Evaluation of Response to loading Doses of Clopidogrel Therapy in Patients Undergoing Percutaneous Coronary Intervention

Thesis

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BY

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List of abbreviations

ACC	American College of Cardiology
ACE	Angiotensin converting enzyme
ACEI	Angiotensin converting enzyme inhibitor
ACS	Acute Coronary Syndrome
ADP	Adenosine diphosphate
AD	Allele discrimination
AHA	American Heart Association
ALT	Alanine Transaminase
BMI	Body mass index
BMS	Bare metal stents
CABG	Coronary Artery Bypass Graft
CAD	Coronary Artery Disease
CAMP	Cyclic adenosine monophosphate
CATH	Catheterization
CAPRIE	Clopidogrel versus aspirin in patients at risk ischemic
CK-MB	Creatine Kinase-myocardial band
CREDO	Clopidogrel for the reduction of event during observation
CRP	C-Reactive Protein
CTO	Chronic total occlusion
CVD	Cardiovascular disease
CURE	Clopidogrel in unstable angina to prevent recurren Events
CVS	Cardiovascular system
DESs	Drug-eluting stents
DM	Diabetes mellitus
ECG	Electrocardiography
ESC	European Society of Cardiology
GI	Gastrointestinal
GP	Glycoprotein
GTP	Guanosine triphosphate
Hb	Heamoglobin
HDL	High density lipoprotein
HPR	High post treatment platelet reactivity
IQR	interquartile range
LD	loading dose
LDL	low density lipoprotein
LMWH	Low molecular weight heparin
LV	Left ventricular
MACE	Major adverse cardiac events
MI,AMI	Myocardial infaraction, acute myocardial infarction
NL	Normal level
NSAID	Non steroidal anti-inflammatory drug
NSTEMI	Non ST segment elevation Infarction
PAD	Peripheral arterial disease
PCI	Percutaneous coronary intervention
PCR	polymerase chain reaction

PFA	Platelet function analyzer
POC	point-of-care
PRINC	Plavix® response in coronary intervention
RPA	Residual platelet aggregation
VASP	Vasodilator-stimulated phosphoprotein

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Abstract

Background : Coronary artery diseases constitute a major health problem in many parts of the world and are an important cause of morbidity and mortality. Clopidogrel is better tolerability profiles and is currently the anti platelet treatment of choice for prevention of stent thrombosis . In addition, in patients undergoing Percutaneous coronary intervention (PCI) , prolonged dual antiplatelet therapy has been associated with better long-term clinical outcomes.

Aim : To evaluate the response to 600 mg loading dose Clopidogrel versus 300 mg loading dose Clopidogrel in patients undergo PCI. Identify whether the loss of function CYP2C19 681G>A *2 polymorphism correlated with high platelet aggregation after administration of either 300 mg loading dose and 600 mg loading dose of Clopidogrel, and impact of poor response to Clopidogrel and clinical outcome in these patients**.**

Patients and methods : Venous blood samples were collected from 51 patients with coronary artery diseases. The selected patients were divided according to loading does to two groups: Patients in Group I consisted of 11 males (64.7%) and 6 females (35.3%). While patients in Group II consisted of 28 males (82.4%) and 6 females (17.6%). The mean age of Group I was 52.9± 7.3 years with maximum of 65 years and a minimum of 44 years. The mean age of Group II was 52.3±7.8 with a maximum of 67 years and minimum of 37 years. There was no significant difference between the two groups regarding age (P=0.790) and sex (P=0.161). All patients were subjected to complete history taking, through clinical examination and coronary angiography. Detection of Clopidogrel response was assessed by measuring of the platelet aggregation percentage and CYP2C19 *2 investigated in both 300mg and 600mg Clopidogrel users groups. Clopidogrel resistance was defined by an arbitrary cut off value of <10% with respect definition as compared to control value.

Results : There were non significant difference in the percentage of platelet aggregation between 300mg and 600mg Clopidogerl users as regarding to good

response, poor response and non response (*P*-value=0.619). The percentage of Clopidogrel response among the studied patients was 29.2% for 300mg Clopidogrel and 70.8% for 600mg Clopidogrel loading dose. In non response group, 1 patients (20.0%), were resistance to 300mg Clopidogrel dose and 4 patients (80.0%) were resistance to 600mg Clopidogrel dose.

Clopidogrel resistance groups were more likely have CYP2C19 genotype (CYP2C19*1/*2) (GA), than Clopidogrel response groups, and highly significant association between good response group ,non response and poor response groups as regarding to genotype CYP2C19*1/*1 (GG) carriers and CYP2C19*1/*2 (GA) carriers.

