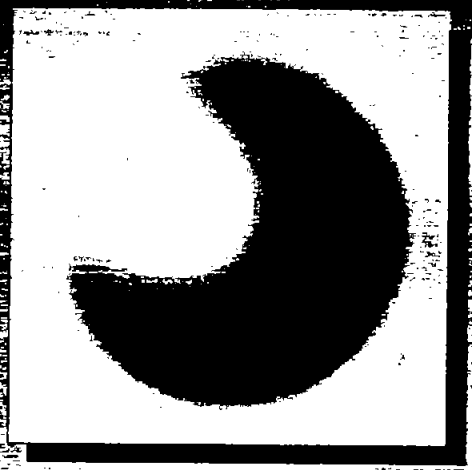
11227

Volume 8 Number 2 April 2002

CLINICAL AND APPLIED THROMBOSIS/HEMOSTASIS



Listed in Index Medicus/MEDLINE

Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis

WESTMINSTER PUBLICATIONS

2004

CLINICAL AND APPLIED THROMBOSIS/HEMOSTASIS

Official Journal of the International Academy of Clinical and Applied Thrombosis/Hemostasis

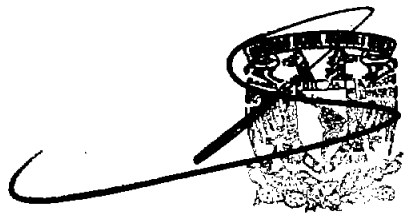
CONTENTS

VOLUME 8, NUMBER 2, APRIL 2002

(continued on next page)

Clinical and Applied Thrombosis/Hemostasis (ISSN: 1076-0296) is published quarterly by Westminster Publications, Inc. Editorial and Business Office, 708 Glen Cove Avenue, Glen Head, NY 11545, USA.

Clin Appl Thrombosis/Hemostasis 8(2):169–177, 2002



Effect of Clopidogrel on Platelet Aggregation and Plasma Concentration of Fibrinogen in Subjects with Cerebral or Coronary Atherosclerotic Disease

R. Izaguirre-Avila, MD,* A. De la Peña-Díaz, MSc,* F. Barinagarrementería-Aldatz, MD,†
H. González-Pacheco, MD,* A. E. Ramírez-Gutierrez, MD,*
J. L. Ruiz-Sandoval, MD,† A. Quiroz-Martínez, MD,* and C. Cantú-Brito, MD†

**Instituto Nacional de Cardiología Ignacio Chávez, México; †Instituto Nacional de Neurología y Neurocirugía, México*

Summary: Acetylsalicylic acid inhibits thromboxane A2 production and reduces the risk of vascular occlusive events by 20% to 25%. Ticlopidine inhibits ADP-dependent platelet aggregation and reduces the same risk by 30% to 35%, but produces some adverse effects. Clopidogrel is a ticlopidin-related antiplatelet drug, with the same mechanism of action; it reduces the expression of the glycoprotein IIb/IIIa, the fibrinogen receptor on the platelet surface. Clopidogrel has the same clinical efficacy of ticlopidin and has a decreased incidence of adverse effects. The effect of one daily dose of 75 mg of clopidogrel on platelet function in 90 subjects was evaluated; 41 with coronary artery disease and 49 with cerebral vascular disease. Before treatment and after 6 and 12 weeks, bleeding time and fibrinogen plasma concentration were also evaluated. There was a reduction in 5-μM ADP-induced platelet aggregation of 38% ± 27% at 6 weeks and 44% ± 29% at 12 weeks in patients with coronary artery disease; 35% ± 41%, 29% ± 59% in the cerebral vascular disease group; and 36% ± 36% and 35% ± 49% in the total group. Reduction of 20 μg/mL collagen-induced platelet aggregation was not significant in any group. Plasma fibrinogen levels did not vary during treatment. Bleeding time was significantly prolonged in all studied groups. There were no hemorrhagic complications; only digestive discomfort in less than 3% of patients. Clopidogrel efficiently reduces ADP-induced platelet aggregation and prolongs bleeding time and is a safe and efficacious antiplatelet drug.

Key Words: Clopidogrel—Antiplatelet drug—Atherosclerotic disease—Coronary and cerebral artery disease.

Occlusive coronary and cerebral events due to atherosclerosis represent one of the main causes of death in numerous countries (1,2). Cardiovascular diseases occupy the first place in mortality rate in 31 of 35 countries in America. In Mexico, 65,603 deaths occurred due to coronary arterial disease (CAD) and 24,344 due to cerebral vascular disease (CVD) in 1996 (3). The platelets have a well-defined role in the etiopathogenesis of this disease in the chronic phase of the development of the arterial lesion (4) and in the acute phase of the thrombosis (5).

