Contribution of Hepatic Cytochrome P450 3A4 Metabolic Activity to the Phenomenon of Clopidogrel Resistance

Wei C. Lau, MD; Paul A. Gurbel, MD; Paul B. Watkins, MD; Charlene J. Neer, BSN, MPA; Amy S. Hopp, BS; David G.M. Carville, PhD; Kirk E. Guyer, BS; Alan R. Tait, PhD; Eric R. Bates, MD

Background—Interindividual variability of platelet inhibition after aspirin or clopidogrel administration has been described. Additionally, aspirin resistance and clopidogrel resistance occur in some individuals. Because the prodrug clopidogrel is activated by hepatic cytochrome P450 (CYP) 3A4, we hypothesized that interindividual variability in clopidogrel efficacy might be related to interindividual differences in CYP3A4 metabolic activity.

Methods and Results—Platelet aggregation was measured before and after clopidogrel treatment in 32 patients undergoing coronary artery stent implantation and in 35 healthy volunteers. The erythromycin breath test was used to measure CYP3A4 activity in vivo in 25 of the healthy volunteers. Individual platelet aggregation was studied in 10 healthy volunteers after the coadministration of clopidogrel and rifampin (a CYP3A4 inducer). Clopidogrel nonresponders, low responders, and responders were defined by a relative inhibition of adenosine diphosphate (20 μmol/L)–induced platelet aggregation of <10%, 10% to 29%, and ≥30%, respectively. Among patients, 22% were clopidogrel nonresponders, 32% were low responders, and 47% were responders. Among volunteers, 16% were nonresponders, 12% were low responders, and 72% were responders. Percent platelet aggregation after clopidogrel inversely correlated with CYP3A4 activity (r = −0.6, P = 0.003). Improved platelet inhibition in volunteers resistant to clopidogrel was observed with the coadministration of clopidogrel and rifampin.

Conclusions—Clopidogrel administration results in interindividual variability in platelet inhibition, which correlates with CYP3A4 metabolic activity. Measurement of antiplatelet drug efficacy with a point-of-care device and alternative antithrombotic strategies for aspirin or clopidogrel nonresponders and low responders could reduce the incidence of thrombotic events that continue to occur despite oral antithrombotic therapy. (Circulation. 2004;109:166-171.)

Key Words: drugs ● platelets ● pharmacology

Clopidogrel, a thienopyridine derivative similar to ticlopidine, is an inhibitor of platelet aggregation induced by adenosine diphosphate (ADP). Clopidogrel was approved by the United States Food and Drug Administration (FDA) in 1997 for the reduction of myocardial infarction, stroke, and vascular death in patients with recent stroke, recent myocardial infarction, or established peripheral arterial disease after the Clopidogrel versus Aspirin in Patients at Risk of Ischemic Events (CAPRIE) trial showed superior reduction of these events with clopidogrel compared with aspirin (annual risk, 5.3% versus 5.8%; P = 0.04). Dual antiplatelet therapy (aspirin plus clopidogrel) for acute coronary syndromes was approved by the FDA in 2002 on the basis of the Clopidogrel in Unstable angina to prevent Recurrent ischemic Events (CURE) trial results, which showed a significant reduction in the 9-month composite end point of cardiovascular death, nonfatal myocardial infarction, or stroke versus aspirin monotherapy (9.3% versus 11.4%, P < 0.001). Additionally, the combination of aspirin and clopidogrel is the standard antiplatelet therapy for coronary stenting although it has not gained formal FDA approval status.

The elucidation of the pharmacological properties of clopidogrel has lagged behind the randomized clinical trial reports. A 75-mg once-daily clopidogrel dose was used in CAPRIE because it produced inhibition of ADP-induced platelet aggregation equivalent to ticlopidine 250 mg twice daily. Only later were dosing studies published, and this work continues. Subsequently, the active metabolite of clopidogrel, a prodrug, was identified, and its noncompetitive inhibition of the platelet P2 Y12 ADP receptor was

© 2004 American Heart Association, Inc.

Circulation is available at http://www.circulationaha.org DOI: 10.1161/01.CIR.0000112378.09325.F9
described. We recently demonstrated that clopidogrel is activated in humans by the hepatic cytochrome P450 (CYP) 3A4 enzyme system and that its platelet inhibition efficacy could be perturbed by CYP3A4 inhibitors, inducers, and substrates.

