment Periods 1 and 3 and at screening and on days 29 and 36 of treatment Period 2.

Batch #s: Clopidogrel 25 mg tablet: batch # RGT01 expiration date August 1 1990.
: batch# RF018 expiration date October 21 1989.
Clopidogrel 50 mg tablet: batch # RGN23 expiration date November 23, 1990.
Batch # RF012 expiration date October 21 1989.
17 beta-estradiol valerate (Progynova) 2 mg tablets
Progesterone (Duphaston 10) 10 mg tablets.

Pharmacokinetic parameters were determined using non-compartmental methods. The influence of estrogens on the pharmacokinetics of SR26334 as well as the effect of gender was studied using a Student's t test. Similar testing procedures were used on platelet aggregation and bleeding times.

Table 2 gives the summary of the steady state values for maximum intensity of platelet aggregation and % inhibition during treatment Period I. It can be seen from the above results that the % inhibition from baseline was greater in males than females (50 % vs 28 % at 5μM ADP). It was observed that in four females there was unusually lower levels of inhibition which might partially explain the differences between males and females. Table 3 gives a comparison of bleeding times during treatment Period I. The results show that unlike inhibition of platelet aggregation, the gender differences that were seen on day 1 disappeared on days 15 and 21. Table 4 gives the steady state values for the maximum intensity of platelet aggregation change from baseline and % inhibition in treatment Period 2 and shows that clopidogrel seems to be less active in women who were receiving estrogen replacement therapy compared to those not on therapy. Table 5 shows the comparison between treatment Period 1 and 3 for the three women that were retested. The results generally show a higher inhibition in Period 3 compared to Period 1. Plot of mean concentration profiles for SR26334 after clopidogrel in men and in women receiving and not receiving estrogen therapy are shown in Figures 1 to 3.

From the above results, it can be concluded that neither estrogen replacement therapy nor gender had any effects on the pharmacokinetics of SR26334.

Comments:

In view of the fact that this study seems to indicate that clopidogrel seems to be less

effective in women taking estrogen replacement therapy, the medical officer should confirm whether the same finding is seen in the clinical trial.

Table [illegible]1 - Schedule of Blood Samples

Period 1	Period 2	Period 3	Time
Day			
1	16	1	0 hours (before study drug intake, while fasting)
			+2 hours (before eating)
			+5 hours (before eating)
2	17	2	0 hours (before study drug intake)
4	19	4	0 hours (before study drug intake)
7	22	7	0 hours (before study drug intake)
11	24	11	0 hours (before study drug intake)
14	29	14	0 hours (before study drug intake, while fasting)
			+2 hours (before eating)
			+5 hours (before eating)
15	30	15	while fasting
21	36	21	while fasting

TABLE 2:- Summary of Steady-State Values for Maximum Intensity of Platelet Aggregation, Change From Baseline, and Percent Inhibition During Treatment Period 1 (5 µM ADP), Mean (SEM)

Parameter	Females	SEM	Males	SEM	p-Value[a]
Mean maximum intensity	51	3.8	39	3.6	not tested
Mean change from baseline	-21	4.5	-40	4.1	0.0050
Mean percent inhibition	28	5.9	50	4.6	not tested

a: Student's t-test

TABLE 3:- Comparison of Mean Bleeding Times During Treatment Period 1 (min:sec)

		Day 1 (Screening)	Day 15	Day 21
Women	Mean	6:00	11:21	6:09
	SEM	0:24	1:17	0:51
Men	Mean	4:48	11:51	5:38
	SEM	0:16	1:10	0:35

