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The Influence of Smoking Status on the Pharmacokinetics and Pharmacodynamics of Clopidogrel and Prasugrel

The PARADOX Study

Paul A. Gurbel, MD,* Kevin P. Bliden, MBA,* Douglas K. Logan, MD,† Dean J. Kereiakes, MD,‡ Kenneth C. Lasseter, MD,§ Alex White, MD,‖ Dominick J. Angiolillo, MD,¶ Thomas D. Nolin, PharmD, PhD,# Jen-Fue Maa, PhD,** William L. Bailey, PharmD,** Joseph A. Jakubowski, PhD,†† Clement K. Ojeh, PhD,†† Young-Hoon Jeong, MD, PHD,* Udaya S. Tantry, PhD,* Brian A. Baker, PharmD**

Baltimore, Maryland; Cincinnati, Ohio; Miami, Port Orange, and Jacksonville, Florida; Pittsburgh, Pennsylvania; Parsippany, New Jersey; and Indianapolis, Indiana

Objectives The goal of this study was to evaluate the effect of smoking on the pharmacokinetics and pharmacodynamics (PD) of clopidogrel and prasugrel therapy.

Background Major randomized trial data demonstrated that nonsmokers experience less or no benefit from clopidogrel treatment compared with smokers (i.e., the "smokers' paradox").

Methods PARADOX was a prospective, randomized, double-blind, double-dummy, placebo-controlled, crossover study of objectively assessed nonsmokers (n = 56) and smokers (n = 54) with stable coronary artery disease receiving aspirin therapy. Patients were randomized to receive clopidogrel (75 mg daily) or prasugrel (10 mg daily) for 10 days and crossed over after a 14-day washout. PD was assessed by using VerifyNow $P2Y_{12}$ and vasodilator-stimulated phosphoprotein phosphorylation assays. Clopidogrel and prasugrel metabolite levels, cytochrome P450 1A2 activity, *CYP2C19* genotype, and safety parameters were determined.

Results During clopidogrel therapy, device-reported inhibition of platelet aggregation (IPA) trended lower in nonsmokers than smokers (least squares mean treatment difference ± SE: 7.7 ± 4.1%; p = 0.062). Device-reported IPA was significantly lower in clopidogrel-treated smokers than prasugrel-treated smokers (least squares mean treatment difference: 31.8 ± 3.4%; p < 0.0001). During clopidogrel therapy, calculated IPA was lower and $P2Y_{12}$ reaction units and vasodilator-stimulated phosphoprotein phosphorylation and platelet reactivity index were higher in nonsmokers than in smokers (p = 0.043, p = 0.005, and p = 0.042, respectively). Greater antiplatelet effects were present after prasugrel treatment regardless of smoking status (p < 0.001 for all comparisons).

Conclusions PARADOX demonstrated lower clopidogrel active metabolite exposure and PD effects of clopidogrel in nonsmokers relative to smokers. Prasugrel was associated with greater active metabolite exposure and PD effects than clopidogrel regardless of smoking status. The poorer antiplatelet response in clopidogrel-treated nonsmokers may provide an explanation for the smokers' paradox. (The Influence of Smoking Status on Prasugrel and Clopidogrel Treated Subjects Taking Aspirin and Having Stable Coronary Artery Disease; NCT01260584) (J Am Coll Cardiol 2013;62:505–12) © 2013 by the American College of Cardiology Foundation

From the *Sinai Center for Thrombosis Research, Baltimore, Maryland; †Medpace Clinical Pharmacology, Cincinnati, Ohio; ‡The Christ Hospital Heart and Vascular Center and The Lindner Research Center, Cincinnati, Ohio; §Clinical Pharmacology of Miami, Inc., Miami, Florida; ‖Progressive Medical Research, Port Orange, Florida; ¶University of Florida College of Medicine–Jacksonville, Jacksonville, Florida; #University of Pittsburgh Schools of Pharmacy and Medicine, Pittsburgh, Pennsylvania; **Daiichi Sankyo, Inc., Parsippany, New Jersey; and the ††Eli Lilly and Company, Indianapolis, Indiana. This study was supported by research grants from Daiichi Sankyo, Inc. (Daiichi Sankyo) and Eli Lilly & Company (Lilly). The sponsors participated in the study design and development of the protocol, and provided logistical support during the trial. Monitoring of the study was performed by contract research organizations under contract with the sponsor. The sponsor maintained the trial database. Statistical analyses were performed by statisticians from the contract research organization with oversight from the statisticians at Daiichi Sankyo, Inc. Dr. Gurbel has served as a consultant for Daiichi Sankyo, Lilly, Pozen, Novartis, Bayer, AstraZeneca, Accumetrics, Nanosphere, sanofi-aventis, Boehringer Ingelheim, Merck, Medtronic, Iverson Genetics, CSL, and Haemonetics; has received grants or grants pending from the National Institutes of Health, Daiichi Sankyo, Lilly, Pozen, CSL, AstraZeneca, sanofi-aventis, Haemoscope, Medtronic, Harvard Clinical Research Institute, and Duke Clinical Research Institute; has received payment for lectures, including service on speaker's bureaus, from Lilly, Daiichi Sankyo, Nanosphere, sanofi-aventis, Merck, and Iverson Genetics; has received payment for the development of educational presentations