Interindividual variation in platelet inhibition by clopidogrel has long been recognized but only recently evaluated in peer-reviewed manuscripts. Clopidogrel resistance, a concept related to aspirin resistance, has also been described. This phenomenon includes both clopidogrel nonresponders and low responders. Possible mechanistic explanations include increased platelet reactivity before clopidogrel dosing, drug–drug interactions inhibiting clopidogrel activation by CYP3A4, drug–drug interactions inhibiting clopidogrel activation by CYP3A4, and the platelet count is determined. This process is repeated with a second 1-mL sample of fresh whole blood in a Plateletworks tube containing K3-EDTA as the anticoagulant. The sample is then passed through the cell counter and the platelet count is determined. This process is repeated with a second 1-mL sample of fresh whole blood in a Plateletworks tube containing both citrate and 20 μmol/L ADP. In the presence of ADP, platelets associate and aggregate. Because the aggregated platelets exceed the threshold limitations for platelet size, they are no longer counted as individual platelets. The ratio of the platelet count between the agonist and reference tubes is calculated as percent platelet aggregation. The results are available within 4 minutes. A previous study comparing this device to light transmission aggregometry using platelet-rich plasma demonstrated a correlation coefficient of 0.83 in 225 paired samples.

Erythromycin Breath Test
The erythromycin breath test (Metabolic Solutions, Inc) was used to measure hepatic CYP3A4 activity in vivo in the second study. A preinjection breath sample was obtained. An intravenous dose of [14C-N-methyl]-erythromycin (3 μCi, 0.01 mmol of erythromycin) was then administered. Subsequently, a single breath sample was collected after 20 minutes. Quantitation of exhaled 14 CO2 provides a selective measure of the instantaneous hepatic CYP3A4 activity.

Statistics
In patients undergoing stent implantation, paired 2-sample t tests were used to compare platelet aggregation between 0 and 5 days. Linear regression plot comparing the baseline CYP3A4 metabolic activity and postclopidogrel CYP3A4 activity with percent platelet aggregation before and after clopidogrel was performed using SPSS statistical analysis. In healthy volunteers taking rifampin, paired 2-sample t tests with Bonferroni’s correction were used to compare platelet aggregation between 0, 6, 20, 24, and 30 days within each group. Nonparametric data that did not conform to a normal distribution were analyzed using Mann-Whitney U tests for unpaired data and Wilcoxon tests for paired data. All values were expressed as mean±SD. P<0.05 was considered statistically significant.

Results
Variable Clopidogrel Response
Interindividual variability in platelet inhibition was demonstrated in both patients and healthy volunteers. In patients, 7 of 32 (22%) were clopidogrel nonresponders, 10 of 32 (32%) were low responders, and 15 of 32 (47%) were responders (Figure 1). In healthy volunteers, 4 of 25 (16%) were clopidogrel nonresponders, 3 of 25 (12%) were low responders, and 18 of 25 (72%) were responders (Figure 2).

Clopidogrel Response Versus CYP3A4 Activity
Baseline CYP3A4 activity was randomly distributed, as measured by the erythromycin breath test, and was not related
to baseline percent platelet aggregation in 25 healthy volunteers (Figure 3A). After clopidogrel (Figure 3B), there was a significant inverse correlation between platelet aggregation and CYP3A4 activity ($r = -0.6; P = 0.003$).

CYP3A4 Induction

Clopidogrel produced interindividual variability in platelet aggregation inhibition in 10 healthy volunteers (Figure 4). Rifampin did not change platelet aggregation, but platelet aggregation inhibition after clopidogrel was enhanced by rifampin (56±20% versus 33±18%; $P = 0.001$), a CYP3A4 inducer. After rifampin, the 3 initial nonresponders and 1 low responder demonstrated enhanced platelet inhibition that then met the definition for a clopidogrel responder (Figure 4).