The meta-analysis performed by Antiplatelet Trialist's Collaboration in 145 clinical studies, in which 100,000 patients were included, reached the conclusion that the platelet antiaggregating drugs are efficacious in the prevention of cerebral infarcts, myocardial infarcts, and deaths due to vascular diseases; it was also demostrated that there was a reduction in the relative risk (RR) of nonfatal cerebral infarct (31%), nonfatal acute myocardial infarct (AMI) (35%), as well as in the mortality rate due to vascular causes (18%) (6). The conclusions of the analysis are: acetylsalicylic acid (ASA) alone is clearly useful. The combination of ASA and dipyridamol is not superior to ASA alone. Sulfinpyrazone is less efficacious. Other studies have demonstrated that ticlopidine is more efficacious than ASA (7,8).

There is, then, sufficient evidence that ASA and ticlopidine are useful in subjects with atherosclerotic disease and that both drugs seem to be more efficacious than any other tested so far; nevertheless, both agents can produce important adverse effects.

Address correspondence and reprint requests to Raúl Izaguirre-Ávila, MD, Instituto Nacional de Cardiología. Departamento de Hematología, Juan Badiano 1, CP 14080, México, DF; e-mail: rizagui@yahoo.com.

Clopidogrel is a recent platelet antiaggregating agent, belonging to the family of thienopyridines, having a molecular structure close to that of ticlopidine. It is a safe and efficacious drug. The CAPRIE study (clopidogrel vs. aspirin in the prevention of recurrent ischemic events) (9) recruited 19,185 patients in 384 centers: 9,599 were treated with clopidogrel and 9,586 with ASA. The follow-up was 1.91 years. Clopidogrel reduced the odds ratio for any terminal vascular event by 8.7% (for the overall population of the CAPRIE study estimated for a combined endpoint, myocardial infarct, ischemic stroke, vascular death), more than ASA ($p = 0.043$; 95% confidence interval, 0.3–16.5). The number of events taken as fatal was 5.32 per 100 patients for clopidogrel and 5.83 per 100 patients for ASA per year.

Our objectives were to define the effect of clopidogrel on ADP- and collagen-induced platelet aggregation, bleeding time (Ivy-Mielke), and fibrinogen level, as well as the tolerability and safety profile at short term, in patients in whom the use of platelet antiaggregating drugs are indicated due to risk of atherothrombotic events.

MATERIALS AND METHODS

We performed an open study that included 90 patients: 41 had a history of coronary arterial disease (CAD), such as acute myocardial infarction (AMI), stable (SA) or unstable (UA) angina; and 49 had cerebral vascular disease (CVD) of thrombotic origin, all candidates for platelet antiaggregating treatment, without having received any drug of this kind for 7 days before inclusion in the study. The diagnosis of ischemic cardiopathy was based on the clinical and electrocardiographic studies as well as on coronary angiography. The diagnosis of cerebral vascular disease was based on the clinical symptoms of transient cerebral ischemia or cerebral infarct, as well as studies such as Doppler and computed tomography. Written consent to participate in the study was requested from all patients, and the study was approved by the ethical committee and the procedures were in accordance with the guidelines of our institutions.

Patients were excluded according to the following criteria: severe neurologic deficit with permanent prostration or dementia, cerebrovascular events of cardiac origin or posterior to carotid endarterectomy or carotid angiography, uncontrolled arterial hypertension, serious hepatic failure, antecedents of coagulopathy, use of oral anticoagulants, antecedents of systemic hemorrhage, neutropenia (<1,200 neutrophils/mm^3) or thrombocytopenia (< 100 × 10^9/L), allergy to ticlopidine, as well as the use of thrombolytic drugs within 48 hours before study inclusion.

Administered Treatment

Each patient received a 75-mg tablet of clopidogrel once a day after breakfast for 12 weeks as the only antiplatelet treatment. A clinical evaluation was made before starting the treatment, then at 6 and 12 weeks, followed by an evaluation at 16 weeks via telephone. A note was taken of any adverse reactions and other medications given concomitantly. If required, analgesics were given in the form of paracetamol or any other agent without platelet antiaggregating effect.

Evaluation of Platelet Function

To evaluate the effect of clopidogrel on platelet function, the following tests were performed both before the start of the treatment (baseline sample) and at each of the follow-up consultations (weeks 6 and 12).