Abbreviations and Acronyms

AM = active metabolite
AUC = area under the curve
CAD = coronary artery disease
CI = confidence interval
C-IPA = calculated inhibition of platelet aggregation
CYP = cytochrome
DR-IPA = device-reported inhibition of platelet aggregation
EM = extensive metabolizers
HPR = high on-treatment platelet reactivity
LS = least squares
ORE = odds ratio estimates
PD = pharmacodynamics
PK = pharmacokinetics
PRI = platelet reactivity index
PRU = $P2Y_{12}$ reaction units
RM = reduced metabolizer
VASP = vasodilator-stimulated phosphoprotein phosphorylation

Clopidogrel efficacy in high-risk coronary artery disease (CAD) has been demonstrated in major trials and recognized by regulatory agencies and in treatment guidelines (1). However, recent analyses of major trials demonstrated a substantial cardiovascular event reduction with clopidogrel therapy in smokers but not in nonsmokers, a phenomenon termed the "smokers' paradox" (2). It has been reported that smoking status influences clopidogrel pharmacodynamics (PD) in healthy volunteers, patients with acute coronary syndromes, and patients treated with percutaneous coronary intervention (PCI) (3–5). An explanation for the smokers' paradox is cytochrome P450 (CYP) 1A2 and CYP2B6 induction by cigarette smoking, resulting in greater clopidogrel active metabolite (AM) generation (6). To the best of our knowledge, there have been no prospective studies evaluating the effect of smoking status on both clopidogrel and prasugrel pharmacokinetics (PK) and PD.

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Drug–drug interactions and carriage of *(CYP) 2C19 loss-of-function* alleles have been implicated in clopidogrel response variability, high on-treatment platelet reactivity (HPR), and adverse clinical outcomes. CYP1A2 is also involved in clopidogrel biotransformation but has received less attention (7).

The goal of the current study was to assess the effect of smoking status on the PK and PD of clopidogrel and prasugrel therapy in aspirin-treated patients with stable CAD. We hypothesized that smoking status influences clopidogrel but not prasugrel AM concentrations and antiplatelet effects, and that prasugrel antiplatelet effects are greater than clopidogrel regardless of smoking status.

Methods

PARADOX was a prospective, randomized, double-blind, double-dummy, placebo-controlled, crossover investigation conducted at 6 centers in the United States between November 18, 2010, and September 21, 2011. The respective investigational review boards approved the study, and patients provided written informed consent.

Study design. Patients (18 to 75 years of age) with documented stable CAD receiving 81 to 325 mg of aspirin therapy daily were enrolled. Exclusion criteria were: weight <60 kg, bare-metal or drug-eluting stenting within 12 months, history of bleeding diathesis, transient ischemic attack, stroke, hepatic disease or HIV, pregnancy, antithrombotic treatment other than aspirin, use of proton pump inhibitors or drugs or dietary products that strongly inhibit or induce CYP within 10 days, nonsteroidal anti-inflammatory drug use >3 doses per week, platelets <100,000/mm^3 or >500,000/mm^3, hemoglobin <10 g/dl, and indication for thienopyridine therapy.

Patients were stratified according to smoking status before randomization (1:1) to receive 10 days of clopidogrel (75 mg daily) or prasugrel (10 mg daily) followed by a 14-day washout and crossover period (Fig. 1). Randomization occurred by an interactive voice response system/Web response system. Compliance was confirmed by a dosing diary and assessment of tablet counts.

Smoking status. Urine cotinine concentrations were determined at screening by using Accutest NicAlert (Jant Pharmacal Corporation, Encino, California). Patients who reported smoking ≥0.5 pack of cigarettes per day with a NicAlert level of 6 were enrolled in the smoking group and those who reported being nonsmokers with a NicAlert level of 0, 1, or 2 were enrolled in the nonsmoking group.

Blood sampling. Blood for PD and genotyping was collected as previously described (5,8,9). PD measurements were performed at baseline (visits 2 and 4) and at the end of active treatment (visits 3 and 5) just before the 10th (last) maintenance dose. PK samples were collected at 0.5, 1, 2, 4, and 6 hours after the 10th maintenance dose.

Platelet function. The VerifyNow $P2Y_{12}$ and vasodilator-stimulated phosphoprotein phosphorylation (VASP-P) (Biocytex, Inc., Marseille, France) assays, which are methods of quantifying $P2Y_{12}$ receptor reactivity, were performed as previously described (5,9).

