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High-Dose Atorvastatin on the Pharmacodynamic Effects of Double-Dose Clopidogrel in Patients Undergoing Percutaneous Coronary Interventions

The ACHIDO (Atorvastatin and Clopidogrel HIgh DOse in stable patients with residual high platelet activity) Study

Mario Leoncini, MD,* Anna Toso, MD,* Mauro Maioli, MD,* Dominick J. Angiolillo, MD, PhD,† Betti Giusti, PhD,‡ Rossella Marcucci, MD,‡ Rosanna Abbate, MD,‡ Francesco Bellandi, MD*

Prato and Florence, Italy; and Jacksonville, Florida

Objectives The goal of this study was to investigate the impact of high-dose atorvastatin on the pharmacodynamic (PD) effects of double-dose clopidogrel in statin-naive patients with stable coronary artery disease (CAD) and high-on-treatment platelet reactivity (HTPR) while on standard-dose clopidogrel before percutaneous coronary intervention (PCI).

Background Patients with HTPR are at increased risk of adverse cardiovascular events after PCI. High-dose statins improve prognosis in high-risk patients by lipid- and nonlipid-related mechanisms, including antithrombotic effects.

Methods The ACHIDO (Atorvastatin and Clopidogrel HIgh DOse in stable patients with residual high platelet activity) study was a randomized PD study of high-dose (80 mg) atorvastatin in addition to double-dose (150 mg) clopidogrel (atorvastatin group, n = 38) versus double-dose clopidogrel alone (control group, n = 38) in patients with HTPR. HTPR was defined as $P2Y_{12}$ reaction units (PRU) ≥235 by the VerifyNow P2Y12 assay. Platelet reactivity was evaluated immediately before PCI and at 10 and 30 days.

Results Patients randomized to atorvastatin had lower PRU values (188 ± 48 vs. 223 ± 53 PRU, $p < 0.01$; primary endpoint) and HTPR rates (16% vs. 42%, $p < 0.01$) at 30 days than patients in the control group. Statin treatment (odds ratio [OR]: 3.8, $p = 0.011$), baseline PRU <298 (OR: 10.7, $p = 0.0001$), noncarrier status of *CYP2C19*2* loss-of-function allele (OR: 2.9, $p = 0.043$), and age (OR: 0.94, $p = 0.032$) were variables significantly associated with optimal PD response (PRU <235) at 30 days. No correlations were found between PRU and lipid fractions.

Conclusions High-dose atorvastatin significantly improved the PD effects of double-dose clopidogrel in our stable CAD patients with HTPR undergoing PCI (Atorvastatin and Clopidogrel HIgh DOse in stable patients with residual high platelet activity [ACHIDO]; NCT01335048). (J Am Coll Cardiol Intv 2013;6:169–79) © 2013 by the American College of Cardiology Foundation

From the *Division of Cardiology, Misericordia e Dolce Hospital, Prato, Italy; †Division of Cardiology, Department of Medicine, University of Florida College of Medicine, Jacksonville, Florida; and the ‡Department of Medical and Surgical Critical Care, University of Florence–Atherothrombotic Diseases Center, Careggi Hospital, Florence, Italy. This study was supported by grants from the Ministry of Health to young investigators GR 2009 1605547 and from Ente Cassa di Risparmio di Firenze to Fiorgen Foundation. Dr. Angiolillo has received honoraria for lectures from Bristol-Myers Squibb, Sanofi-Aventis, Eli Lilly & Co., Daiichi Sankyo, AstraZeneca, and Abbott Vascular; consulting fees from Bristol-Myers Squibb, Sanofi-Aventis, Eli Lilly & Co., Daiichi Sankyo, The Medicines Company, Portola, Novartis, Medicure, Accumetrics, Arena Pharmaceuticals, Abbott Vascular, AstraZeneca, Merck & Co., and Evolva; and research grants from GlaxoSmithKline, Otsuka, Eli Lilly & Co., Daiichi Sankyo,

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The long-term prognostic benefits of 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitors in high-risk patients are well established, and their use is strongly recommended for secondary prevention in patients with coronary artery disease (CAD) (1). The clinical benefit of statins is attributed to multiple mechanisms that go beyond their lipid-lowering effects and include antithrombotic properties (2). In fact, statins inhibit adenosine diphosphate (ADP) and thrombin-induced platelet aggregation in both healthy subjects and patients with CAD (3,4). Although a pharmacodynamic (PD) interaction between clopidogrel and cytochrome CYP3A4–metabolized statins has been described in the acute phase of clopidogrel treatment with standard loading-dose regimens (5,6), most studies show a lack of interaction that has been consistently shown when using high clopidogrel dosing regimens as well as once patients are in the maintenance phase of treatment (7–11). Moreover, PD studies appear to show some synergy between clopidogrel and atorvastatin (3,4). Atorvastatin effects seem to be dose related and additive to that exerted by clopidogrel (4).

Abbreviations and Acronyms

ADP = adenosine diphosphate
BASE = iso-thrombin receptor activating peptide reaction unit
CAD = coronary artery disease
CI = confidence interval
CK-MB = creatine kinase MB fraction
HTPR = high-on-treatment platelet reactivity
IPA = percent $P2Y_{12}$ inhibition
iso-TRAP = iso-thrombin receptor activating peptide
OR = odds ratio
PCI = percutaneous coronary intervention
PD = pharmacodynamic
PRU = $P2Y_{12}$ reaction units
ULN = upper limit of normal

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High on-treatment platelet reactivity (HTPR) has been shown to be an independent risk factor for recurrent ischemic events, particularly in patients undergoing percutaneous coronary intervention (PCI) (12). Double maintenance doses of clopidogrel may be a possible strategy to overcome HTPR (13–15). However, the impact of this treatment, when used in association with high-dose atorvastatin, has not been explored. The aim of this prospective, randomized study was to evaluate the effects of high-dose (80 mg/day) atorvastatin on PD profiles of double-dose clopidogrel (150 mg/day) in statin-naive patients with stable CAD and HTPR undergoing PCI.