Platelet aggregation was determined by a standard method described. For each test, blood was obtained by venipuncture and collected in polypropylene tubes (Nalgene) containing 3.8% sodium citrate in the proportion of 9:1. Platelet-rich plasma (PRP) was prepared by centrifugation at 140 × g for 4 minutes at room temperature, from the remaining blood, platelet-poor plasma (PPP) was obtained by centrifuging at 1000 × g for 15 minutes. The concentration of platelets was adjusted at 200 × 10^9/L. The tests were carried out within 2 hours after blood withdrawal, by means of a Lumino-aggregometre (Chronolog) at 37°C (11). Platelet aggregation was induced with 5-μM adenosine-diphosphate or 20-μg/mL collagen (Sigma Chemicals). The Chronolog equipment was attached to a computer that calculates the results as percentages of aggregation. To avoid variability, platelet aggregation was determined in a mixture of PRP from four healthy donors, taken as 100% aggregation, against which the results from each patient were compared.

Bleeding time was measured according to the Ivy technique, using disposable lancets (Simplate, Organon Teknica), which make 1-mm deep and 5-mm-long incisions. These were made on the ventral side of the superior third part of

the forearm, after applying a pressure of 40 mm Hg by means of a bracelet and a baumanometer. The bleeding time was measured with a chronometer. The reference range for healthy volunteers was 3 to 7 minutes.

The concentration of plasma fibrinogen was measured by means of the technique described by Clauss on the dates of the clinical consultations previously mentioned.

In parallel with the tests for platelet function, several other tests were carried out on each patient, including: complete blood cell count in a Coulter cell counter model S-Plus, blood chemistry, total bilirubin, alkaline phosphatase, pyruvic and oxalacetic transaminase levels, lactate dehydrogenese, and plasma proteins.

Percentage variations were evaluated between basal values for platelet aggregation, bleeding time in minutes, and plasma fibrinogen levels, and those observed during the follow-up (weeks 5 and 12). Statistical significance was defined when the p value was less than 0.05.

RESULTS

Ninety patients were included and evaluated (41 CAD and 49 CVD). Table 1 shows demographic characteristics. It is worth noting that there is approximately a 5 year difference in age between the population with CAD and CVD, and a predominance (77%) of males in the total patient population. Table 2 shows the personal pathologic background details of the population studied. Those more frequently reported were diabetes mellitus type II (26.8% in CAD and 38.7% in CVD patients), hyperlipidemia (approximately 50%), essential arterial hypertension (41.4% in CAD and 63.2% in CVD patients), and angina (84.8% in CAD and 8.1% in CVD patients).

As part of the safety evaluation procedure, systolic and diastolic blood pressures were registered; these readings showed no changes during the study period.

The most frequent diseases were diabetes mellitus, arterial hypertension, coronary cardiopa-

TABLE 1. Main Population Characteristics

Variable	CAD	CVD	Total
Patients	41	49	90
Age (years)			
Mean	57.73	63.59	60.92
Standard deviation	11.07	10.31	11.00
Minimum	36.00	28.00	28.00
Median	56.00	66.00	62.00
Maximum	77.00	85.00	85.00
Evolution time (yr)			
Mean	4.60	1.91	3.14
Standard variation	5.27	2.31	4.15
Minimum	0.00	0.00	0.00
Median	3.00	1.00	2.00
Maximum	24.00	10.00	24.00
Body weight (kg)			
Mean	73.46	67.51	70.15
Standard variation	11.39	12.10	12.10
Minimum	49.00	40.50	40.50
Median	73.00	67.00	69.00
Maximum	99.50	90.00	99.50
Height (cm)			
Mean	164.00	159.14	161.29
Standard variation	8.34	8.65	8.80
Minimum	145.00	139.00	139.00
Median	164.00	160.00	162.00
Maximum	182.00	176.00	182.00

TABLE 2. Pathological Personal History

Variable	CAD*		CVD*		Total	
	N	%	N	%	N	%
Diabetes mellitus	11	26.83	19	38.78	30	33.33
Peripheal arterial disease	1	2.44			1	1.11
Hyperlipidemia	22	53.66	23	46.94	45	50.00
Essential arterial hypertension	17	41.46	31	63.27	48	53.33
Secondary arterial hypertension			1	2.04	1	1.11
Acute myocardial infarct	5	12.20	1	2.04	6	6.67
Congestive cardiac failure	2	4.88	1	2.04	3	3.33
Angina	28	84.84	4	8.16	32	38.09
Chronic nephropathy	2	4.88	1	2.04	3	3.33

CAD, coronary artery disease; CVD, cerebral vascular disease.
*The percentage of the subjects is given taking the total patients in each group.