Pharmacokinetics. Prasugrel and clopidogrel metabolite concentrations were determined in stabilized plasma samples by using high-performance liquid chromatography with mass spectrometric detection as previously described (10,11).

CYP1A2 activity. CYP1A2 activity was determined by the ratio of paraxanthine/caffeine 6 hours after oral administration

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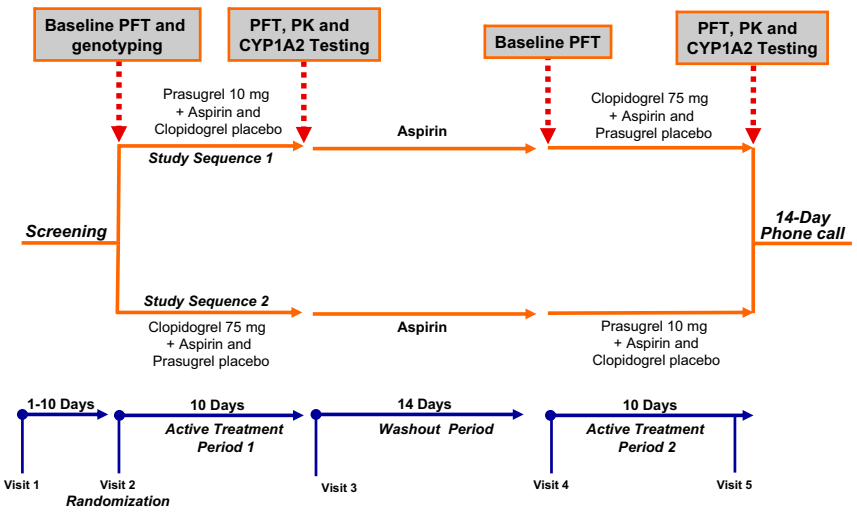

Figure 1 Study Design

Subjects were stratified according to smoking status in a 1:1 ratio and randomly assigned to 1 of 2 double-blind treatment sequences. CYP1A2 = cytochrome P450 1A2; PFT = platelet function testing; PK = pharmacokinetics. All patients received the same aspirin dose from randomization throughout the study.

of 100 mg of caffeine (JET-ALERT, Bell Pharmaceuticals, Minneapolis, Minnesota) (12). Caffeine was administered with the 10th maintenance dose of study medication, and all caffeine intake was prohibited ≤24 hours before administration of caffeine. Plasma concentrations of paraxanthine and caffeine were determined by using a high-performance liquid chromatography with mass spectrometric method.

***CYP2C19* genotyping.** Common *loss-of-function* variants of *CYP2C19* (**2,*3*) and the common *gain-of-function* variant (**17*) were identified by using a TaqMan assay, and CYP2C19 metabolizer status was categorized as either extensive metabolizers (EMs) *(*1/*1, *1/*17, *17/*17)* or reduced metabolizers (RMs) *(*2/*2, *1/*2)* (8).

Safety. An adverse event within 14 days after the last dose was any untoward event. Treatment-emergent adverse events were adverse events that started or worsened in severity on or after the first dose of study medication.

Statistical methods. PRIMARY ANALYSIS. The first co-primary endpoint was device-reported inhibition of platelet aggregation (DR-IPA) in smokers versus nonsmokers after 9 days of clopidogrel therapy. The second co-primary endpoint was DR-IPA in prasugrel-treated smokers versus clopidogrel-treated smokers. A linear mixed-effects model for the crossover design with fixed effects of smoking status, sequence, treatment, period, smoking status × treatment, and a random effect of subject (smoking × sequence) was used for comparisons between groups.

SECONDARY ANALYSES. The secondary analyses included: 1) DR-IPA in prasugrel-treated smokers versus prasugrel-treated nonsmokers, in prasugrel-treated nonsmokers versus clopidogrel-treated nonsmokers, and in prasugrel-treated nonsmokers versus clopidogrel-treated smokers; and 2) all of these comparisons and the comparisons between clopidogrel-treated smokers and nonsmokers and between prasugrel-treated smokers and clopidogrel-treated smokers. The analyses included calculated inhibition of platelet aggregation (C-IPA), $P2Y_{12}$ reaction units (PRU), and platelet reactivity index (PRI) after 9 days of therapy. A post hoc analysis was conducted by using C-IPA to compare clopidogrel-treated smokers and nonsmokers and prasugrel-treated smokers and clopidogrel-treated smokers. For the secondary endpoints, summary statistics and 95% confidence intervals (CIs) were used for between-group comparisons. A logistic regression analysis of responder rate according to treatment and smoking status was conducted by using a generalized mixed-effects model for the crossover design, with fixed effects of smoking status, sequence, treatment, period, and smoking status × treatment, and a random effect of subject (smoking status × sequence). Significance for statistical tests was evaluated at the $p = 0.05$ level. All analyses were conducted by using SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina).