Methods

Population and study protocol. The ACHIDO (Atorvastatin and Clopidogrel HIgh DOse in stable patients with residual high platelet activity) study was a prospective, randomized, active-control, PD trial. From April 2011 to December 2011, all consecutive statin-naive patients (N = 209) with angiographically documented CAD undergoing PCI with stent implantation at the Division of Cardiology, Misericordia e Dolce Hospital (Prato, Italy) were considered for enrollment in the present study. Per our institutional protocol, all stable patients scheduled to undergo elective angiography are prescribed with aspirin (100 mg/day) and clopidogrel (600 mg loading dose followed by a maintenance dose of 75 mg/day) for at least 7 days. Exclusion criteria included: contraindications to statin treatment; acute renal failure or end-stage renal failure requiring dialysis; active liver disease or liver cirrhosis; unexplained transaminase increase >2 times the upper limit of normal (ULN); peripheral muscle disease or creatine kinase >2.5 times ULN; treatment with a glycoprotein IIb/IIIa inhibitor within the past week; current treatment with proton-pump inhibitors and/or omega-3; active bleeding or recent bleeding diathesis (within the past month); platelet function disorder or platelet count $<150 \times 10^3/\mu l$; hemoglobin <10 g/dl; need for warfarin treatment; previous hemorrhagic stroke; malignancy; and refusal of consent. Of the 209 patients screened for the study, 90 (43%) presented HTPR immediately before PCI. HTPR was defined as $P2Y_{12}$ reaction units (PRU) ≥235 according to the VerifyNow $P2Y_{12}$ platelet function test (Accumetrics, San Diego, California) (16–19). Of these, 12 had exclusion criteria, and thus a total of 78 patients were enrolled in this study. Patient disposition is summarized in Figure 1. Per study protocol, all enrolled patients were treated with a double maintenance dose of clopidogrel (150 mg/day). This regimen was chosen, given its efficacy in reducing platelet reactivity in patients with HTPR as observed in other PD investigations (13–15). More potent treatment strategies in overcoming HTPR, such as by the novel $P2Y_{12}$ receptor inhibitors prasugrel or ticagrelor, were not considered since these drugs have an indication for use only in the setting of acute coronary syndromes, and our population was composed of stable CAD patients.

Randomization (1:1) to the atorvastatin or control arms was performed immediately after PCI by computerized open-label assignments in consecutive blinded envelopes: 39 patients were assigned to receive clopidogrel at the maintenance dose of 150 mg/day (8 AM) and atorvastatin 80 mg/day (10 PM) (atorvastatin group) and 39 patients to receive only clopidogrel at 150 mg/day (control group). Antiplatelet and statin regimens were maintained un-

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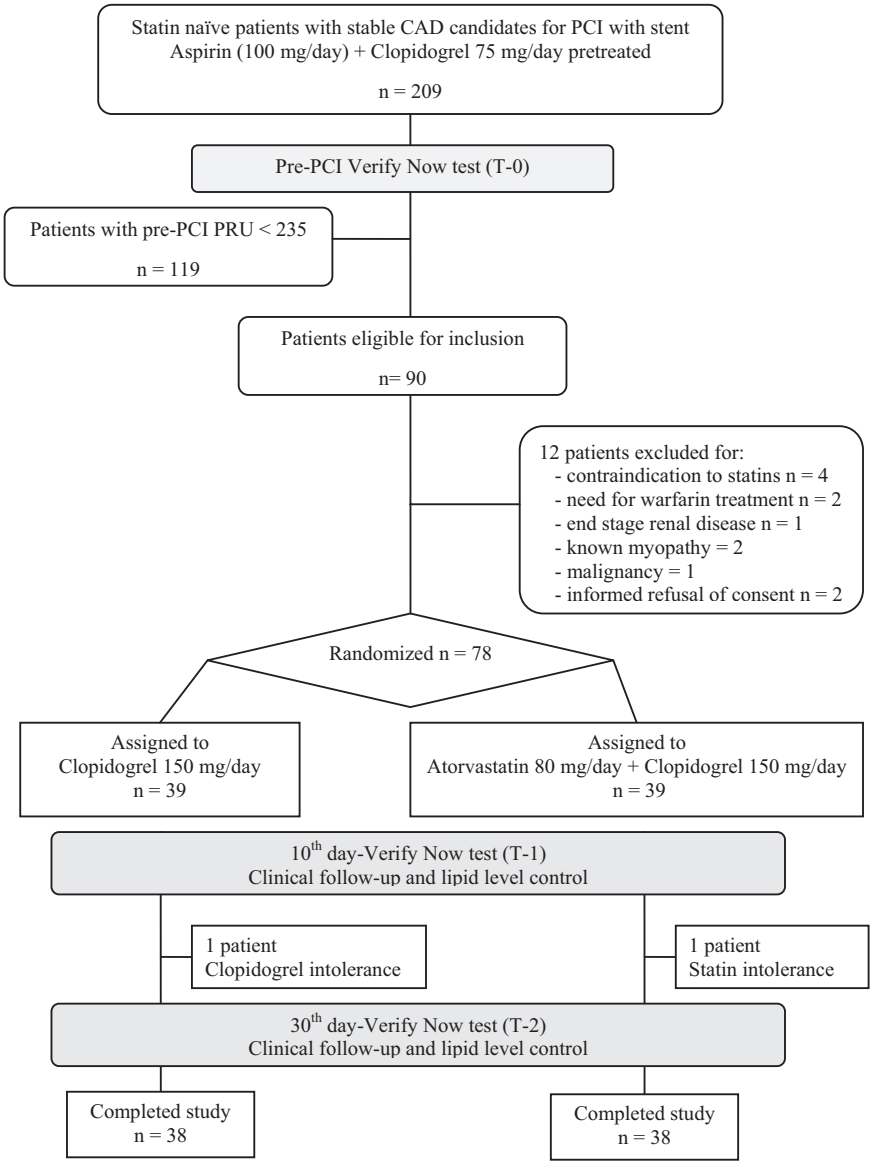