Platelet aggregation after 600mg Clopidogrel in both CYP2C19*1/*1 (GG) carriers and CYP2C19*1/*2(GA) carriers is a statistically non significant with basal aggregation, at cath lab (before PCI) and inhibition percentage after PCI. While there were significant difference with pre-discharge (day after PCI), and highly significant difference with inhibition percentage before PCI. Platelet aggregation after 300mg Clopidogrel in both CYP2C19*1/*1 (GG) carriers and CYP2C19*1/*2(GA) carries was statistically non significant with basal aggregation,before PCI, day after PCI, and inhibition percentage after PCI. While there was significant difference with inhibition percentage before PCI.

The presence of atherothrombotic risk factors (hypertension, smoker, BMI), age, sex were comparable in both 600mg, 300mg users patients with no statistically differences between them (*P*>0.05). however, there were statistically significant differences of 300mg users and 600mg users Clopidogrel with diabetes (11.8% vs 43.8%) and with anticonverted enzyme inhibitor (ACEI) (64.7% vs 34.4%).

The results obtained from measuring Adenosine diphosphate (ADP)-induced platelet aggregation percentage were significantly correlated with those results obtained from CYP2C19*2 investigated, therefor we concluded that both methods

(light transmission aggregometry and TaqMan real time polymerase chain reaction (PCR), can be used for measuring Clopidogrel response and more preferred to use both methods together.

Conclusion: As a consequence, There was no variable individual response to the antiplatelet effect of the loading dose (300mg) and higher loading dose (600mg) Clopidogrel therapy used in clinical practice for the protection against recurrent thrombotic in patients with coronary heart disease. Also, there was a highly significant association between Clopidogrel poor response and CYP2C19*2 genotype, revealing the importance of identifying the CYP2C19*2 genotype that may contribute to Clopidogrel poor response,

Key words : Clopidogrel, loading dose, Percutaneous Coronary Intervention, Clinical pharmacy.

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Contents

Coronary artery disease (CAD) is a condition in which the vascular supply to the heart is impeded by atheroma, thrombosis or spasm of coronary arteries. This may impair the supply of oxygenated blood to cardiac tissue sufficiently to cause myocardial ischaemia which, if severe or prolonged, may cause the death of cardiac muscle cells, i.e. a myocardial infarction (MI) ***(Scott and Dwight, 2007).***

Percutaneous coronary intervention (PCI) has become the most frequently used form of coronary revascularization. Since the mid-1990s, stent deployment has been the gold standard to reduce the rate of acute closure and in-stent restenosis ***(Bonello et al.,2008).*** PCI can be considered a valuable initial mode of revascularization in all patients with stable CAD and objective large ischaemia in the presence of almost every lesion subset, with exception of chronic total occlusion (CTO) that cannot be crossed ***(Silber et al.,2005).***

Antiplatelet medications (aspirin, thienopyridines, and platelet glycoprotein IIb/IIIa inhibitors) are commonly used alone or in combination with other antithrombotic medications in patients with acute coronary syndrome (ACS) undergoing PCI ***(Parikh and Keeley,2009).***

Aspirin is the most widely used antiplatelet agent for preventing and treating vascular events. The thienopyridine derivatives, ticlopidine and Clopidogrel, are a suitable alternative in patients who are intolerant to aspirin, and Clopidogrel exhibits better tolerability than ticlopidine. The available evidence from randomized trials indicates that dual therapy with Clopidogrel and aspirin is modestly but significantly more effective than aspirin in preventing serious vascular events. It is also associated with a favorable benefit–risk profile in patients at high risk (especially in acute coronary syndromes and after stenting) ***(Eshaghian et al.,2007).***

The concept of aspirin and Clopidogrel as dual antiplatelet therapy in the secondary prevention of cardiovascular events. Dual platelet inhibition demonstrated a reduction of cardiovascular events from 28 days to up to 1 year in patients undergoing elective or urgent percutaneous coronary intervention or post-myocardial infarction with or without ST-elevation. ***(De Oliveira and Bhatt, 2006)***.

However, not all patients benefit to the same extent from these improvements in antiplatelet therapy, and some continue to suffer ischemic recurrences, including stent thrombosis, which are associated with significant mortality and morbidity. The pathophysiology of recurrent ischemic events is multifactorial, although numerous reports have demonstrated that Clopidogrel resistance is a major precipitating factor ***(Cuisset et al.,2006).***

Clopidogrel is a platelet adenosine diphosphate P2Y12- receptor antagonist that is widely used to prevent vascular events across a wide spectrum of atherothrombotic cardiovascular disease ***(Eshaghian et al.,2007)***. Clopidogrel is rapidly absorbed from the intestine and extensively converted by hepatic cytochrome P450 isoenzymes (CYP3A4, CYP3A5, CYP2C19) to an active thiol metabolite . This short lived active metabolite binds to the P2Y12 receptor via a disulfide bridge between the reactive thiol group and two cysteine residues (cys17 and cys270) present in the extracellular domains of the P2Y12 receptor. Thus, the binding of Adenosine diphosphate (ADP) to the P2Y12 receptor is permanently inhibited ***(Ding et al.,2003).***