Sample size. For the co-primary endpoints' sample size calculations, no type I error rate was adjusted, and both co-primary endpoints were tested at the 0.05 level by using a 2-sample Student *t* test to demonstrate a significant difference between groups (clopidogrel-treated smokers vs. clopidogrel-treated nonsmokers). We estimated a 15% difference in DR-IPA between smokers and nonsmokers. A sample size of 108 patients (54 smokers and 54 nonsmokers) was required given the assumption of an SD of 24%, power of 80%, dropout rate of 25%, and equal stratification between groups.

Results

Patients. Among 170 patients screened, 110 were randomized; 97% completed treatment period 1, 88% completed treatment period 2, and 88% completed both

Table 1 Patient Demographics, Medical History, Concomitant Medications, and Baseline Laboratory Data

Variable	Total Group (n = 110)	Smokers (n = 54)	Nonsmokers (n = 56)
Demographics			
Age (yrs)	59.4 ± 8	58 ± 8	61 ± 8
Male	79 (72)	37 (68)	42 (75)
BMI (kg/m^2)	31 ± 6	30 ± 5	32 ± 6
Ethnicity			
White	85 (77)	41 (76)	44 (79)
African American	23 (21)	11 (20)	12 (21)
Other	2 (2)	2 (3.7)	0 (0)
Medical history			
Smoking (current)	54 (49)	54 (100)	0 (0)
Cardiovascular history	105 (95)	53 (98)	52 (93)
Hypertension	76 (69)	40 (74)	36 (64)
Hyperlipidemia	103 (94)	52 (96)	51 (91)
Diabetes	31 (28)	13 (24)	18 (32)
Previous MI	50 (45)	30 (56)	20 (36)
Previous CABG	42 (38)	15 (28)	27 (48)
Previous PCI	93 (85)	49 (91)	44 (79)
Baseline medications			
Statins	76 (69)	37 (68)	39 (70)
ACE inhibitors	40 (36)	19 (35)	21 (37)
Beta-blockers	65 (59)	29 (54)	36 (64)
Organic nitrates	10 (9)	4 (7)	6 (11)
PPI	11 (10)	5 (9)	6 (11)
Baseline laboratory data			
WBC (×1,000/mm^3)	7.4 ± 2.0	8.3 ± 2.0	6.6 ± 2.0
Platelets (×1,000/mm^3)	229 ± 56	239 ± 60	219 ± 53
Hematocrit (%)	43 ± 3	44 ± 4	42 ± 3
Creatinine (μmol/l)	94 ± 23	91 ± 23	96 ± 23
Total cholesterol (mg/dl)	178 ± 42	185 ± 47	170 ± 36
Uric acid (mg/dl)	6.2 ± 1.5	6.0 ± 1.2	6.6 ± 1.7

Values are mean ± SD or n (%).
ACE = angiotensin-converting enzyme; BMI = body mass index; CABG = coronary artery bypass graft; CAD = coronary artery disease; Hb = hemoglobin; MI = myocardial infarction; PCI = percutaneous coronary intervention; PPI = proton pump inhibitor; WBC, white blood cells.