Figure 1. Study Flow Chart

Enrollment criteria and trial flow. CAD = coronary artery disease; PCI = percutaneous coronary intervention; PRU = $P2Y_{12}$ reaction units.

changed for 30 days. One patient in the atorvastatin group and 1 in the control group withdrew from the study due to drug intolerance. Thus, 76 patients completed the study.

All patients continued treatment with aspirin (100 mg/day) indefinitely. At the end of the study, the choice of antiplatelet and statin dosage was left at the discretion of the treating physician. PCI was performed according to current standard guidelines; the type of stent implanted and the periprocedural use of antithrombotic therapy, including glycoprotein IIb/IIIa inhibitor use and choice of anticoagulant, were left to the discretion of the operator.

Periprocedural myocardial infarction was defined as creatine kinase-MB (CK-MB) mass elevation ≥3 times ULN within 24 h after PCI or the development of new Q waves in 2 or more contiguous electrocardiographic leads with CK-MB greater than ULN (1).

Clinical, biochemical, angiographic, and genotyping data were recorded for all patients in a dedicated database. The protocol was approved by our institutional ethics committee, and all patients gave written informed consent.

Platelet reactivity. Platelet reactivity was evaluated immediately before PCI (T-0) and at 10 (T-1) and 30 (T-2) days using the VerifyNow $P2Y_{12}$ assay. This point-of-care assay

measures the inhibition of $P2Y_{12}$ ADP receptors on platelets. Technical details of the assay have been described previously (20). Blood samples for platelet function testing were collected just before PCI and 2 to 4 h after the ingestion of the last clopidogrel dose. We note that the first blood sample (T-0) was drawn before administration of glycoprotein IIb/IIIa inhibitors. The VerifyNow instrument reports 3 different parameters for each assay: 1) PRU, which express the extent of ADP-mediated aggregation specific to the $P2Y_{12}$ receptors; 2) iso-TRAP reaction units (BASE) which represents total platelet function, despite $P2Y_{12}$ receptor blockage, in response to iso-thrombin receptor activating peptides (iso-TRAP) specific to the thrombin protease-activated receptor-1 (PAR-1) and PARS-4; and 3) percent platelet $P2Y_{12}$ inhibition (IPA) calculated based on the PRU and BASE results according to the formula: $[(\text{BASE} - \text{PRU}) \div \text{BASE}] \times 100$ (20). We calculated absolute and relative changes over time in PRU and BASE values. The quality controls for our laboratory have been reported elsewhere (18). Clinical follow-up and plasma lipid levels were also carried out at T-0, T-1, and T-2. Compliance and adverse events were assessed by the attending physician based on interview, pill count, and laboratory evaluation.

Genotyping. Genomic DNA was isolated from venous peripheral blood using Tecan, Freedom EVO liquid handler (Tecan Group, Männedorf, Switzerland) and the magnetic bead based GeneCatcher gDNA Blood kit (Invitrogen, Carlsbad, California). DNA purity and concentration were determined by NanoDrop spectrophotometer (Thermo Scientific, Wilmington, Delaware). Genotyping of the *CYP2C19*2* loss-of-function polymorphism (681G > A, rs4244285) was performed using TaqMan validated Drug Metabolism Genotyping assay with the 7900HT Sequence Detection System (Applied Biosystems, Foster City, California) (21).

Study endpoints and sample size calculation. The primary endpoint of the study was the PRU after 30 days (T-2) of treatment. The sample size was calculated by assuming mean baseline PRU values of 285 ± 47 in patients with HTPR, defined as PRU ≥235 while on standard-dose clopidogrel, and a mean PRU reduction of 24% after 30 days of 150-mg clopidogrel (15). Thus, 74 patients were required (37 per treatment group) to detect a further 15% relative reduction in mean PRU values in the atorvastatin compared with the control group, with 80% power at the conventional, 2-sided significance level of 5%. Our protocol required that each group comprise at least 39 patients to allow a 5% dropout rate. Additional exploratory endpoints were: 1) the percentage of patients with optimal response at 30 days, defined as PRU <235; 2) the changes over time in PRU and BASE; and 3) correlations with lipid levels.

Statistical analysis. Variables were analyzed for a normal distribution with the Kolmogorov-Smirnov test, and variables are presented as mean ± SD or as median (interquartile range). Categorical variables were presented as counts and percentages, and compared by chi-square or Fisher exact test. Continuous variables were compared by t test for normally distributed values; the Mann-Whitney *U* test was used alternatively. The repeated-measure analysis of variance was used to evaluate platelet reactivity and lipid fraction changes over time. Pairwise comparisons within group were approached with *t* test for paired samples and Bonferroni adjustment (yielding a significance threshold of 0.016). The Pearson correlation coefficient was used to measure the relationship between PRU and BASE and lipid fraction values. The univariate analysis was used to identify the variables significantly associated with optimal response (PRU <235) at 30 days. For baseline PRU values, the optimal cutoff values were calculated by receiver-operating characteristic curve analysis as the closest to the upper-left corner. The association with optimal response at 30 days was expressed as the odds ratio (OR), and the 95% confidence interval (CI) was also reported. All p values are 2-tailed, and statistical significance was defined as $p < 0.05$. All analyses were performed with SPSS statistical software, version 19.0 (SPSS, Chicago, Illinois).