TABLE 3. Family History

Variable	CAD*		CVD		Total	
	N	%	N	%	N	%
Coronary cardiopathy	19	46.34	13	26.53	32	35.56
Coagulation disorders						
Diabetes mellitus	22	53.66	27	55.10	49	54.44
Dyslipidemia	4	9.76	8	16.33	12	13.33
Peripheral arterial disease	2	4.88	5	10.20	7	7.78
Previous cerebral arterial ischemia	10	24.39	14	28.57	24	26.67
Collagen disease						
Arterial hypertension	16	39.02	24	48.98	40	44.44
Hematologic neoplasias	1	2.44	—	—	1	1.11

CAD, coronary artery disease; CVD, cerebral vascular disease.
*The percentage of the subjects is given taking the total patients in each group as 100%.

thy, and previous cerebral arterial ischemia (Table 3). The incidence of diabetes was reported to be 50% to 55% among the relatives of the subjects from both CAD and CVD groups. Coronary cardiopathy was found in 46.3% of relatives of the CAD group and 26.5% of the CVD group. Regarding smoking habits, low frequency was remarkable—among the smokers and ex-smokers (7.78%) a long duration of the habit was noticed, as well as a high consumption of cigarettes (greater than 5 times/week and greater than 10 cigarettes per day).

Use of platelet antiaggregating agents were stopped until 1 week before the start of the study. All subjects in the CAD group had received previous treatment with ASA. In the group CVD, four subjects did not have any antiaggregating drug (only naproxen), 24 had ASA, 17 had ticlopidine, one had a combination of antiaggregants (ASA plus ticlopidine), another had a combination of ASA and acenocoumarin, and two subjects had no medication at all.

More than 75% of patients from both groups required concomitant medication. The most frequently administered drugs were diuretics, antihypertensives, glucose- or lipid-lowering and anti-angina drugs. The treatment was given for the associated diseases found in this group of patients (arterial hypertension, diabetes mellitus and hyperlipidemia).

Platelet Function and Fibrinogen

There was a progressive diminution in ADP-induced (5 mM) platelet aggregation during the 12 weeks of treatment. This effect was significant ($p < 0.001$) at 6 and 12 weeks, as compared to the basal value both in CAD and CVD subjects (Fig. 1). The percentage of reduction in ADP-dependent platelet aggregation, after the administration of the drug is shown in Table 4 and confirms that there was a significant diminution, between 33% and 41%. There was a diminution in platelet aggregation induced by collagen 120 mg/mL during the treatment in both CAD and CVD subjects but it was not significant (10% at 12 weeks of treatment) (Fig. 2).

Bleeding time was significantly prolonged from 3:4 ± 1:5 to 14:3 ± 14:2, from 5:2 ± 4:1 to 15:3 ± 15:4, and from 4:3 ± 3:2 to 15:1 ± 15:0 minutes, in groups CAD, CVD, and in the total population, respectively, which represented an increase of 3.3 to 4.12 times as compared to the basal value (Fig. 3).

No significant modifications in the level of fibrinogen were observed during the treatment (Table 5).

Safety Parameters

In all, six (6.66 %) subjects had to be withdrawn from the study before completion. In the CAD group the events that lead to withdrawal were nausea, gastric acidosis sensation, pruritus, angina at rest, hypertensive crisis, cephalea, and dizziness. In group CVD, the suspension was due to intracerebral hemorrhage and focal erythema in the thigh.

In group CAD, there was a case of pruritus, nausea, pyrosis, and dizziness. In group CVD, there was a case of vomiting, abdominal distention, and diarrhea. The adverse events that were possibly, probably, or definitely related to the drug under study appeared in four subjects. None of the reported events had a frequency above 3% (Table 6).