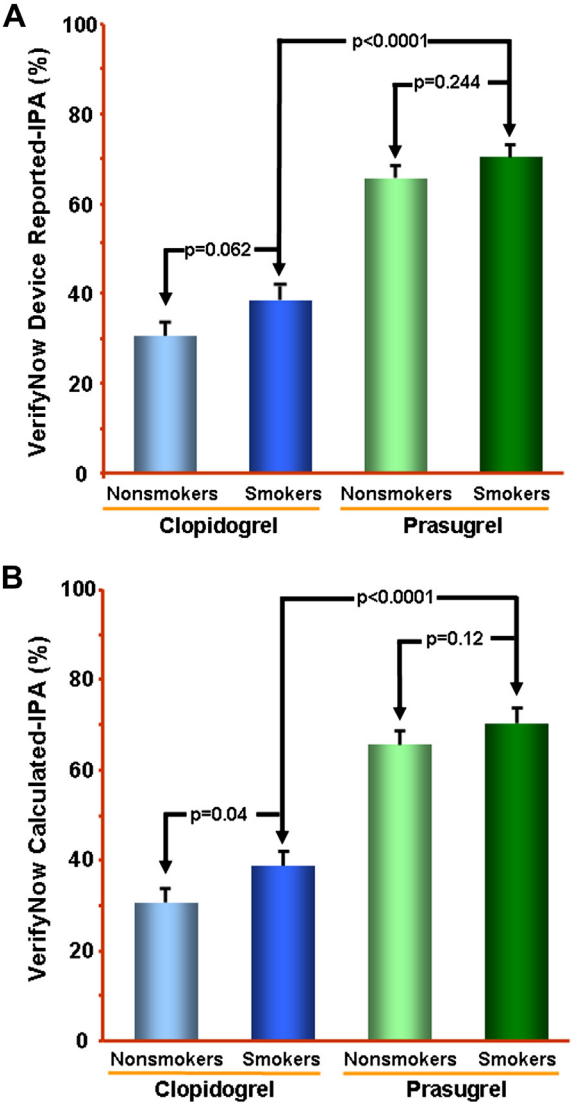

Figure 2 Inhibition of Platelet Aggregation by Treatment and Smoking Status

(A) Device-reported inhibition of platelet aggregation (IPA) and **(B)** calculated IPA. Data are presented as least squares (LS) mean ± SE. Device reported-inhibition of platelet aggregation (DR-IPA) is reported using post-dosing BASE ($BASE_t$) and PRU (PRU_t): DR-IPA (%) = 100 × [($BASE_t$ – PRU_t)/$BASE_t$]. Calculated inhibition of platelet aggregation (C-IPA), using pre-dosing PRU (PRU_b) and post-dosing PRU (PRU_t) was determined as follows: C-IPA (%) = 100 × [(PRU_b – PRU_t)/PRU_b]. In the VerifyNow assay, the second channel contains fibrinogen-coated polystyrene beads, 3.4 mM iso-thrombin receptor activating peptide [iso-TRAP: protease-activated receptor (PAR)-1 agonist] and PAR-4 activating peptide (PAR-4 AP); this channel estimates maximal platelet function independent of $P2Y_{12}$ receptor blockade and is reported as "BASE".

treatment periods. Eight patients discontinued due to a protocol violation, 4 because of an adverse event, and 1 withdrew consent. Demographic characteristics and concomitant medications were similar between groups except for a higher body mass index in the nonsmokers ($p = 0.035$) (Table 1). Aspirin doses were 81 mg daily (61%) and 325 mg daily (39%). Overall compliance was 99.8%.

Platelet function. In clopidogrel-treated patients, baseline PRU was 298 ± 56 and 314 ± 45 in smokers and nonsmokers, respectively. In prasugrel-treated patients, baseline PRU was 310 ± 50 and 309 ± 48 in smokers and nonsmokers.

After 9 days of clopidogrel therapy, DR-IPA trended to be lower in nonsmokers than smokers ($p = 0.062$). DR-IPA was higher in prasugrel-treated smokers than in clopidogrel-treated smokers ($p < 0.0001$) (Fig. 2A, Table 2).

There was a significant increase in platelet inhibition according to C-IPA in clopidogrel smokers compared with clopidogrel-treated nonsmokers ($p = 0.043$) as well as in prasugrel-treated smokers compared with clopidogrel-treated smokers ($p < 0.0001$) (Fig. 2B, Table 2). PRU and PRI were lower in clopidogrel-treated smokers than in nonsmokers ($p = 0.0048$ and $p = 0.042$, respectively). PRU and PRI were lower in prasugrel-treated smokers than in clopidogrel-treated smokers ($p < 0.0001$ for both).

Prasugrel treatment was associated with a greater antiplatelet response compared with clopidogrel as determined

Table 2 Mean Treatment Differences for DR-IPA, C-IPA, PRU, and VASP-PRI

Analysis/Treatment Comparison	DR-IPA (%)		C-IPA (%)		PRU		VASP-PRI (%)	
	LS Mean Treatment Difference	p Value	LS Mean Treatment Difference	p Value	LS Mean Treatment Difference	p Value	LS Mean Treatment Difference	p Value
Co-primary endpoints								
Clopidogrel-treated smokers versus clopidogrel-treated nonsmokers	7.7 ± 4.1	0.062	9.1 ± 4.4	0.043	−36.2 ± 12.7	0.0048	−7.6 ± 3.7	0.042
Prasugrel-treated smokers versus clopidogrel-treated smokers	31.8 ± 3.4	<0.0001	32.7 ± 4.0	<0.0001	−93.8 ± 11.5	<0.0001	−22.7 ± 3.2	<0.0001
Secondary endpoints								
Prasugrel-treated smokers versus prasugrel-treated nonsmokers	4.8 ± 4.1	0.244	6.9 ± 4.4	0.120	−21.2 ± 12.5	0.0924	−5.8 ± 3.7	0.118
Prasugrel-treated nonsmokers versus clopidogrel-treated nonsmokers	34.7 ± 3.2	<0.0001	34.9 ± 3.8	<0.0001	−108.8 ± 10.9	<0.0001	−24.5 ± 3.0	<0.0001
Prasugrel-treated nonsmokers versus clopidogrel-treated smokers	27.0 ± 4.1	<0.0001	25.8 ± 4.5	<0.0001	−72.6 ± 12.7	<0.0001	−16.9 ± 3.8	<0.0001

Values are mean ± SE.

C-IPA = calculated inhibition of platelet aggregation; DR-IPA = device-reported inhibition of platelet aggregation; LS = least squares; PRU = $P2Y_{12}$ reaction units; VASP-PRI = vasodilator-stimulated phosphoprotein phosphorylation-platelet reactivity index.