Results

The mean age for the entire study cohort was 67 ± 11 years, and 43% of patients were over 70 years old. Forty-two percent of patients had estimated creatinine clearance <60 mL/min, 36% had diabetes mellitus, and 64% multivessel coronary disease. Clinical, biochemical, and procedural variables were comparable in the 2 treatment arms. In particular, advanced age, diabetes mellitus, poor renal function, and higher total cholesterol levels were evenly distributed in the 2 groups. No significant differences were observed in *CYP2C19*2* loss-of-function allele frequencies (Table 1).

Platelet reactivity. The mean time intervals between the loading dose of clopidogrel and T-0 were 10 ± 3 and 10 ± 2 days in the atorvastatin and control groups, respectively ($p = 0.68$). All patients enrolled in the study underwent all the PD testing as scheduled, and the time intervals were similar for both groups (Table 2). Results of platelet reactivity at each time point for both groups are presented in Table 2. The PRU values decreased significantly from baseline to 10 days and from baseline to 30 days in both treatment arms. However, patients in the atorvastatin group achieved significantly lower PRU than patients in the control group at 30 days (188 ± 48 vs. 223 ± 53, respectively; $p < 0.01$; primary endpoint).

The distribution of mean PRU values over time in both groups is shown in Figure 2A. PRU values in the atorvastatin group decreased consistently for 30 days, whereas in the control group, PRU values showed a marked reduction in the first 10 days and a slight increase thereafter. The extent of relative PRU reduction mirrors this trend over

Table 1. Clinical Characteristics, and Biological and Angiographic Parameters of the Study Groups

	Atorvastatin Group (n = 38)	Control Group (n = 38)	p Value
Age, yrs	66 ± 11	68 ± 11	0.58
Age ≥70 yrs	16 (42)	17 (45)	0.82
Men	30 (79)	27 (71)	0.59
Risk factors			
Hypertension	24 (63)	29 (76)	0.31
Diabetes mellitus	12 (32)	15 (39)	0.63
Active smoking	14 (37)	9 (24)	0.31
BMI, kg/m^2	26 [24–29]	28 [25–30]	0.23
Total cholesterol >200 mg/dl	11 (29)	10 (26)	1.00
Creatinine clearance <60 ml/min	16 (42)	16 (42)	1.00
Previous MI	9 (24)	9 (24)	1.00
Previous PCI or CABG	10 (26)	9 (24)	1.00
Baseline LV ejection fraction, %	51 ± 10	54 ± 5	0.07
Laboratory variables			
Hemoglobin, mg/dl	13.9 ± 0.9	13.6 ± 1	0.15
RBCs, $10^3/\mu l$	46 ± 0.5	4.5 ± 0.3	0.44
WBCs, $10^3/\mu l$	6.6 ± 1.7	6.8 ± 1.8	0.74
Platelet count, $10^3/\mu l$	203 ± 51	208 ± 48	0.61
MPV, $10^9/\mu l$	9 ± 1	9 ± 1	0.84
hs-CRP, mg/l	0.28 ± 0.5	0.24 ± 0.2	0.52
Creatinine clearance, ml/min	67 ± 18	66 ± 22	0.83
Baseline cTnI, ng/ml	0.03 ± 0.04	0.02 ± 0.03	0.14
Baseline CK-MB, ng/ml	2.4 ± 1.1	2.3 ± 0.8	0.74
Angiographic and PCI data			
Multivessel disease	26 (68)	23 (61)	0.63
Multivessel PCI	17 (45)	14 (37)	0.64
Lesion location			0.82
Left anterior descending	21 (37)	19 (35)	
Left circumflex artery	17 (30)	17 (31)	
Right coronary artery	16 (28)	17 (31)	
Left main	2 (4)	1 (2)	
Saphenous vein graft	1 (2)	0	
Mean number of stents	2.5 ± 1.3	2.5 ± 1.6	0.93
Total stent length, mm	47 ± 28	44 ± 25	0.63
Stent type			0.51
Drug-eluting stents	31 (82)	34 (89)	
Bare-metal stents	7 (18)	4 (11)	
GP IIb/IIIa inhibitor*	13 (34)	9 (24)	0.44
*CYP2C19*2* polymorphism genotype			
*1*1	27 (71)	27 (71)	1.00
*1*2	9 (24)	10 (26)	
*2*2	2 (5)	1 (3)	
Concomitant medications at T-0			
Beta-blockers	20 (51)	15 (41)	0.35
Calcium channel blockers	8 (21)	6 (16)	0.76
ACE inhibitors	24 (64)	20 (54)	0.49
ARB	4 (10)	5 (13)	0.99
Nitrates	10 (26)	12 (31)	0.80
Diuretic	6 (15)	3 (10)	0.47
Insulin	4 (10)	4 (10)	1.00

Values are mean ± SD n(%), or median [interquartile range]. *Only eptifibatide was used.

ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blockers; BMI = body mass index; CABG = coronary artery bypass graft; CK-MB = creation kinase-MB; cTnI = cardiac troponin I; GP = glycoprotein; hs-CRP = high sensitivity C-reactive protein; LV = left ventricular; WBC = white blood cell; MI = myocardial infarction; MPV = mean platelet volume; PCI = percutaneous coronary intervention; RBC = red blood cell; T-0 = enrollment time.