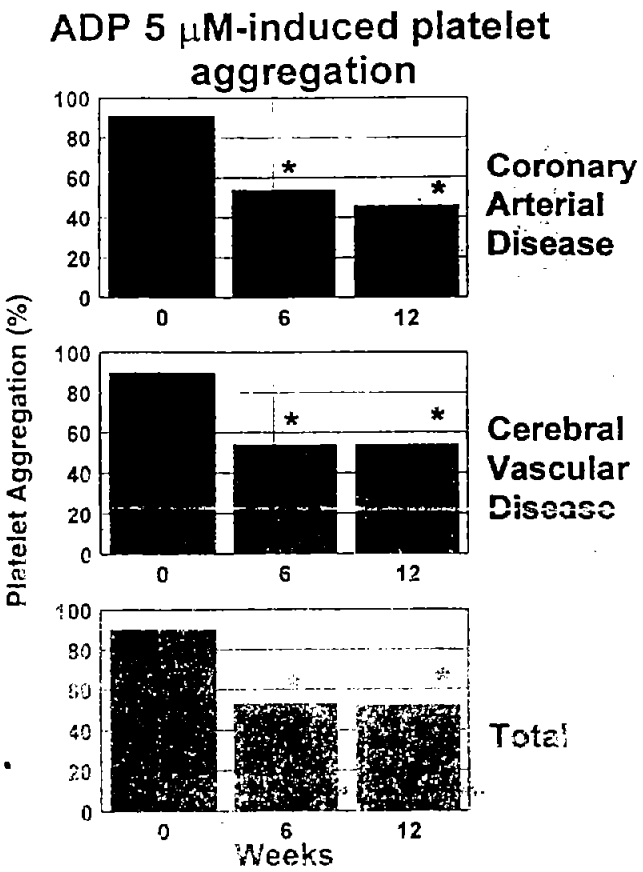


FIG. 1. Statistical significance vs. week 0 ($p < 0.01$).

DISCUSSION

In the present study, we investigated the inhibitory effect of clopidogrel on platelet aggregation induced by ADP or collagen in a population of patients with atherosclerosis, which had manifested by coronary or cerebrovascular arterial events in patients who had a high risk or recurrent atherothrombotic events.

TABLE 4. Percentage Decrease in ADP-dependent Platelet Aggregation

Variable	CAD		CVD		Total	
	Mean (%)	SD	Mean (%)	SD	Mean (%)	SD
Difference week 0–6	−38.60	27.31	−35.59	41.51	−36.83	36.17
Difference visit 0–12	−44.43	29.55	−29.14	59.2	−35.33	49.72

CAD, coronary artery disease; CVD, cerebrovascular disease.