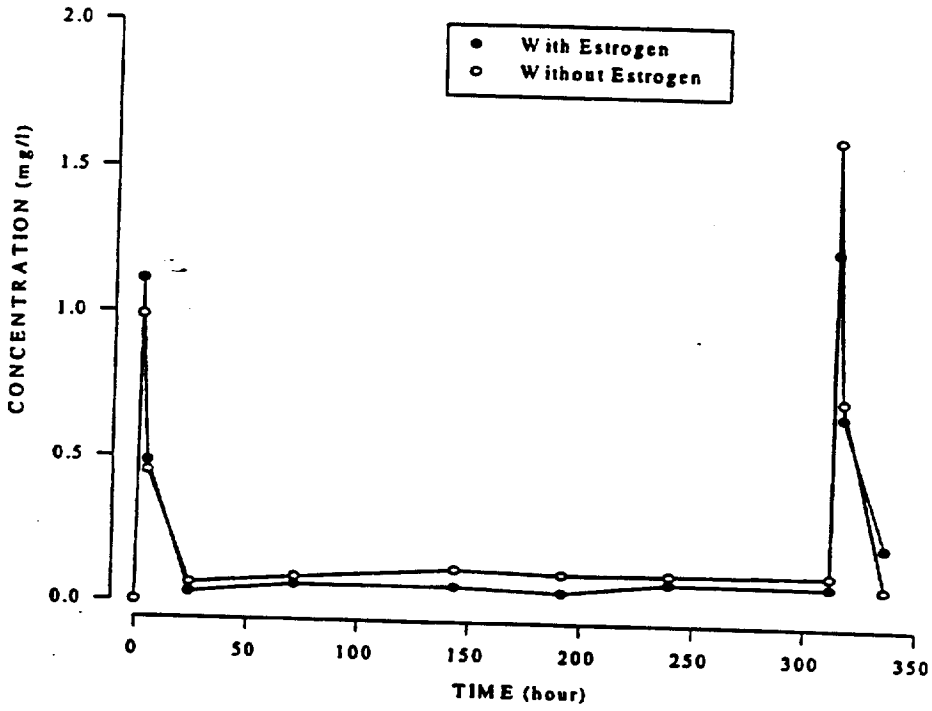
TABLE 4:- Steady-State Values for Maximum Intensity of Platelet Aggregation, Change From Baseline, and Percent Inhibition During Treatment Period 2 (5 µM ADP)

Parameter	Mean Value	SEM	p-Value[a]
Mean maximum intensity	55	3.8	
Mean change from baseline	-11	4.7	0.0626
Mean percent inhibition from baseline	16	7.3	
Change from P1 (Day 1, Time = 0 hours)	17	4	0.1719
% Inhibition from P1 (Day 1, Time = 0 hours)	23	6	

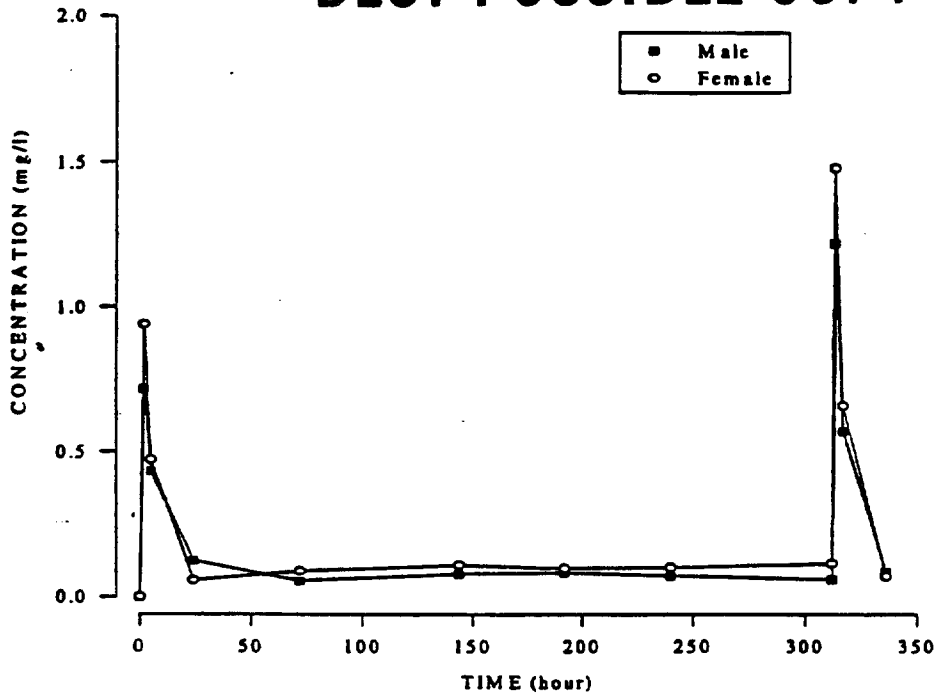
a: p-value from paired Student's t-test.