Table 2. Distribution of Platelet Function Parameters Over Study Time Course

	Atorvastatin Group			Control Group		
	T-0	T-1	T-2	T-0	T-1	T-2
Time to determination, days		11 ± 2	32 ± 4		10 ± 1	32 ± 4
PRU	290 ± 35	200 ± 50*	188 ± 48*†‡	292 ± 37	210 ± 58*	223 ± 53*†
Absolute difference from T-0		−90 ± 58	−102 ± 54‡		−82 ± 53	−69 ± 51
Relative difference from T-0, %		−31 ± 17	−35 ± 16‡		−28 ± 17	−23 ± 16
Absolute difference from T-1			−12 ± 35‡			13 ± 49
Relative difference from T-1, %			−4 ± 20‡			12 ± 31
BASE	312 ± 39	290 ± 35*‡	298 ± 34†	316 ± 29	313 ± 37	310 ± 20
Absolute difference from T-0		−23 ± 29‡	−14 ± 46		−3 ± 34	−6 ± 30
Relative difference from T-0, %		−7 ± 8‡	−3 ± 13		−1 ± 11	−1 ± 9
Absolute difference from T-1			9 ± 35			−3 ± 30
Relative difference from T-1, %			4 ± 12			−0.05 ± 10
IPA, %	8 ± 7	31 ± 17*	37 ± 14*†‡	10 ± 7	33 ± 16*	28 ± 15*†

Values are mean ± SD. *p < 0.01 versus baseline (T-0); †p < 0.01 within group (Bonferroni adjustment); ‡p < 0.01 atorvastatin versus control group.
BASE = iso-TRAP reaction units; IPA = percent $P2Y_{12}$ inhibition; PRU = $P2Y_{12}$ reaction units.

time, and the difference between the 2 groups becomes significant only at 30 days (35 ± 16% vs. 23 ± 16%, p < 0.01) (Table 2, Fig. 2B). Iso-TRAP–mediated platelet reactivity, expressed by BASE values, decreased slightly over time in the control group. Atorvastatin-treated patients showed a distinctive pattern of BASE reduction with a significant marked decrease in the short term (T-1) and a later increase (T-2) to levels similar to that of controls (Table 2, Fig. 3). Based on the results of PRU and BASE, the mean IPA was significantly higher after 1 month of treatment with atorvastatin (37 ± 14% vs. 28 ± 15%, respectively; p < 0.01) (Table 2).

High residual platelet reactivity. A higher number, albeit not significant, of patients randomized to atorvastatin changed their response status, becoming optimal responders at T-1 (10 days) compared with patients in the control arm (74% vs. 63%, p = 0.1). This number further increased at T-2 (30 days), reaching statistical significance (84% vs. 58%, p = 0.02) (Fig. 4). Baseline variables significantly associated with 30-day optimal response at univariate analysis included age (OR: 0.94 [95% CI: 0.89 to 0.99]; p = 0.032), noncarrier status of the *CYP2C19*2* allele (OR: 2.9 [95% CI: 1.01 to 8.39]; p = 0.043), baseline higher PRU values (OR: 0.98 [95% CI: 0.96 to 0.99]; p = 0.005), and use of atorvastatin (OR: 3.8 [95% CI: 1.3

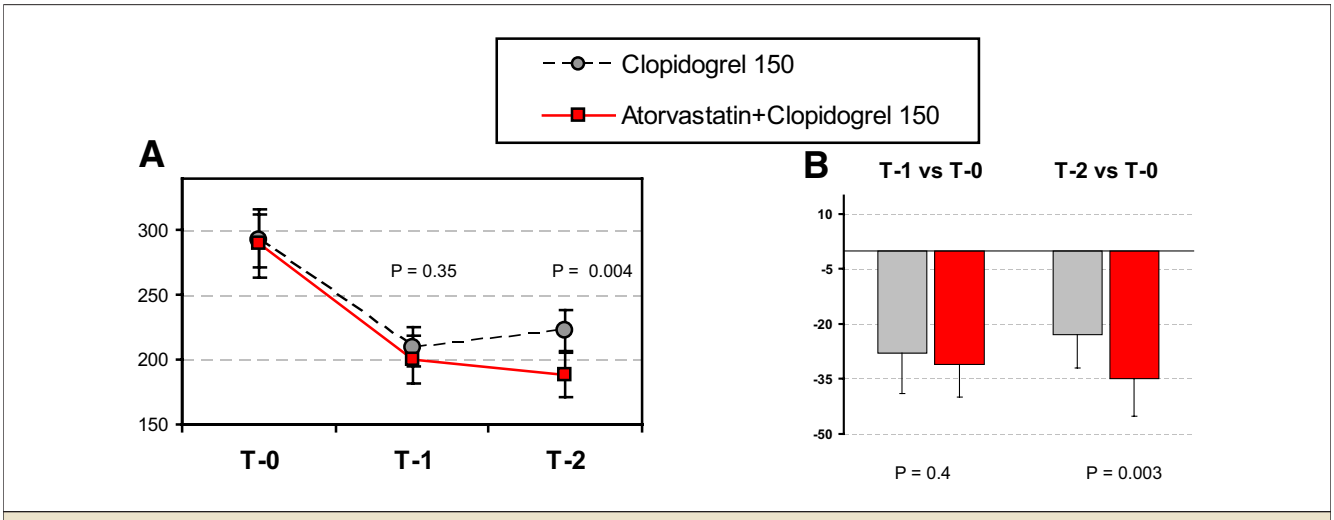

Figure 2. Differences in ADP-Induced Platelet Reactivity as Expressed by PRU

(A) Mean $P2Y_{12}$ reaction units (PRU) values at baseline (T-0), 10 days (T-1) and 30 days (T-2) in atorvastatin+clopidogrel 150 mg **(squares)** and clopidogrel 150 mg patients **(circles)**. **(B)** Differences in mean relative PRU reduction from T-0 to T-1 and from T-0 to T-2: atorvastatin+clopidogrel 150 mg are **red,** and clopidogrel 150 mg patients are **gray**. ADP = adenosine diphosphate.

