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Cigarette Smoking Is Associated With a Dose-Response Effect in Clopidogrel-Treated Patients With Diabetes Mellitus and Coronary Artery Disease

Results of a Pharmacodynamic Study

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Objectives This study sought to assess the presence of a dose-response effect of cigarette smoking and its impact on high on-treatment platelet reactivity (HPR) in patients with diabetes mellitus treated with clopidogrel.

Background Cigarette smoking is an inducer of cytochrome P450 1A2, a hepatic enzyme involved in clopidogrel metabolism. If cigarette smoking is associated with a dose-response effect on pharmacodynamic measures in clopidogrel-treated patients is unknown.

Methods A total of 134 type 2 diabetes mellitus patients on maintenance aspirin and clopidogrel therapy were studied. Patients were divided into 3 groups according to cotinine levels: <3 ng/ml (nonsmokers), 3 to 199 ng/ml (light smokers), and ≥200 ng/ml (heavy smokers). Platelet function was assessed by light transmittance aggregometry, VerifyNow P2Y12 assay (Accumetrics, San Diego, California), and vasodilator-stimulated phosphoprotein. Rates of HPR were defined using established cutoff values.

Results A dose-response effect was observed for all pharmacodynamic parameters tested. Serum cotinine levels were inversely associated with platelet reactivity as assessed by light transmittance aggregometry using 5 and 20 μmol/l adenosine diphosphate ($p < 0.0001$ for all). Accordingly, platelet disaggregation increased with levels of serum cotinine ($p < 0.0001$). Similar results were found with $P2Y_{12}$ reaction units ($p < 0.0001$) and inhibition of platelet aggregation ($p = 0.005$) as defined by VerifyNow P2Y12 testing, and platelet reactivity index ($p = 0.002$) as assessed by vasodilator-stimulated phosphoprotein. Higher serum cotinine levels were significantly associated with lower rates of HPR, as defined according to various pharmacodynamic cutoff measures.

Conclusions Cigarette smoking is associated with a dose-response effect on clopidogrel-induced antiplatelet effects and lower rates of HPR in diabetes mellitus patients. (J Am Coll Cardiol Intv 2012;5:293–300) © 2012 by the American College of Cardiology Foundation

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Numerous investigations have shown a broad variability in clopidogrel-induced antiplatelet effects, and patients with high on-treatment platelet reactivity (HPR) have an increased risk of recurrent atherothrombotic events (1,2). Multiple factors have been associated with the degree of platelet inhibition induced by clopidogrel. Among these, genetic and environmental factors modulating hepatic metabolism of clopidogrel appear to have a pivotal role (1,2). Clopidogrel is a prodrug that requires a 2-step oxidation by cytochrome P450 (CYP) isoenzymes to generate an active metabolite that in turn irreversibly inhibits the platelet $P2Y_{12}$ receptor (3). Cigarette smoking is a known inducer of CYP1A2, which is the predominant isoenzyme responsible for the first oxidative step in the conversion of clopidogrel into its active metabolite (4,5). Pharmacodynamic (PD) and clinical studies have shown that smokers treated with clopidogrel have enhanced platelet inhibition and derive higher relative benefit, as assessed by angiographic and clinical outcomes, than nonsmokers do (6–9). However, these studies identified the aforementioned effects in smokers consuming above a certain threshold of number of cigarettes and were not able to determine a dose-response effect in a continuous way. This may be attributed to the fact that these investigations were based on self-reported smoking, which is not an objective measure of the amount of nicotine exposure, as it depends for instance on the type and brand of cigarettes and smokers' habit (e.g., deep inhalation). In addition, because baseline characteristics are associated with variations in clopidogrel metabolism, it cannot be excluded that patient selection may have had an impact on these findings.

Abbreviations and Acronyms

ADP = adenosine diphosphate
CI = confidence interval
CYP = cytochrome P450
DM = diabetes mellitus
HPR = high on-treatment platelet reactivity
IPA = inhibition of platelet aggregation
LPA = late values of on-treatment platelet aggregation
LTA = light transmittance aggregometry
MFI = mean fluorescence intensity
MPA = maximal values of on-treatment platelet aggregation
OR = odds ratio
PD = pharmacodynamic
PGE_1 = prostaglandin E_1
PRI = platelet reactivity index
PRP = platelet-rich plasma
PRU = $P2Y_{12}$ reaction units
VASP-P = phosphorylation of vasodilator-stimulated phosphoprotein

In the present investigation, the impact of cigarette smoking on clopidogrel-induced antiplatelet effects was assessed by means of a more objective assessment based on levels of serum cotinine, the major stable degradation product of nicotine metabolism (10). Because clopidogrel metabolism is reduced among patients with diabetes mellitus (DM), which may contribute to their high prevalence of HPR while on clopidogrel therapy (11), this population was identified to test our study hypothesis. The aim of the present investigation was to assess if there is a dose-response effect of cigarette smoking, as assessed by serum cotinine levels, and how this affects rates of HPR in patients with DM on maintenance clopidogrel therapy.

Methods

Patient population. The present investigation is a cross-sectional observational study that evaluated the association between cigarette smoking and PD effects of clopidogrel. A database of patients who had undergone platelet function assessments at our Thrombosis Research Laboratory (University of Florida College of Medicine–Jacksonville) between 2006 and 2010 was used to identify eligible subjects for this investigation. Patients meeting study inclusion criteria, who also had a serum sample collected at the time of platelet function assessment to enable cotinine measurement, were identified. All patients had undergone percutaneous coronary intervention with stent implantation and were treated with dual antiplatelet therapy per standard of care. In particular, patients were eligible for the study if they had type 2 DM and were clinically stable while on maintenance dual antiplatelet therapy with aspirin (81 mg daily) and clopidogrel (75 mg daily) for at least 1 month. Patients needed to be on maintenance dual antiplatelet therapy for at least 1 month as prior investigations have shown that platelet reactivity is subject to variability in the earlier phases of treatment and reaches a steady-state phase following 1 month of therapy (12–14). Type 2 DM patients also needed to have been medically managed (oral or insulin therapy) for at least 2 months without changes in hypoglycemic treatment regimen. General major exclusion criteria included: known allergies to aspirin or clopidogrel; left ventricular ejection fraction <30%; blood dyscrasia; active bleeding or bleeding diathesis; gastrointestinal bleed within last 6 months; hemodynamic instability; cerebrovascular accident within 3 months; any malignancy; concomitant use of other antithrombotic drugs (oral anticoagulants, dipyridamole, ticlopidine, or cilostazol); recent treatment (<30 days) with a glycoprotein IIb/IIIa antagonist; platelet count $<100 \times 10^3/\mu l$; liver disease (baseline alanine transaminase >2.5× the upper limit of normal).

Patients were recruited at the Division of Cardiology of the University of Florida College of Medicine–Jacksonville. The study complied with the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Florida College of Medicine–Jacksonville. All subjects provided written informed consent for platelet function assessments and for storage of serum samples. The authors had full access to the data and take full responsibility for its integrity. All authors have read and agreed to the manuscript as written.

Blood sampling and functional assessments. Peripheral venous blood samples were drawn with a loose tourniquet to