Table 3 Prevalence of Patients With High Platelet Reactivity According to Treatment and Smoking Status

Assay	HPR Cutoff	Nonsmokers				Smokers			
		Clopidogrel (%) (n = 54)	Prasugrel (%) (n = 52)	Odds Ratio Estimate (95% CI)	p Value	Clopidogrel (%) (n = 47)	Prasugrel (%) (n = 50)	Odds Ratio Estimate (95% CI)	p Value
PRU	>235	38.9	3.8	17.11 (3.13–93.65)	0.001	23.4	2.0	16.54 (1.85–148.23)	0.013
	>208	53.7	3.8	29.65 (5.72–153.76)	<0.0001	38.3	4.0	15.32 (3.07–76.55)	0.001
PRI	>50%	55.6	17.3	11.71 (2.95–46.52)	0.0006	48.9	4.0	58.64 (6.80–505.58)	0.0003
	>60%	46.3	11.5	8.17 (2.41–27.67)	0.0009	31.9	2.0	29.19 (3.15–270.48)	0.003

CI = confidence interval; HPR = high platelet reactivity; other abbreviations as in Table 2.

by DR-IPA, C-IPA, PRU, and PRI, regardless of smoking status ($p < 0.0001$ for all comparisons) (Table 2). There were no significant differences in the antiplatelet response to prasugrel associated with smoking according to DR-IPA, C-IPA, PRU, and PRI ($p > 0.05$ for all). Although the interaction between smoking status and treatment was not significant, the study was not powered to show a difference.

Sensitivity analyses. Results of the analyses of mean DR-IPA, PRU, and VASP-PRI that adjusted for differences in baseline characteristics by adding all potential covariates to the mixed-effects model were similar to those obtained for the full PD population (data not shown).

High on-treatment platelet reactivity. Among clopidogrel-treated patients, smoking was associated with a 2.06 (95% CI: 0.80 to 5.32) and 1.86 (95% CI: 0.81 to 4.29) times lower odds ratio estimate (ORE) for HPR as determined by >235 PRU and >208 PRU cutoff values, respectively, and 1.34 (95% CI: 0.49 to 3.67) and 1.88 (95% CI: 0.74 to 4.75) times lower ORE as determined by >50% PRI and >60% PRI cutoff values, respectively (data not shown). Using all 4 definitions, the odds of occurrence of HPR were significantly lower for prasugrel-treated patients compared with clopidogrel-treated patients regardless of smoking status ($p < 0.05$ for all comparisons) (Table 3).

CYP1A2 activity. Overall, smokers had higher median CYP1A2 activity than nonsmokers (2.2 vs. 1.1; $p = 0.01$). The median CYP1A2 activity was 2.1 and 2.3 for clopidogrel- and prasugrel-treated smokers, respectively, and 1.1 and 1.0 for clopidogrel- and prasugrel-treated nonsmokers (median difference 0.9 [$p = 0.0136$ for clopidogrel treatment] and 1.1 [$p = 0.0024$ for prasugrel treatment]). No difference in CYP1A2 activity was observed between prasugrel-treated patients and clopidogrel-treated patients. For a given therapy, there were no significant differences in least squares (LS) mean DR-IPA, PRU, or PRI between CYP1A2 activity categories (median or lower vs. higher than the median, or between quartiles) (data not shown).

Pharmacokinetics. Geometric LS mean systemic exposure (area under the curve $[AUC]_{0-last}$) to AM was higher for prasugrel compared with clopidogrel in both nonsmokers (48.4 h · ng/ml vs. 16.2 h · ng/ml) and smokers (53.6 h · ng/ml vs. 19.2 h · ng/ml). Smokers had 10.7% higher exposure to the prasugrel AM and 18.4% higher exposure to the clopidogrel AM (Table 4). Conversely, mean clopidogrel inactive metabolite exposure was 13.6% lower in smokers. The median time to maximum concentration of the prasugrel AM was 0.5 hour in both smokers and nonsmokers, and for clopidogrel AM, it was 0.8 hour for smokers and 1.0 hour for nonsmokers.

After dose–weight adjustment, the AUC_{0-last} geometric LS mean ratios (90% CI) were: clopidogrel AM 111.4 (95.0 to 130.6); clopidogrel-inactive metabolite 80.3 (68.5 to 94.3); and prasugrel AM 102.7 (87.9 to 119.9). These data indicate that the exposure to clopidogrel AM and the inactive metabolite were not equivalent between smokers and nonsmokers (the CIs for clopidogrel AM and the inactive metabolite were outside the 0.80 to 1.25 interval required for equivalence), whereas exposure to prasugrel AM was equivalent in smokers and nonsmokers. The comparisons of the log-transformed PK parameters of the prasugrel AM and the clopidogrel AM and inactive metabolite between CYP1A2 categories (median or lower vs. higher than the median or CYP1A2 quartiles) were not significant (data not shown).