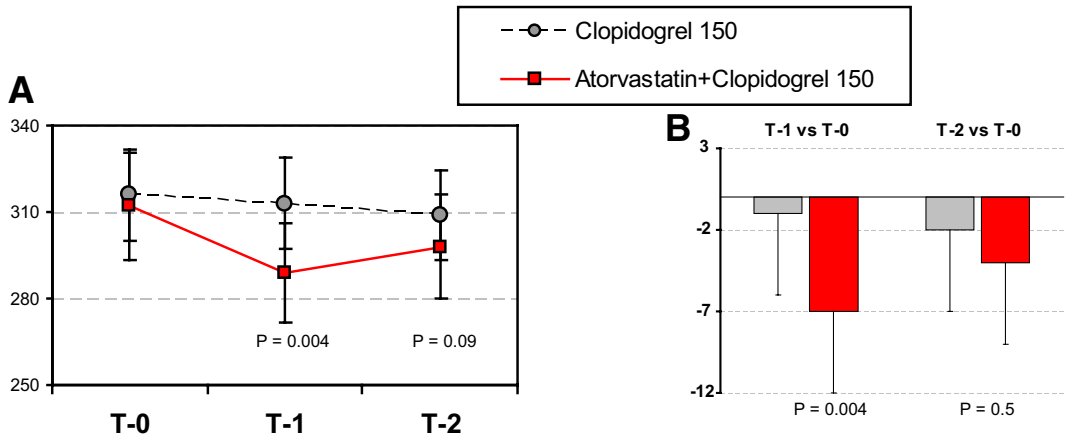

Figure 3. Differences in iso-TRAP–Induced Platelet Reactivity as Expressed by BASE

(A) Mean iso-thrombin receptor activating peptide (iso-TRAP) reaction unit (BASE) values at baseline (T-0), 10 days (T-1), and 30 days (T-2) in atorvastatin+clopidogrel 150 mg **(squares)** and clopidogrel 150 mg patients **(circles)**. **(B)** Differences in mean relative BASE reduction from T-0 to T-1 and from T-0 to T-2: atorvastatin+clopidogrel 150 mg is indicated by the **red**, and clopidogrel 150 mg by the **gray**.

to 11.5]; p = 0.011), (Table 3). The receiver-operating characteristic curve analysis showed that pre-procedural PRU significantly discriminate between patients with and without HTPR at 30 days, with an area under the curve of 0.77 (95% CI: 0.6 to 0.9, p = 0.001). A PRU value <298 was identified as the optimal cutoff point to predict optimal response at 30 days (OR: 10.7 [95% CI: 3.3 to 34.8]; p = 0.0001).

Lipid levels. Patients treated with high-dose atorvastatin showed a significant reduction in all fractions of cholesterol and triglycerides at 10 and 30 days. In particular, low-density lipoprotein cholesterol levels were markedly lower in patients randomized to treatment with atorvastatin at both time points (Table 4). However, no significant correlations were found between the different lipid fractions and PRU and BASE values.

Adverse events and follow-up. Clinical follow-up was completed in all patients. Overall, the occurrence of periprocedural myocardial infarction was 18%, without significant differences between the 2 groups (atorvastatin, 16%, vs. control, 21%, p = 0.7); the mean peak CK-MB values were 7 ± 8 ng/ml versus 10 ± 17 ng/ml in the atorvastatin and control groups, respectively (p = 0.2). No other ischemic events, major bleeding, or transfusions were reported during the follow-up.

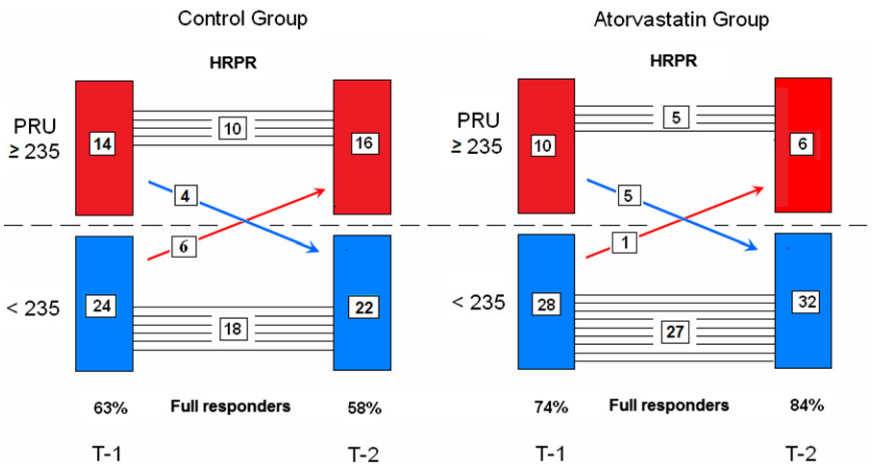

Figure 4. Responsiveness Status at 10 and 30 Days

Responsiveness status at 10 days (T-1) and 30 days (T-2). $P2Y_{12}$ reaction units (PRU) values ≥235 (high on-treatment platelet reactivity [HTPR]) are indicated by the **red**, and PRU values <235 (optimal responders) by the **blue**. **Continuous lines** show patients with stable reactivity between T-1 and T-2. **Arrows** indicate patients who change their reactivity between T-1 and T-2. Responsiveness status at baseline is not represented: all patients had PRU values ≥235.