TABLE 5:- Maximum Intensity and Percent Inhibition of 5μM ADP-Induced Platelet Aggregation at Baseline and Steady State for Three Subjects for Treatment Periods 1 and 3

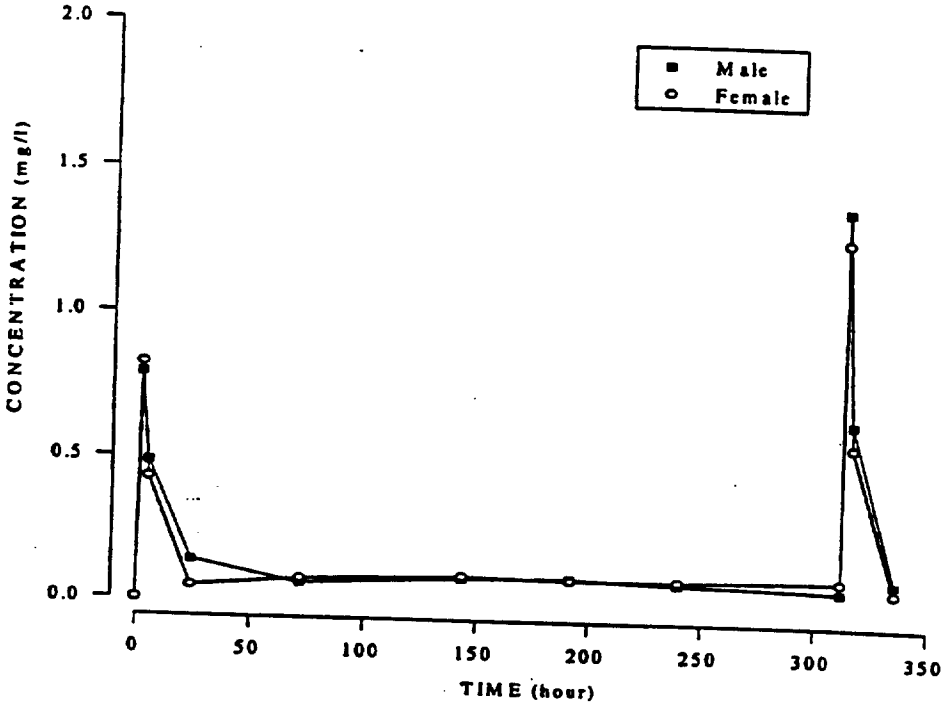
Subject No.	Period	Baseline Value	Mean Value (Steady State)	Difference	Percent Inhibition From Baseline
5	1				
	3				
9	1				
	3				
10	1				
	3				



Primary Figure 1 - Mean of SR26334 Plasma Levels Obtained After Oral Administration of 75 mg of SR25990C to Nine Postmenopausal Women Without and With Estrogen-Replacement Therapy



Primary Figure 2 - Mean of SR26334 Plasma Levels Obtained After Oral Administration of 75 mg of SR25990C to Men and Postmenopausal Women



Primary Figure 3 - Mean of SR26334 Plasma Levels Adjusted for 70 kg Body Weight, Obtained After Oral Administration of 75 mg of SR25990C to Men and Postmenopausal Women

STUDY ENZ2556: (DRUG INTERACTION STUDY WITH PHENOBARBITAL)

EFFECT OF PHENOBARBITAL (INDUCER OF METABOLISM) ON THE PHARMACOKINETIC AND THE PHARMACOLOGICAL ACTIVITY OF SR25990C AFTER REPEATED ORAL ADMINISTRATION IN HEALTHY VOLUNTEERS.