Cuisset et al., (2006), reported in a recent prospective, randomized, single-center study that increasing the Clopidogrel loading dose from 300 to 600 mg is likely to improve clinical outcome after coronary angioplasty. However, despite the 600-mg loading dose, some patients remained Clopidogrel-resistant and the rate of major adverse cardiac events (MACE), observed at 1 month, although decreased, was still 5%. The superiority of a high dose regimen in reducing ischemic events and the associated risk profile compared to a standard dose has yet to be established in large scale clinical trials ***(Smith et al.,2006).***

The loading dose may need to be individually adjusted according to the patient's biological response to Clopidogrel to decrease the rate of MACE after stenting (***Bonello et al.,2008).***

Matetzky., et al (2004), studied Clopidogrel responsiveness in patients undergoing stenting for acute ST-elevation myocardial infarction and found that patients who exhibited the highest quartile of ADP induced aggregation had a 40% probability for a recurrent cardiovascular event within 6 months**.**

The concept of Clopidogrel and aspirin resistance has been the subject of much recent attention. Numerous potential mechanisms have been proposed, including general pharmacokinetic and pharmacodynamic variability, variability in compliance, underdosing, drug– drug interactions, genetic polymorphisms, and upregulation of other platelet activation pathways. The reported prevalence ranges from 4% to 30%, depending on the clinical indication, dose of Clopidogrel, timing of assessment, and the type of agonist or platelet function test that is used . Although there have been no prospective studies directly linking the degree of platelet inhibition to clinical outcomes, a few observational reports have shown an association between variability in platelet responsiveness to Clopidogrel and thrombotic events in patients undergoing elective PCI ***(Eshaghian et al.,2007***).

In the recent small studies, heightened platelet reactivity or Clopidogrel nonresponsiveness in patients who were on Clopidogrel 300 mg loading dose treatment was associated with adverse thrombotic events including stent thrombosis. The primary reason has been attributed to the suboptimal generation of active metabolite secondary to potential limitation in intestinal absorption, drug–drug interaction at CYP3A4 and genetic polymorphism of hepatic cytochrome P450 isoenzymes ***(Gurbel and Tantry, 2007).***

In the latter study, 53–63% of patients were resistant to Clopidogrel 300 mg loading dose treatment at 2 h post- stenting; ∼30% were resistant at day 1 and day 5 post-stenting; and 13%–21% were resistant at day 30 post-stenting. Therefore, Clopidogrel resistance might be related to the inadequacy of a 300 mg loading dose to provide sufficient active metabolite generation to arrest platelet reactivity in selected

patients, and that these resistant patients may be at particular risk for thrombotic complications including periprocedural infarction and stent thrombosis ***(Gurbel et al.,2003).***

The optimal definition of resistance or nonresponsiveness to an antiplatelet agent is the failure of the antiplatelet agent to inhibit the target of its action. The identification of resistance would therefore utilize a laboratory technique that detects residual activity of the target. Therefore, Clopidogrel resistance is best demonstrated by evidence of residual post- treatment P2Y12 activity by measuring ADP-induced platelet aggregation before and after treatment ***(Gurbel and Tantry ,2007).***

Cytochrome P4502C19 (abbreviated CYP2C19), a member of the cytochrome P450 mixed-function oxidase system, the CYP2C19 protein is encoded by the CYP2C19 gene ***(Gray et al.,1995).*** Patients carrying at least one CYP2C19*2 allele are more prone to high-on Clopidogrel platelet reactivity, which is also associated with poor clinical outcome after coronary stent placement ***(Trenk et al.,2008).***

The prodrug Clopidogrel has to be converted into the active metabolite that selectively and irreversibly binds to the P2Y12 receptor on the platelet membrane . Conversion is achieved by the highly polymorphic hepatic cytochrome P450 (CYP) system in a 2-step process ***(Trenk et al.,2008).***

Hulot et al (2006). investigated polymorphisms of CYP 2C19, 2B6, 1A2, and 3A4/5 with known functional consequences on enzyme activity. Among the polymorphisms investigated, only the loss-of-function CYP2C19 681G_A polymorphism (*2) was associated with blunted antiplatelet responses to Clopidogrel.

The role of the *2 polymorphism of CYP2C19 in healthy volunteers was subsequently confirmed ***(Brandt et al., 2007).*** However, in a study of 79 patients with coronary artery disease ***(Smith et al 2007).*** The investigators were unable to demonstrate an impact of the CYP2C19 genotype on the antiplatelet effect of a 600-mg bolus dose. Thus, in the clinical setting, the role of the CYP2C19*2 polymorphism on the loss-of-function is currently unclear.