Table 3. Univariate Analysis of Predictors of Clopidogrel Response at 30 Days

	Optimal Responders (Pry <235) (n = 54)	HTPR (Pry ≥235) (n = 22)	p Value
Age, yrs	65 ± 11	72 ± 10	0.027
Age ≥70 yrs	20 (37)	13 (59)	0.08
Male	41 (76)	16 (73)	0.77
Diabetes mellitus	16 (30)	11 (50)	0.09
Hypertension	38 (70)	15 (68)	0.84
BMI, kg/m^2	26 [24–29]	27 [25–29]	0.26
Laboratory variables			
Hemoglobin, mg/dl	13.8 ± 1	13.7 ± 1	0.74
RBCs, 10^3/μl	4.5 ± 0.4	4.6 ± 0.3	0.99
WBCs, 10^3/μl	6.8 ± 1.9	6.4 ± 1.3	0.38
Platelet count, 10^3/μl	210 ± 51	196 ± 45	0.33
MPV, 10^9/μl	9.5 ± 1.4	9.3 ± 1.1	0.53
Creatinine clearance, ml/min	69 ± 20	60 ± 18	0.12
hs-CRP, mg/l	0.24 ± 0.6	0.31 ± 0.4	0.45
Creatinine clearance <60 ml/min	20 (37)	12 (55)	0.16
Baseline LV ejection fraction, %	52 ± 9	55 ± 6	0.21
*CYP2C19*2* polymorphism genotype			0.008*
*1*1	42 (78)	12 (55)	
*1*2	12 (22)	7 (32)	
*2*2	—	3 (14)	
Allocation to high-dose atorvastatin	32 (59.3)	6 (27.3)	0.02
Total cholesterol, mg/dl	182 ± 36	181 ± 34	0.90
LDL-cholesterol, mg/dl	118 ± 1	116 ± 35	0.83
HDL-cholesterol, mg/dl	40 ± 9	37 ± 8	0.32
Triglycerides, mg/dl	124 ± 55	139 ± 63	0.33
Baseline (T-0) PRU	283 ± 35	311 ± 29	0.002
Baseline (T-0) BASE	314 ± 35	313 ± 32	0.87

Values are mean ± SD, n(%), or median [interquartile range]. *Values were compared using the p for trend test.
HDL = high-density lipoprotein; LDL = low-density lipoprotein; other abbreviations as in Table 1.

At 30 days, 2 patients, both randomized to atorvastatin, presented a transaminase increase >2 × ULN.

Discussion

The results of this prospective, PD, randomized study show that treatment with high-dose atorvastatin associated with double-dose clopidogrel reduces platelet reactivity significantly more than double-dose clopidogrel alone in statin-naive patients with stable CAD and HTPR before PCI. In particular, the antiplatelet effects of high-dose atorvastatin occur early and persist over time; they are additive to those of clopidogrel and do not correlate with the degree of lipid reduction observed during the study period.

Statins are known to have multiple nonlipid-lowering ("pleiotropic") effects. They have vasoprotective, anti-inflammatory, antioxidant, immunomodulating properties, and also inhibit platelet function (2). In particular, statins

Table 4. Change in Lipid Profile Over Time in Atorvastatin and Control Group

	Atorvastatin Group			Control Group		
	T-0	T-1	T-2	T-0	T-1	T-2
Total cholesterol, mg/dl	183 ± 42	146 ± 38*†	134 ± 24*†‡	181 ± 28	170 ± 33	165 ± 39*‡
LDL-cholesterol, mg/dl	117 ± 34	91 ± 30*†	81 ± 17*†‡	117 ± 29	109 ± 29	105 ± 37‡
HDL-cholesterol, mg/dl	40 ± 8	35 ± 8*	36 ± 10*‡	38 ± 10	35 ± 9	36 ± 8
Triglycerides, mg/dl	127 ± 54	95 ± 39*†	88 ± 34*†‡	127 ± 55	132 ± 61	119 ± 55

Values are mean ± SD. *p < 0.01 versus baseline (T-0); †p < 0.01 atorvastatin versus control group; ‡p < 0.01 within group (Bonferroni adjustment).
Abbreviations as in Table 3.

have been shown to reduce platelet aggregation, dense granule release, and platelet-mediated thrombus formation, all rapidly and in a dose-dependent manner (3,4,22,23). Moreover, the combination of standard-dose clopidogrel (75 mg) with atorvastatin (40 mg), leads to further reduction in ADP- and TRAP-induced platelet activation and TRAP-induced platelet aggregation (4). This additive effect is already evident with low-dose (20 mg) atorvastatin and persists over time (3). Although PD interaction between clopidogrel and atorvastatin, both substrates of cytochrome CYP3A4, has been described in the early phase of clopidogrel treatment (standard loading dose) (5,6), a lack of PD interaction has been shown with high clopidogrel doses as well as once patients are in the maintenance phase of treatment, which was the setting of our study (7–11).

Our study expands upon previous data showing that the addition of high-dose atorvastatin (80 mg) in statin-naive HTPR patients simultaneously with increased doses of clopidogrel (150 mg) is associated with enhanced platelet inhibitory effects, in particular with more potent $P2Y_{12}$ receptor inhibition at 30 days compared with controls. The reduction of ADP-mediated reactivity is greater in our study than in previous studies that showed minimal effect on ADP-induced aggregation (4). This could depend on various factors, including the very high dose (80 mg) of atorvastatin administered in our study, the relatively low efficacy of high-dose clopidogrel in HTPR patients, and/or the synergy in inhibition of ADP-mediated and iso-TRAP–mediated platelet aggregation pathways (24). In fact, only atorvastatin-treated patients had significant early inhibition of iso-TRAP–-induced aggregation. This effect is less appreciated at 30 days when the values of BASE were similar in the 2 study groups. We could assume that in the early period following PCI, when thrombin levels increase, atorvastatin exerts a protective role (17) as supported by the inhibition of iso-TRAP–mediated aggregation.