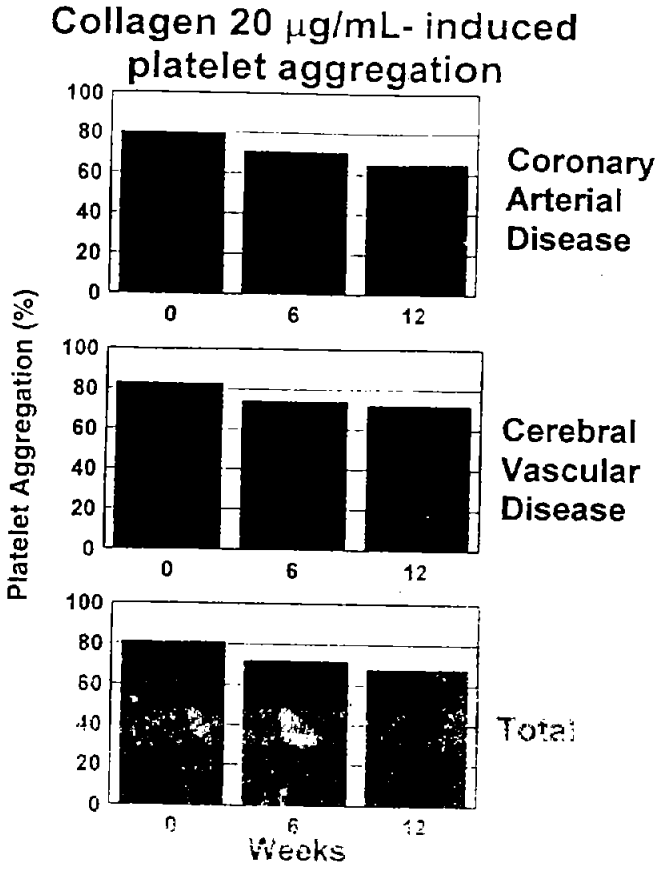


FIG. 2. No statistical significance

In this disease, vascular episodes occur due to the presence of a complicated atherosclerotic plaque whose rupture conduces the rapid formation of an occluding thrombus, through a series of complex physiopathologic events in which platelet reactivity plays a determinant role. Clinical syndromes are produced, such as unstable angina, acute myocardial infarct, sudden death, transitory cerebral ischemia or cerebral infarct; also thrombosis in distal arteries. The events begin when the plaque exposes tissue factor and hemostasis becomes activated. Platelets are recruited and activated via three mechanisms: secretion of ADP, production of thromboxane A2, and generation of thrombin, which conduce to amplification reactions that will rapidly produce a thrombus, the growth of which, if not limited, will occlude an arterial vessel. In patients with atherosclerosis, the administration of ASA inactivates the production of thromboxane A2 irreversibly, which reduces the risk of occluding vascular events by 20% to 25%. Ticlopidine, while inhibiting ADP-dependent platelet aggregation, reduces the risk by 30% to 35%. Several studies have demonstrated that ticlopidine is more efficacious than ASA for reducing cerebral vascular events (7,8). Nevertheless, this drug caused adverse reactions such as neutropenia and skin eryhema, which justified changing the molecule to diminish unwanted effects while maintaining its larger efficacy.

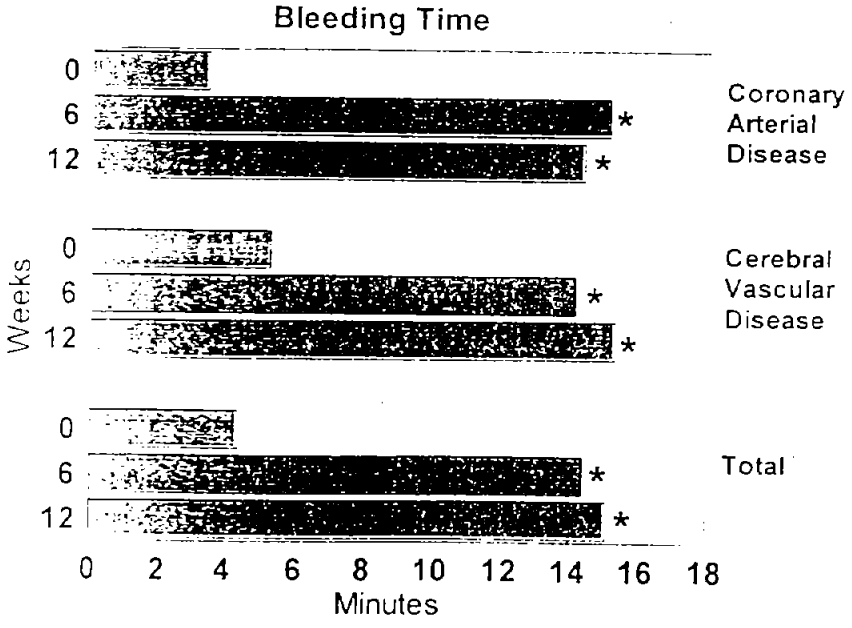


FIG. 3. Statistical significance vs. week 0 ($p < 0.01$).

TABLE 5. Plasma Fibrinogen (g L)

Week	CAD		CVD		Total	
	Mean	SD	Mean	SD	Mean	SD
0	2.99	0.56	3.14	0.70	3.07	0.81
6	2.70	0.33	3.09	0.58	2.93	0.53
12	2.71	0.48	2.98	0.59	2.87	0.57

CAD, coronary artery disease; CVD, cerebrovascular disease.

TABLE 6. Adverse Effects

EVENT	CAD*		CVD*		Total*	
	N	%	N	%	N	%
Visit 2						
Nausea	1	2.4%	—	—	1	1.11
[illegible]sis	1	2.4	—	—	1	1.11
Pruritus	1	2.4	—	—	1	1.11
Dizziness	1	2.4	—	—	1	1.11
Vomit	—	—	1	2.0	1	1.11
Abdominal distention	—	—	1	2.0	1	1.11
Visit 4						
Diarrhea	—	—	1	2.0	1	1.11

*The percentage of the subjects is given taking the total of patients in each group as 100%.
CAD, coronary artery disease; CVD, cerebrovascular disease.

Clopidogrel specifically inhibits ADP-dependent platelet activation (12,13) by acting on one of the receptors, P2Y, related to the inhibition of adenylate cyclase and to the expression of the fribrinogen receptor, the glycoprotein IIb/IIIa (14–16). In this manner, the expansive reaction of the ADP-dependent platelet activation is inhibited and the interaction of platelet and fibrin during the formation of a thrombus is avoided. This mechanism explains its larger efficacy as compared to aspirin in reducing the risk of vascular occlusive events. The CAPRIE study demonstrated a reduction in vascular atherothrombotic events by more than 8.7% in the group treated with clopidogrel compared with the group treated with aspirin (9) and considering only secondary prevention. In the different clinical studies, the clinical efficacy of clopidogrel has been corroborated, although no routine tests of platelet function have been performed to document the diminution in ADP-dependent aggregation, or the increase in bleeding time. There are no studies to evaluate the effect of the drug on the plasma concentration of fibrinogen.