Reference: Volume 71-75
Investigator:

Study Location:

Objective:

1. To assess the influence of phenobarbital on SR26334 pharmacokinetic parameters and on platelet aggregation in subjects receiving repeated administration of 75 mg clopidogrel (SR25990).

Study design:

This is an open label randomized multiple dose design study in 12 healthy male volunteers of age 18-35 years. The participants received 75 mg clopidogrel once daily from day 1 up to and including day 7 during treatment Period A. During treatment Period 2 , subjects took one 100 mg phenobarbital tablet from days 1 to days 20. Additionally, after an overnight fast, subjects took 75 mg tablet of clopidogrel on days 15 to 21. A 21 day washout period was allowed between the two treatment. Plasma samples were collected on day 1 of clopidogrel administration and on the last day of clopidogrel administration for each treatment period according to the following time schedule: 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36 and 48 hours after administration.. ADP induced thrombocyte aggregation was assessed by measuring the maximum intensity of platelet aggregation (expressed as a %) and the velocity (expressed as %/min). Values were recorded at baseline during the clopidogrel period only on days 1, day 7 at 8:00 , 9:30 and 11:00 am and on day 21 and during the clopidogrel + phenobarbital on days 1, 15 and 21 at 8:00, 9:30 and 11:00 am and at the end of the study.

Batch #s: Clopidogrel 75 mg tablet: batch # 102D5 expiration date May 31, 1996.

Phenobarbital 100mg tablet (Phenaemal^{R}): batch# 940023335 expiration date December 31, 1997.

Pharmacokinetic parameters were determined using non-compartmental methods. All statistical tests were performed at the 0.05 significance level using a two tailed test. 90 % confidence intervals of the ratios of the results on day 20 to day 14 were calculated. A non parametric Wilcoxon rank sum test was used to assess the treatment effect on CMAX for SR 25990.

Plot of mean concentration profiles for clopidogrel and its metabolite SR26334 after clopidogrel administration with and without the administration of phenobarbital is shown in Figure 1 while the corresponding pk parameters are summarized in Table 1.

Table 1

	SR26334		Clopidogrel	
	Clopidogrel	Clopidogrel +phenobarbital	Clopidogrel	Clopidogrel +phenobarbital
CMAX (mg/l)	1.93	2.44 (1.07-1.48)	2.07	.81
T1/2	7.19	7.55		
TMAX (hours)	0.75	.69	.82	.78
AUC_{0-24} (mg*hr/l)	6.11	6.64(0.84-1.26)		

( ) = 90% confidence intervals on the ratio of the geometric means.

Table 2 gives the summary of the % inhibition of platelet aggregation induced by ADP while Table 3 summarizes the analysis of maximum platelet aggregation induced by ADP. Table 4 gives the analysis of bleeding time prolongation factor.
It can be seen from the above results that coadministration of 100 mg phenobarbital with 75 mg clopidogrel decreased the CMAX of the parent drug clopidogrel by 60 % most probably due to the induction of Phase I metabolic enzymes. This decrease in the plasma levels of the parent drug was accompanied by a relatively moderate increase of CMAX (27 %) and AUC (8.6 %) of the metabolite SR26334. The sponsor speculates that since there was no change in the half-life of clopidogrel with the coadministration of phenobarbital, the observed increases in CMAX and AUC are not probably due to the inducing properties of phenobarbital.
Moreover, the results show that coadministration of phenobarbital increased the mean inhibition of ADP induced platelet aggregation by clopidogrel on day 7 from 41.6 % to 49.1 %. However this increase in pharmacological activity was not translated to an increase in bleeding time.