***CYP2C19* genotype.** Eighty-six patients (80%) were EMs (45 nonsmokers and 41 smokers) and 20 (19%) were RMs (10 nonsmokers and 10 smokers). Genotype information was missing in 1 patient. There were no differences in genotype distribution between treatment groups (data not shown).

CYP2C19 genotype did not influence exposure to the AMs of either prasugrel or clopidogrel (EM/RM [95% CI]:

Table 4 Comparison of PK Parameters of the Active Metabolite of Prasugrel and the Active and Inactive Metabolites of Clopidogrel Between Smokers and Nonsmokers

Parameter	Smokers Geometric LS Means	Nonsmokers Geometric LS Means	Ratio of Geometric LS Means (%)	90% CI for Ratio (%)
Prasugrel active metabolite				
C_{max} (ng/ml)	42.6	36.1	117.9	94.4–147.3
AUC_{0-last} (h · ng/ml)	53.6	48.4	110.7	93.7–130.8
Clopidogrel active metabolite				
C_{max} (ng/ml)	13.5	10.9	123.8	98.6–155.4
AUC_{0-last} (h · ng/ml)	19.2	16.2	118.4	99.8–140.4
Clopidogrel inactive metabolite				
C_{max} (ng/ml)	2,080.9	2,349.8	88.6	70.6–111.1
AUC_{0-last} (h · ng/ml)	5,025.5	5,816.6	86.4	72.8–102.5

A mixed-effects model for the crossover design was performed on log-transformed pharmacokinetic (PK) parameters. The model included fixed effects for smoking status, treatment sequence, treatment, treatment period, and smoking status × treatment and a random effect for subject (smoking status × treatment sequence). Geometric LS means are the LS means obtained from the mixed-effects model presented after back-transformation to the original scale. The 90% CIs are presented after back-transformation to the original scale. All mean prasugrel and clopidogrel C_{max} and AUC_{0-last} values had 90% CIs that fell outside of the 80% to 125% interval.

AUC_{0-last} = area under the curve up to the last sampling time; C_{max} = maximum plasma concentration; other abbreviations as in Tables 2 and 3.

Table 5 DR-IPA by Treatment, Smoking, and CYP2C19 Genetically Determined Metabolizer Status

	n	LS Mean (SE)	LS Mean ± SE Difference Between Treatment	p Value
Reduced metabolizers				
Clopidogrel-treated smokers	10	20.2 ± 5.79	−9.1 ± 8.40	0.285
Clopidogrel-treated nonsmokers	9	29.3 ± 6.08		
Prasugrel-treated smokers	10	68.1 ± 5.79	47.9 ± 6.38	<0.0001
Clopidogrel-treated smokers	10	20.2 ± 5.79		
Extensive metabolizers				
Clopidogrel-treated smokers	36	44.0 ± 3.39	12.8 ± 4.54	0.006
Clopidogrel-treated nonsmokers	45	31.2 ± 3.05		
Prasugrel-treated smokers	39	70.8 ± 3.26	26.8 ± 3.71	<0.0001
Clopidogrel-treated smokers	36	44.0 ± 3.39		

CYP = cytochrome P450; other abbreviations as in Table 2.

prasugrel 0.98 [0.79 to 1.23]; clopidogrel 1.17 [0.84 to 1.64]). LS mean DR-IPA was lower in clopidogrel-treated nonsmokers compared with smokers with respect to EM ($p = 0.0056$) but did not differ in RMs ($p = 0.29$) (Table 5). The difference in LS mean DR-IPA between prasugrel-treated smokers and clopidogrel-treated smokers was significant in both EMs (26.8%; $p < 0.0001$) and RMs (47.9%; $p < 0.0001$).

Safety. Here were no serious bleeding events. Patients treated with prasugrel had a greater occurrence of ecchymoses (2.8% vs. 0%).

Discussion

PARADOX is the first prospective study to evaluate the influence of smoking status on the PK and PD profile of clopidogrel and prasugrel. Nonsmokers had reduced responsiveness to clopidogrel compared with smokers who reported smoking ≥0.5 pack cigarettes per day and had a NicAlert level of 6. Smoking was associated with approximately 2-fold decreased ORE for HPR during clopidogrel therapy. However, smoking did not significantly influence the antiplatelet response to prasugrel, and a greater antiplatelet effect and a markedly lower prevalence of HPR were present after prasugrel treatment regardless of smoking status. Smoking enhanced bioactivation of clopidogrel as reflected by greater AM exposure after adjustment for weight and lower inactive metabolite exposure.