In healthy volunteers, it has been demonstrated that a daily dose of 75 mg of clopidogrel reduces ADP-dependent platelet aggregation by 52%, after the sixth day, and prolongs bleeding time 2.2 times the basal value (17). The effect on platelet aggregation is observed after 6 hours of the first dose, but not to a significant degree; in another study on healthy volunteers, a reduction between 55% and 57% in platelet aggregation was found after 7 days, which did not vary with the age of the subject (18). This prolonged latency period should be taken into account in some special clinical situations in which a rapid effect is required (19). When a dose of 300 to 400 mg is administered, the antiaggregating effect is achieved within a shorter time (20). This

means that in those cases when clopidogrel needs to be administered chronically, a daily dose of 75 mg is sufficient, whereas a larger dose is advisable when a rapid effect is required, for instance after the insertion of intravascular stents or aorto-coronary grafts.

The combination of clopidogrel and ASA increases the efficacy to reduce the relative risk of atherothrombotic events, by inhibiting the two most important pathways in the amplification reactions of platelet aggregation. In primates, such a combination is more efficacious that each of the drugs administered individually, both to inhibit platelet activation and to reduce the deposits of platelets and fibrin (10). The same results have been obtained in studies performed in rabbits (21) and in clinical studies (22). The safety and efficacy of ASA and clopidogrel in the prevention of occlusion of intracoronary stents is similar to that observed with ASA and ticlopidine, although the secondary effects are reduced when clopidogrel is used; the events of occlusion of stents that have been reported during the initial stages of the treatment with clopidogrel could be related to a lack of a loading dose (23,24).

In this study, there is a high risk of recurrent atherothrombotic events; therefore, antiplatelet treatment is indicated. With the dose of 75 mg of clopidogrel daily, we have confirmed that there is a significant reduction in ADP-dependent platelet aggregation from 90.7% ± 13.2% to 49.2% ± 23.7%, from 89.7% ± 23.3% to 55.1% ± 26.1%, and from 90.2% ± 19.3% to 52.7% ± 25.2% in groups CAD, CVD, and total patient population, respectively. We studied a group of atherosclerotic patients with manifestations in arterial coronary and cerebral areas, which represents a diminution in platelet aggregation of 35% to 41% in CAD group, 35% to 29% in CVD group, and 36% to 35% in the total patient group. As occurs with other platelet antiaggregating agents, clopidogrel has no effect on collagen-induced platelet aggregation. In this study, the mean levels in the platelet collagen-aggregation curve decreased from 67.2% ± 39.3% to 60.6% ± 30.7%, from 76.1% ± 35.4% to 75.6% ± 31.8%, and from 72.2% ± 37.2% to 69% ± 32.0% in groups CAD, CVD, and total population, respectively, representing a nonsignificant approximate reduction of 10%.

The bleeding time, measured according to the Ivy technique, was significantly prolonged up to 4.12 times the basal value, which is larger than that reported for volunteer subjects.

This efficient prolongation did not increase bleeding complications. On the other hand, in this study we observed that fibrinogen levels remained unchanged during the 12 weeks of treatment, which permits us to confirm that it does not add an undesirable hemorrhagic effect. The normal fibrinogen concentration during the treatment with clopidogrel contributes to maintain another variable within safe levels.

We conclude from these data that clopidogrel produces a significant reduction of ADP-dependent platelet aggregation in patients with clinically proven atherosclerosis, related to the increase in bleeding time; this could explain the larger efficacy of the drug demonstrated by the clinical studies. Despite the reduction in platelet function and the increase in bleeding time, the profile of hemostatic safety was adequate.

ACKNOWLEDGMENT

The authors express their sincere gratitude to Guadalupe MacCarty, PhD, for review of the manuscript.