Conclusions:

Coadministration of 100 mg of phenobarbital for 21 days with 75 mg clopidogrel decreased the CMAX of clopidogrel by 60 % with a corresponding slight increase of 27 % in

CMAX and 8.6 % in AUC of SR 26334. This pharmacokinetic interaction was accompanied by an increased inhibition of ADP induced platelet aggregation of clopidogrel from 41.6 to 49.1 % with no effect on bleeding time.

TABLE (2) :-

Summary of Analysis of Bleeding Time Prolongation Factor by Day*

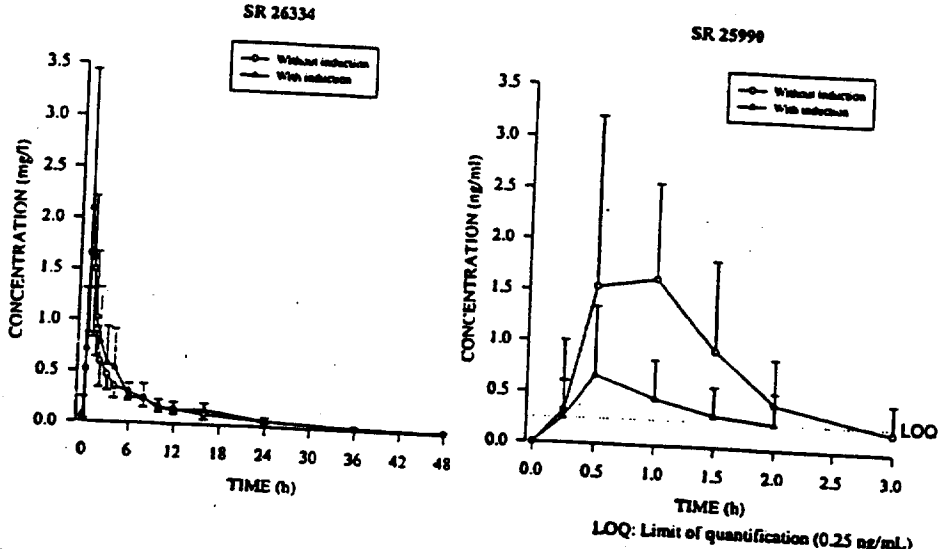
Time Day 7*	Geometric Mean (log transformed) of Treatments		p-Values		
	Clopidogrel	Phenobarbital + Clopidogrel	Treatment	Sequence	Period
8:00	1.96	1.81	0.545	0.823	0.966
9:30	1.89	1.83	0.802	0.840	0.561
11:00	2.00	1.83	0.489	0.693	0.911

* Day 7 is the seventh day post-clopidogrel administration (Day 21 of the clopidogrel + phenobarbital period).

TABLE (3) :- **Summary of Analysis of Maximum Platelet Aggregation Induced by ADP (5 µM): Treatment Group Differences By Time**

Parameter	Time Day 7*	Mean (90% CI) of Treatment Difference (Phenobarbital + Clopidogrel Minus Clopidogrel)	p-Values		
			Treatment	Sequence	Period
Intensity (%)	8:00	-6.6 (-11.8, -1.4)	0.045	0.925	0.802
	9:30	-5.0 (-10.3, 0.4)	0.122	0.880	0.694
	11:00	-7.9 (-11.8, -4.0)	0.004	0.466	0.039
Velocity (%/min)	8:00	-13.9 (-22.1, -5.8)	0.011	0.963	0.493
	9:30	-11.5 (-24.5, 1.5)	0.141	0.936	0.937
	11:00	-22.0 (-33.3, -10.7)	0.006	0.378	0.147

* Day 7 is the seventh day post-clopidogrel administration (Day 21 of the clopidogrel + phenobarbital period).