Discussion

The variable platelet inhibition response to clopidogrel has been recognized by all who have tested clopidogrel efficacy by platelet aggregometry, and clopidogrel resistance has recently been described.18,23,24 In a previous report, the incidence of clopidogrel nonresponders was $\approx 10\%$ and the incidence of low responders was $\approx 20\%$.24 These frequencies are similar to those reported for aspirin resistance using platelet aggregometry.19 Others have demonstrated higher frequencies for clopidogrel resistance, reporting an absolute, rather than a relative, change in platelet aggregation.18 This is an important differentiation, because it may be argued that aspirin might influence the prevalence of clopidogrel nonresponders. However, because the response to clopidogrel in this study was reported as a relative change in platelet aggregation, any change can be attributed to clopidogrel administration alone. We previously demonstrated that clopidogrel, a prodrug, was activated in humans by CYP3A4.14,15 In the present study, we offer additional evidence in support of that observation and describe 1 mechanism to explain clopidogrel resistance: individual variability in the metabolic activity of CYP3A4.

In our earlier study,14 we showed that the pharmacological manipulation of CYP3A4 metabolic activity affected the ability of clopidogrel to inhibit platelet aggregation, as measured by platelet aggregometry. Erythromycin and troleandomycin, CYP3A4 inhibitors, decreased clopidogrel efficacy, as did atorvastatin lactone, a competitive CYP3A4 substrate. In contrast, rifampin, a CYP3A4 inducer, increased clopidogrel efficacy.

There exists substantial interindividual variation in CYP3A4 expression that cannot be accounted for by known inducers and inhibitors.31 A genetic basis for this variation has not been identified, but it is possible to use the erythromycin breath test to determine an individual’s CYP3A4 activity. In our second study performed in healthy volunteers, the baseline distribution of individual CYP3A4 metabolic activity was randomly distributed (Figure 3A). CYP3A4 activity levels correlated with platelet aggregation values...
after clopidogrel, and the correlation was inverse, as expected (ie, the lower the CYP3A4 activity, the less clopidogrel was activated [Figure 3B]). Variation in hepatic CYP3A4 activity accounted for approximately one third ($R^2/\text{H}11005 = 0.36$) of the interindividual variability in response. In a patient population receiving potential inducers and inhibitors of CYP3A4 and who may have varying degrees of liver dysfunction because of disease, variation in CYP3A4 activity would be significantly greater than in our healthy volunteers. A logical hypothesis is that variation in CYP3A4 activity would account for significantly more than one third of the variability in clopidogrel response in patients.

Our data also show that the role of CYP3A4 in clopidogrel activation can be exploited to convert nonresponders. In Figure 4, it can be seen that clopidogrel efficacy in 3 nonresponders and 1 low responder was improved by coadministration of rifampin. This suggests that agents that induce the expression of CYP3A4 metabolic activity can decrease the incidence of clopidogrel resistance.

The different definitions of antiplatelet drug resistance are empiric. Muller et al\textsuperscript{24} defined clopidogrel nonresponse as a relative inhibition of ADP-induced platelet aggregation of <10%. Low responders were identified by an inhibition of 10% to 29%. Whereas platelet receptor P2 $\gamma_12$ genetic polymorphisms\textsuperscript{25} or defects in signaling pathways downstream from the receptor would represent durable mechanisms for clopidogrel resistance, the observation that some patients with initial clopidogrel resistance become more responsive to clopidogrel over time\textsuperscript{23} could be explained either by subsequent induction of CYP3A4 expression and increased metabolism of the prodrug or decreasing platelet reactivity after stenting. Increased loading (>300 mg) or maintenance (>75 mg) doses would be expected to decrease clopidogrel resistance because of baseline platelet activation.\textsuperscript{17,18} The Intracoronary Stenting and Antithrombotic Regimen–Rapid Early Action for Coronary Treatment (ISAR-REACT) study\textsuperscript{7} demonstrated no difference between glycoprotein IIb/IIIa receptor inhibitors and placebo in low-risk patients undergoing coronary stent implantation treated with a 600-mg loading dose of clopidogrel.

