การเปรียบเทียบผลของราเบพราโซลและโอเมพราโซลต่อฤทธิ์ยับยั้งการเกาะกลุ่มของเกล็ดเลือดของโคลพิโดเกรลในผู้ป่วยที่ใช้แอสไพริน

นางสาวสุขศิริ ศิริสว่างวัฒน์

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COMPARISON OF THE EFFECT OF RABEPRAZOLE AND OMEPRAZOLE ON ANTIPLATELET ACTION OF CLOPIDOGREL IN PATIENTS RECEIVING ASPIRIN

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A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Clinical Pharmacy
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Thesis Title	COMPARISON OF THE EFFECT OF RABEPRAZOLE AND OMEPRAZOLE ON THE ANTIPLATELET ACTION OF CLOPIDOGREL IN PATIENTS RECEIVING ASPIRIN
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สุขศิริ ศิริสว่างวัฒน์ : การเปรียบเทียบผลของราเบพราโซลและโอเมพราโซลต่อฤทธิ์ยับยั้งการเกาะกลุ่มของเกล็ดเลือดของโคลพิโดเกรลในผู้ป่วยที่ใช้แอสไพริน. (COMPARISON OF THE EFFECT OF RABEPRAZOLE AND OMEPRAZOLE ON ANTIPLATELET ACTION OF CLOPIDOGREL IN PATIENTS RECEIVING ASPIRIN) อ. ที่ปรึกษา: รองศาสตราจารย์ ดร. ดวงจิตต์ พนมวัน ณ อยุธยา, อ.ที่ปรึกษาร่วม: พันเอก นายแพทย์ นครินทร์ ศันสนยุทธ: 85 หน้า.

แนวทางการรักษาผู้ป่วยกล้ามเนื้อหัวใจขาดเลือดเฉียบพลันหรือผู้ป่วยที่ได้รับการถ่างขยายหลอดเลือดหัวใจในปัจจุบันแนะนำให้ใช้ยาต้านการเกาะกลุ่มของเกล็ดเลือดสองชนิดร่วมกันคือแอสไพรินและโคลพิโดเกรล และส่วนใหญ่จะได้รับยาในกลุ่ม proton pump inhibitors (PPIs) เพื่อป้องกันอาการข้างเคียงในทางเดินอาหาร วัตถุประสงค์ในการศึกษาครั้งนี้คือเปรียบเทียบผลของราเบพราโซลและโอเมพราโซลต่อฤทธิ์ยับยั้งการเกาะกลุ่มของเกล็ดเลือดของโคลพิโดเกรลในผู้ป่วยที่ใช้แอสไพริน ศึกษาความชุกของผู้ไม่ตอบสนองต่อยาโคลพิโดเกรลในกลุ่มผู้ป่วยหัวใจและหลอดเลือด และเปรียบเทียบอัตราการไม่ตอบสนองต่อยาโคลพิโดเกรลก่อนและหลังได้รับยาในกลุ่ม PPIs ซึ่งประกอบด้วยผู้ป่วยที่ได้รับโคลพิโดเกรล 75 มก./วัน ติดต่อกันอย่างน้อย 5 วัน และ ผู้ป่วยที่ได้รับการเตรียมถ่างขยายหลอดเลือดหัวใจ ซึ่งจะได้รับโคลพิโดเกรล 300 มก.ร่วมกับแอสไพริน 300-325 มก.ก่อนการถ่างขยายหลอดเลือดหัวใจ ผู้ป่วยแต่ละคนจะได้รับแอสไพริน 81-325 มก. อย่างน้อย 7 วันก่อนเข้าวิจัย โดยต้องไม่ได้รับยาลดกรดในกระเพาะกลุ่ม PPIs ภายใน 2 สัปดาห์ก่อนเข้าการวิจัย และมีค่าครีแอทินินไม่เกิน 1.5 มก./ดล. ผู้ป่วยจะถูกสุ่มแบ่งเป็นสองกลุ่มเพื่อได้รับโอเมพราโซล 20 มก./วัน หรือราเบพราโซล 20 มก./วัน ติดต่อกันอย่างน้อย 2 สัปดาห์การประเมินผลของโคลพิโดเกรลจะวัดค่าการเกาะกลุ่มของเกล็ดเลือดหลังจากถูกกระตุ้นด้วยเอดีพี 20 ไมโครโมล ด้วยวิธีการส่องผ่านของแสง การศึกษานี้ได้จำกัดความการไม่ตอบสนองต่อยาโคลพิโดเกรล หมายถึงเมื่อถูกกระตุ้นด้วยเอดีพี 20 ไมโครโมล แล้วมีค่าการเกาะกลุ่มของเกล็ดเลือดมากกว่าร้อยละ 50 ค่าเกาะกลุ่มของเกล็ดเลือดจะถูกประเมินก่อนและหลังได้รับยากลุ่ม PPIs