LOQ: Limit of quantification (0.25 ng/mL)

Figure (illegible) 1 - Time course of mean plasma concentrations of SR26334 (left) and of SR25990 (right) following repeated administration of 75 mg of clopidogrel alone and after phenobarbital induction *(n=12)*

STUDY P1512: (DRUG INTERACTION STUDY WITH ATENOLOL/NIFEDIPINE)

DOUBLE BLIND STUDY OF PHARMACODYNAMIC INTERACTIONS BETWEEN SR25990C AND ATENOLOL/NIFEDIPINE IN PATIENTS WITH PERIPHERAL ARTERIAL DISEASE OR CORONARY ARTERY DISEASE.

Reference: Volume 76
Investigator:

Objective:
1. To assess the pharmacodynamic activity of SR25990C on the basis of inhibition of ADP induced platelet aggregation in patients with peripheral arterial disease or coronary arterial disease including patients who were stable following a myocardial infarction.
2. To assess the possible effects on other pharmacodynamic variables and to assess the effects of coprescription of atenolol and/or nifedipine on the plasma levels of SR25990C and its main metabolite. Plasma levels of atenolol and nifedipine were also measured for comparison.

Study design:
This was a double blind placebo controlled crossover study . Treatment was either clopidogrel 75 mg every day or placebo for seven days followed by a washout period of two weeks followed by placebo or clopidogrel 75 mg every day for seven days. A total of 24 patients were recruited in this study (8 in each standard therapy group) males aged over 35 years or females who were post menopausal or who had undergone surgical sterilization.
All blood samples for platelet aggregation measurements were taken 120 minutes post dose for simple studies at screening and on days 1, 22 and 36. Extended studies were done on days 7 and 28. Blood samples for the measurement of clopidogrel and its main metabolite, atenolol were collected at 120 minutes post dose on days 1, 7, 22 and 28.
Batch #s: Clopidogrel 25 mg tablet: batch # RHE31, RGT01.
Placebo: batch # XRFN25

Analysis of variance was used to compare the effects of placebo and clopidogrel on platelet aggregation at day 1 and day 7. No formal pharmacokinetic or statistical analysis was performed on the plasma levels of SR26334, nifedipine or atenolol. The purpose of plasma level measurements was to determine compliance.

Results:

Table 1 gives the summary of the % inhibition of platelet aggregation induced by ADP for all the three different treatments. It can be seen from the results that coadministration of nifedipine and/or atenolol to patients with peripheral arterial disease or coronary artery disease did not have any effects on the pharmacological activity of clopidogrel.