REFERENCES

1. Murray CJ, Lopez AD. Mortality by cause for eight regions of the world: Global Burden of Disease Study. *Lancet* 1997;349(9061):1269.
2. Olvera S. *Epidemiología de la ateroesclerosis coronaria.* En: Posadas C (ed). Dislipidemias y Ateroesclerosis. Interamericana-McGraw Hill, México, 1995:29.
3. Chávez-Domínguez R. Factores de riesgo lipídicos. Epidemiología general de la ateroesclerosis en México. En: Alcocer-Díaz-Barreiro L (ed). Pautas en Cardiología Preventiva. *Ediciones de la Sociedad Mexicana de Cardiología. Grupo MIND*, México, 1999:1.
4. Ware JA, Heistad DD. Platelet-Endothelium interactions. *New Engl J Med* 1993;328:628.
5. Fischer A, Gutstein DE, Fuster V. Thrombosis and coagulation abnormalities in the acute coronary syndromes. *Cardiol Clin* 1999;17(2):283.
6. Antiplatelet Trialists' Collaboration. Colaborative overview of randomised trials of antiplatelet therapy I. Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. *BMJ* 1994;308:81.
7. Hass WK, Easton JD, Adams JP, et al. A randomized trial comparing ticlopidine hidrochloride with aspirin for the prevention of stroke in high-risk patients. *New Engl J Med* 1989;321:501.
8. Gent M, Blakely JA, Easton JB. The Canadian American Ticlopidine Study (CATS) in thromboembolic stroke. *Lancet* 1989;1:1215.
9. CAPRIE Steering Commitee. A randomised, blinded trial of clopidogrel versus aspirin in patients at risk of ischemic events (CAPRIE). *Lancet* 1996;348:1329.

10. Harker LA, Marzec UM, Kelly AB, et al. Clopidogrel inhibition of stent, graft, and vascular thrombogenesis with antithrombotic enhancement by aspirin in nonhuman primates. *Circulation* 1998;22:2461.

11. Miller JL. Platelet function testing: An improved approach utilizing lumi-aggregation and interactive computer system. *Am J Clin Pathol* 1984;81:471.

12. Hechler B, Eckly A, Ohlmann P, et al. The PSY1 receptor, necessary but no sufficient to suport full ADP-induced platelet aggregation, is not the target of the drug clopidogrel. *Br J Hematol* 1998;103:858.

13. Weber AA, Reimann S, Schor K. Specific inhibition of ADP-induced platelet aggregation by Clopidogrel in vitro. *Br J Pharmacol* 1999;126:415.

14. Mills DC, Puri R, Hu CJ, et al. Clopidogrel inhibits the binding of ADP analogues to the receptor mediating inhibition of platelet adenylate cyclase. *Arterioscler Thromb* 1992;124:430

15. Takahashi O. Characteristics or rat platelets and relative contributions of platelets and blood coagulation to hemostasiss. *Food Chem Toxicol* 2000;38:203.

16. Schwarz UF, Geiger J, Walter U, et al. Flow citometry analysis of intracellular VASP phosphorylation, the assessment of activating and inhibitory signal transduction pathways in human platelets-definition and detection of ticlopidine/clopidogrel effects. *Thromb Haemost* 1999; 82:1145.

17. Thebault JJ, Kieffer G, Lowe GD, et al. Repeated-dose pharmacodynamics of clopidogrel in healthy subjects. *Semin Thromb Hemost* 1999;25 (suppl 2):9.

18. Denninger MH, Necciari J, Serre Lacroix, et al. Clopidogrel antiplatelet activity is independent of age and presence of atherosclerosis. *Semin Thromb Hemost* 1999;25 (suppl 2):41.

19. David JL, Limet R. Antiplatelet activity of clopidogrel in coronary artery bypass graft surgery patients. *Thromb Haemost* 1999;82:1417.

20. Savcic M, Hauert J, Bachmann F, et al. Clopidogrel loading dose regimens: Kinetic profile of pharmacodynamic response in healthy subjects. *Semin Thromb Hemost* 1999;25 (suppl 2):15.

21. Herbert JM, Dol F, Bernat A, et al. The antiaggregating and antithrombotic activity of clopidogrel is potentiated by aspirin in several experimental models in the rabbit. *Thromb Haemost* 1998;80:512.

22. Berger PB, Bell MR, Rihal CS, et al. Clopidogrel versus ticlopidine after intracoronary stent placement. *J Am Coll Cardiol* 1999;34:1891.

23. Müller C, Büttner HJ, Petersen J, et al. A randomized comparison of clopidogrel and aspirin versus ticlopidine and aspirin after the placement of coronary artery stents. *Circulation* 2000;101:590.

24. Mishkel GJ, Aguirre FV, Ligon RW, et al. Clopidogrel as adjunctive antiplatelet therapy during coronary stenting. *J Am Coll Cardiol* 1999;34:1884.