Aspirin resistance has been defined either as the failure to prevent individuals from clinical thrombotic complications or as the failure to produce an expected response on a laboratory measurement of platelet activation or aggregation.\textsuperscript{22} Unfortunately, the incidence varies from 5% to 50%, depending on the definition used, the test used, and methodological differences among laboratories. Nevertheless, the phenomenon appears real, however defined, and 4 reports suggest that patients with aspirin resistance are at increased risk for thrombotic complications.\textsuperscript{20,21,32,33} Additionally, the concomitant administration of ibuprofen seems to antagonize the irreversible platelet inhibition induced by aspirin\textsuperscript{34} and may also be associated with increased thrombotic complications.\textsuperscript{35,36}

Clopidogrel offers added antiplatelet efficacy to aspirin in patients with acute coronary syndromes\textsuperscript{3} or after stent implantation.\textsuperscript{6} It is possible, however, that the clinical benefit of clopidogrel is more complementary than additive. In the CAPRIE study,\textsuperscript{2} clopidogrel reduced the annual absolute risk of vascular death, myocardial infarction, and stroke by 0.5% (5.3% versus 5.8%) compared with aspirin. In the CURE trial,\textsuperscript{3} although the relative risk reduction for 30-day death or myocardial infarction with aspirin/clopidogrel versus aspirin alone was 21%, the absolute reduction was <1% (3.9% versus 4.8%), an effect that easily could be accounted for by clopidogrel efficacy in patients who were aspirin resistant or taking concomitant ibuprofen with aspirin. If aspirin and clopidogrel were proven to be complementary rather than additive agents, the potential of limiting clopidogrel use to patients who do not respond to aspirin would offer major

![Figure 4. Percent platelet aggregation after clopidogrel, rifampin, and clopidogrel plus rifampin.](image)
cost-effectiveness advantages that would justify the cost of measuring platelet function in patients receiving antiplatelet therapy.

Subacute stent thrombosis continues to occur in 1% to 3% of patients despite dual antiplatelet therapy. Future investigations need to determine whether these patients are aspirin resistant, clopidogrel resistant, or both. Additionally, the possibility that drug–drug interactions between aspirin and ibuprofen or clopidogrel and atorvastatin contribute to these events needs to be evaluated. In view of the recent controversy that the Cypher sirolimus-eluting coronary stent (Cordis Corporation) may be associated with an increased risk of subacute stent thrombosis, it should also be noted that sirolimus promotes platelet aggregation. Therefore, the efficacy of clopidogrel therapy in patients receiving these stents needs to be defined.

Our study has several potential limitations. First, it could be argued that measuring platelet aggregation is instrument dependent and laboratory dependent. However, the same interindividual variability was seen in this study with light transmission aggregometry in platelet-rich plasma as with point-of-care aggregometry in whole blood, and other investigators using the same instruments have produced similar results. Second, one measure of platelet function may not be sufficient to diagnose clopidogrel resistance. Nevertheless, resistance was defined by only 1 measurement in each of the studies correlating aspirin resistance with increased thrombotic complications. Third, the clinical importance of low responders can be disputed. However, the AU–Assessing Ultegra (GOLD) study suggested that suboptimal platelet function inhibition with a glycoprotein IIb/IIIa antagonist, as measured by a point-of-care assay, was associated with increased thrombotic complications after percutaneous coronary intervention.

In conclusion, interindividual variations in platelet inhibition by both clopidogrel and aspirin exist, and some patients are resistant to these antiplatelet agents because of biological variability or drug–drug interactions. With clopidogrel, interindividual variation in platelet inhibition in part reflects variation in CYP3A4 activity, and we have shown that this is true even in healthy volunteers not treated with known CYP3A4 inducers or inhibitors. Consistent definitions for aspirin resistance and clopidogrel resistance are needed that can be documented by reliable laboratory testing and associated with increased risk for thrombotic complications. In the future, measurement of antiplatelet drug efficacy with a point-of-care device and alternative antithrombotic strategies for nonresponders or low responders could reduce the incidence of thrombotic events that continue to occur despite oral antiplatelet therapy.

Acknowledgments
This study was supported by grants from the Clinical Research Center, University of Michigan (No. M01-00042 to Dr Lau) and National Institutes of Health (GM61971 to A.R. Tait and NIH GM 38149 59 to Dr Watkins).

References
Contribution of Hepatic Cytochrome P450 3A4 Metabolic Activity to the Phenomenon of Clopidogrel Resistance

Circulation. 2004;109:166-171; originally published online January 5, 2004;
doi: 10.1161/01.CIR.0000112378.09325.F9
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/2/166

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/