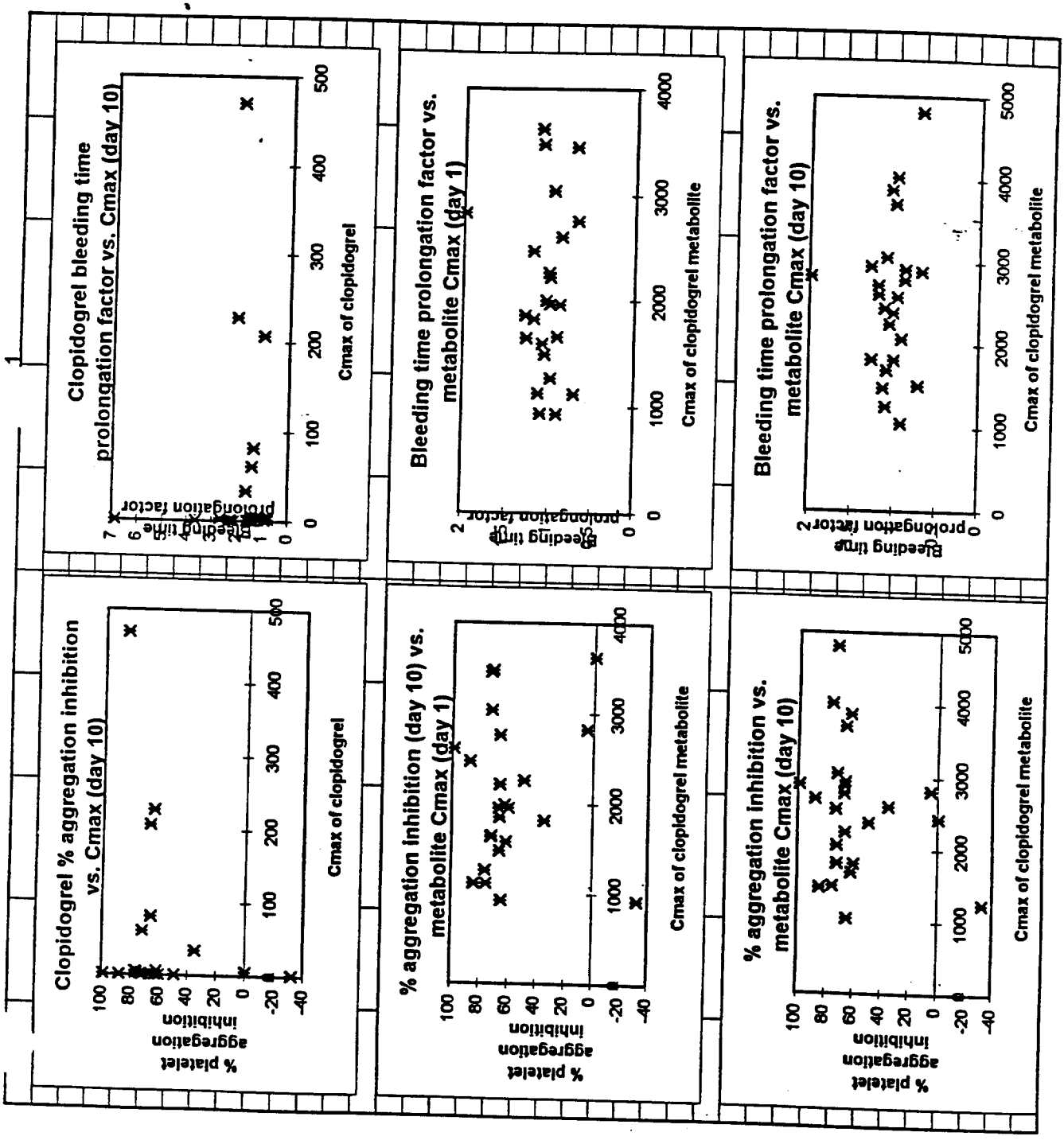
TABLE (1)