Our series of stable CAD patients, undergoing PCI and treated with clopidogrel (75 mg) for at least 7 days, presented a relatively high incidence (43%) of HTPR, but overall consistent with other studies (25). This could be due to the advanced age and high prevalence of diabetes mellitus and renal dysfunction in our study population, all clinical factors associated with HTPR (13,26,27). The association of high-dose atorvastatin and double-dose clopidogrel in the present study significantly reduced 30-day HTPR rates compared with clopidogrel 150 mg alone (16% vs. 42%, $p < 0.02$). We note that the percentage of 30-day optimal responder patients attributable to high-dose atorvastatin (~26%) was similar to that reported with the switch to a non–CYP3A4-metabolized statin (~24%) in stable patients with HTPR while on chronic coadministration of low-dose (10 mg) atorvastatin and standard-dose clopidogrel in the ACCEL-STATIN (Accelerated Platelet Inhibition by Switching From Atorvastatin to a Non-CYP3A4-Metabolized Statin in Patients With High Platelet Reactivity) study (28). The 2 studies were different for design, clinical and genetic characteristics of patient populations, and strategy: in this ACHIDO study, high-dose atorvastatin was added in separate administration (atorvastatin 10 PM, clopidogrel 8 AM) to double-dose clopidogrel in statin-naive HTPR patients already on clopidogrel (75 mg).

The minimal impact on HTPR with only 150-mg clopidogrel is consistent with findings of other studies (13,15,29). Furthermore, similar to previous studies using standard (75 mg) maintenance dose of clopidogrel, this study shows a temporal variability in platelet reactivity even with 150-mg clopidogrel (30). The late slight increase of PRU values (from T-1 to T-2) in the control group may be due to the metabolic adaptation to the higher dose of clopidogrel. This effect does not take place in the atorvastatin group as reflected in the further increase in the number of optimal responders at T-2. This may have clinical relevance because prior investigations show that the predictive value of platelet reactivity was higher when evaluated 30 days after PCI (30).

Age, carrier status of *CYP2C19*2* loss-of-function allele and higher PRU values are the baseline clinical characteristics associated with HTPR at 30 days. Baseline high PRU values (≥298) are the most powerful covariate and indicate the possibility for early identification of risk of persistent high nonresponse even to higher doses of clopidogrel (15,31). We must also point out that treatment with high-dose atorvastatin had a predictive value similar to that of noncarrier status of *CYP2C19*2* loss-of-function allele in this study.

To date, the exact biological mechanisms involved in the statin modulation of platelet function are not fully understood. Both lipid-lowering and nonlipid-related effects probably contribute, because patients with hypercholesterolemia have hyperreactive platelets that may normalize after lipid-lowering treatment (32). By contrast, statins have antithrombotic effects through mechanisms that are prevalently cholesterol independent (33). In this study, high-dose atorvastatin significantly decreased lipid levels with a pattern similar to that of decreasing PRU values. However, we did not find any correlations between lipid fractions and PRU and BASE values, suggesting that atorvastatin may also have nonlipid-related antithrombotic mechanisms.

The clinical implications of our study can only be speculative. Larger randomized clinical studies are needed to define whether the significant enhancement of clopidogrel antiplatelet activity exerted by high-dose atorvastatin may provide biological support for a beneficial impact on outcome in patients with HTPR undergoing PCI. The lack of clinical benefits with high-dose conventional antiplatelet therapy (e.g., GRAVITAS [Gauging Responsiveness With A VerifyNow Assay–Impact on Thrombosis And Safety] study) currently does not support such a therapeutic strategy

in patients with stable CAD and HTPR identified by a single function test (25). We also know that regardless of PD results, ample clinical studies have shown better cardiovascular outcomes in patients treated with clopidogrel and statin compared with those who received clopidogrel alone, independent of the type of statin administered (CYP3A4- or non–CYP3A4-metabolized statin) (34). These results suggest that improved outcome with statins in patients treated with clopidogrel is due to more than platelet inhibition.

Study limitations. First, only the VerifyNow $P2Y_{12}$ assay was used to evaluate platelet function. Second, it had an open-label design, although platelet reactivity and genotyping were blindly evaluated. Third, the small sample size does not allow definitive conclusions about the biological link between platelet reactivity and achieved lipid levels. Fourth, we only looked for the *CYP2C19*2* polymorphism due to its consistent association with poor clopidogrel metabolizing status and clopidogrel response. Indeed, other polymorphisms could have been considered (e.g., *CYP3A4/5*, *ABCB1*); however, PD studies have found inconsistent association with clopidogrel response (35). Finally, given the pilot nature of this study, it was not designed to evaluate clinical outcomes, which would require larger populations.

Conclusions

In stable CAD patients with HTPR undergoing PCI, the addition of high-dose atorvastatin for 30 days significantly improves the pharmacodynamic effects of high-dose clopidogrel and is associated with improved rates optimal clopidogrel response. Further studies are necessary to demonstrate the clinical impact of this strategy.

Reprint requests and correspondence: Dr. Mario Leoncini, Division of Cardiology, Misericordia e Dolce Hospital, Via Cavour, 2 Prato, Italy. E-mail: leoncini.mario@tiscali.it.

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