In a post-hoc analysis, current smoking was associated with lower platelet reactivity and greater clopidogrel-induced inhibition that was dependent on the number of cigarettes smoked per day (4). Subsequently, similar findings were demonstrated in many, but not all, PD studies (3,13,14). A nonsignificant decrease ($p = 0.062$) in DR-IPA observed in PARADOX may be related to use of the BASE value, instead of a true pre-treatment PRU value for IPA determination. In addition, in the calculation of DR-IPA, negative IPA is considered zero inhibition and may underestimate the degree of clopidogrel nonresponsiveness. Therefore, DR-IPA may not be an optimal surrogate for IPA determined with a true baseline PRU measurement. In line with our observations, the manufacturer of VerifyNow has recently chosen to delete the DR-IPA and BASE recordings from their device. Furthermore, C-IPA, determined with actual pre-treatment and post-treatment PRU values, was significantly greater and consistent with the observations of significantly lower PRU and VASP-PRI values in clopidogrel-treated smokers.

The relation of platelet reactivity to ischemic event occurrence may be sigmoidal, with the risk increasing greatly above a certain threshold (7). The large body of translational research data linking clopidogrel PD to clinical outcomes has been based on PRU and PRI thresholds defining HPR. In PARADOX, a poorer antiplatelet response in nonsmokers during clopidogrel therapy was indicated by a significant difference of 36 PRUs ($p = 0.0048$) and 7.6% PRI ($p = 0.042$) and an approximately 2-fold greater ORE for HPR. A 4% increase in the primary ischemic endpoint for every 10-unit increase in PRU (hazard ratio: 1.04 [95% CI: 1.03 to 1.06]; $p < 0.0001$) has been reported (15). In another study of clopidogrel-treated PCI patients, an increase in 21 PRUs translated into a 1.6 times increase in HPR prevalence and an increase in periprocedural myocardial infarction (16). These results suggest that the modest increase in platelet reactivity and HPR prevalence observed in clopidogrel-treated nonsmokers in PARADOX may influence clinical outcomes.

In PARADOX, the overall AM exposure (AUC_{0-last}) was numerically higher for prasugrel compared with clopidogrel in both nonsmokers and smokers. A good correlation between AM exposure and PD has been demonstrated for both prasugrel and clopidogrel such that greater IPA during therapy reflects increased AM generation (17). The mean maximum concentration and mean AUC_{0-last} of clopidogrel inactive metabolite were greater in nonsmokers and are consistent with other findings indicating less bioactivation in nonsmokers (6). Importantly, after dose–weight normalization, clopidogrel AM levels remained lower in nonsmokers.

The PK results of PARADOX support the hypothesis that smoking enhances clopidogrel AM exposure and PD response.

A sigmoidal maximum effect model to describe the PK/PD relationship for prasugrel and clopidogrel has been suggested (17). In PARADOX, an 18.4% increase in clopidogrel AM exposure in smokers was associated with significantly lower PRU, PRI, and C-IPA values. Given that exposure to clopidogrel AM resides on the steep portion of the dose–response curve, relatively small changes in exposure would be expected to result in more significant changes in PD. Given that prasugrel AM typically resides on the flat portion of the dose–response curve, consistent with the sigmoidal maximum effect model, a 10.7% increase in AUC_{0-last} for prasugrel AM in smokers was associated with a nonsignificant increase in antiplatelet effects.

Study limitations. PARADOX was powered at 80% to detect a statistically significant difference of 15% (assumed SD of 24%) in DR-IPA between clopidogrel-treated smokers and clopidogrel-treated nonsmokers. The actual effect size (7.7 ± 22% [approximate]) was smaller than predicted. However, the significantly higher PRUs and VASP-PRI, and lower C-IPA and a higher prevalence of HPR, in clopidogrel-treated nonsmokers support our conclusions.

Greater CYP1A2 activity in smokers has been shown and is consistent with our findings (41). The limited number of patients (n = 45) with evaluable CYP1A2 data in PARADOX may have precluded our ability to demonstrate a significant relation between CYP1A2 activity and PK. Cigarette smoking may affect other factors involved in clopidogrel metabolism that were not assessed in PARADOX. CYP2B6 is involved in both clopidogrel and prasugrel metabolism, and a potential influence of smoking on CY2B6 should be explored further (18). Finally, clopidogrel AM exposure was not significantly influenced by CYP2C19 genotype and may be due to the small CYP2C19 RM sample size.

Conclusions

PARADOX is the first prospective study to demonstrate that clopidogrel-treated nonsmokers have an attenuated PK and PD response compared with clopidogrel-treated smokers and that prasugrel therapy is associated with a greater antiplatelet effect compared with clopidogrel therapy regardless of smoking status. The poorer antiplatelet response in clopidogrel-treated nonsmokers may provide an explanation for the reduced clinical benefit of clopidogrel treatment in nonsmokers observed in major randomized trials and deserves further investigation.

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Reprint requests and correspondence: Dr. Paul A. Gurbel, Sinai Center for Thrombosis Research, Cardiac Catheterization Laboratory, 2401 West Belvedere Avenue, Baltimore, Maryland 21215. E-mail: pgurbel@lifebridgehealth.org.

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