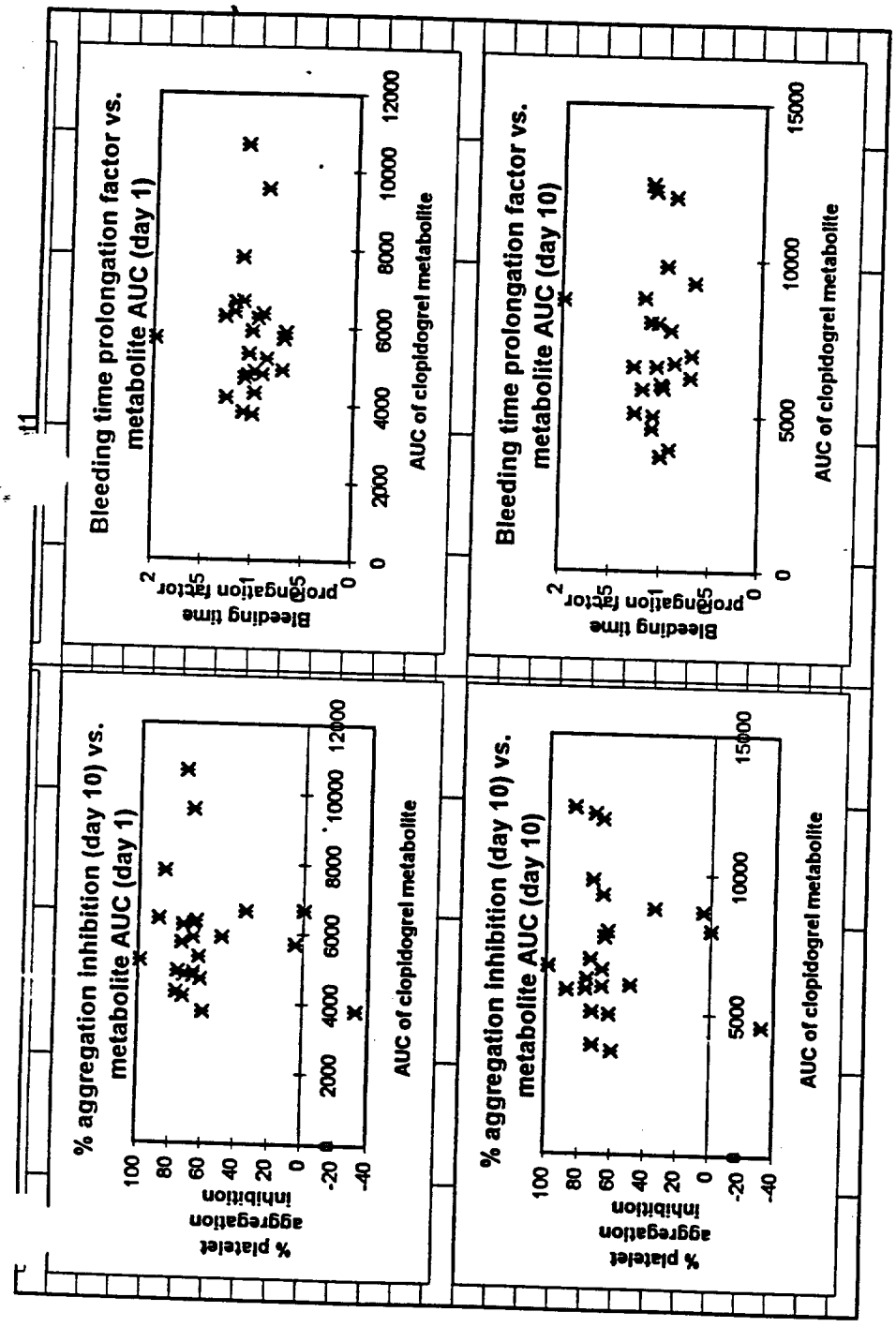
Mean (sd) 5 μmol/l ADP induced Platelet Aggregation (%)

Standard Treatment Group	Mean (sd) Platelet Aggregation (%)					
	Pre-study	Clopidogrel Day 1	Clopidogrel Day 7	Placebo Day 1	Placebo Day 7	Post study
PAD, nifedipine [n]	73.2 (4.5) [6]	81.0 (4.1) [6]	50.7 (21.9) [6]	78.6 (5.5) [5]	76.2 (9.8) [6]	84.7 (9.8) [6]
CAD, atenolol [n]	80.3 (15.6) [10]	82.2 (7.6) [8]	48.6 (20.1) [8]	82.4 (7.0) [10]	74.8 (10.7) [9]	78.1 (4.7) [8]
CAD, atenolol + nifedipine [n]	78.0 (5.9) [8]	85.7 (6.0) [8]	56.5 (10.7) [8]	81.2 (10.1) [8]	86.1 (8.2) [8]	76.7 (5.6) [8]
All patients [n]	77.7 (10.9) [24]	83.2 (6.3) [22]	52.0 (17.3) [22]	81.2 (7.8) [23]	79.1 (10.6) [23]	79.4 (7.2) [22]

PAD = peripheral arterial disease, CAD = coronary artery disease

APPENDIX II





17 pages

PURGED

(DRAFT Labeling)