จากการรวบรวมผู้ป่วยจำนวน 87 คนในช่วงระหว่างเดือนสิงหาคม 2551 ถึง มีนาคม 2552 ผู้ป่วย 43 คนได้รับยาโอเมพราโซลและ 44 คนได้รับยาราเบพราโซล ร้อยละ 18 เป็นผู้ป่วยกลุ่มเตรียมถ่างขยายหลอดเลือดหัวใจ และร้อยละ 82 เป็นผู้ป่วยโรคหัวใจและหลอดเลือดที่ได้รับโคลพิโดเกรลติดต่อกันมากกว่า 5 วัน การได้รับโอเมพราโซลหรือราเบพราโซลร่วมกับแอสไพรินและโคลพิโดเกรลทำให้ค่าการเกาะกลุ่มของเกล็ดเลือดเพิ่มขึ้นอย่างมีนัยสำคัญร้อยละ 32.5 และร้อยละ 14 ตามลำดับ โดยการเพิ่มขึ้นไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติระหว่างยาโอเมพราโซลและราเบพราโซล ($p = 0.519$) ค่าเฉลี่ยของการเกาะกลุ่มของเกล็ดเลือดก่อนและหลังได้รับยากลุ่ม PPIs อยู่ในช่วงร้อยละ 38.53 ± 20.16 และ 52.05 ± 19.46 ตามลำดับ ไม่พบข้อมูลค่าพื้นฐานประชากรหรือข้อมูลทางคลินิกค่าใดที่แตกต่างกันในผู้ป่วยทั้งสองกลุ่มยกเว้นค่ามวลกาย พบผู้ที่ไม่ตอบสนองต่อยาโคลพิโดเกรลร้อยละ 34 และเพิ่มขึ้นเป็นร้อยละ 59 เมื่อได้รับยาลดกรดกลุ่ม PPIs การศึกษานี้พบว่าโรคเบาหวานเป็นปัจจัยเสี่ยงของการไม่ตอบสนองต่อยาโคลพิโดเกรล (OR = 2.93, 95%CI = 1.17-7.29, $p = 0.019$)

ภาควิชา.....เภสัชกรรมปฏิบัติ.....ลายมือชื่อนิสิต.....สุขศิริ ศิริสว่างวัฒน์.....

สาขาวิชา.....เภสัชกรรมคลินิก.....ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ปีการศึกษา.....2551.....ลายมือชื่ออ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5076600533 : MAJOR CLINICAL PHARMACY
KEYWORDS: CLOPIDOGREL/ PLATELET AGGREGATION / OMEPRAZOLE / RABEPRAZOLE / NONRESPONDER / CORONARY ARTERY DISEASE

SUKSIRI SIRISWANGVAT: COMPARISON OF THE EFFECT OF RABEPRAZOLE AND OMEPRAZOLE ON ANTIPLATELET ACTION OF CLOPIDOGREL IN PATIENTS RECEIVING ASPIRIN. THESIS ADVISOR: ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUTHAYA, THESIS CO-ADVISOR: COLONEL NAKARIN SANSANAYUDH, M.D., 85 pp.

Current guidelines recommend dual antiplatelet of aspirin and clopidogrel for patients who have acute coronary syndrome (ACS) or undergo percutaneous coronary intervention (PCI) those patients commonly prescribed proton pump inhibitors (PPIs) to prevent gastrointestinal side effects. The purposes of this study were to determine the effect of rabeprazole and omeprazole on the antiplatelet action of clopidogrel plus aspirin, to investigate the prevalence of clopidogrel nonresponder in patients with coronary artery disease (CAD) and compare the rate of clopidogrel nonresponsiveness before and after receiving each PPI. All consecutive patients with angiographic diagnosis of CAD were recruited. The study consisted of patients who had taken clopidogrel 75 mg/day at least 5 days and patients who received loading dose (LD) of clopidogrel 300 mg and aspirin 300-325 mg before underwent PCI. All patients were treated with aspirin 81-325 mg/day at least 7 days prior to the study. Exclusion criteria included previous treatment with PPIs within 2 weeks and serum creatinine > 1.5 mg/dl. The patients were randomized into two treatment groups: 20 mg/day of omeprazole or 20 mg/day of rabeprazole for at least 2 weeks. Effect of clopidogrel on platelet aggregation was measured by light transmission aggregometry using ADP 20 μM as agonist. Clopidogrel nonresponder was defined as ADP 20 μM-induced maximal platelet aggregation (MPA) > 50%. Platelet aggregation test was assessed before and after receiving PPIs for at least 2 weeks.

Of 87 patients were enrolled during August 2008 to March 2009, 43 patients took omeprazole while 44 patients took rabeprazole. Overall, 18% were scheduled for elective PCI patients and 82% were stable CAD patients. This study found that concomitant use of omeprazole or rabeprazole in patients receiving aspirin plus clopidogrel were significantly increased platelet aggregation from baseline (32.5% and 14%, respectively) but not significantly different between the two drugs (p = 0.519). Average values of MPA after ADP 20 μM stimuli before and after receiving PPIs were 38.53 ± 20.16% and 52.05 ± 19.46%, respectively. There was no significant difference in demographic, clinical characteristics and co-medications between the two groups except for body mass index. This study found high prevalence of clopidogrel nonresponder, 34%, prior to taking PPIs and increased to 59% after receiving PPIs in CAD patients. Univariate analysis, showed that diabetes mellitus to be a risk factor for clopidogrel nonresponsiveness (OR = 2.93, 95%CI = 1.17-7.29, p = 0.019).

Department.....Pharmacy Practice.....Student's Signature.....
Field of Study.....Clinical Pharmacy.....Thesis Advisor's Signature.....
Academic Year..... 2008.....Thesis Co-Advisor's Signature.....

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LIST OF ABBREVIATIONS

a	Chi-square test or Fisher's exact test.
ACEIs	Angiotensin converting enzyme inhibitors
ACS	Acute coronary syndrome
ADP	Adenosine 5-diphosphate
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
ARB	Angiotensin receptor blockers
ATP	Adenosine triphosphate
b	Independent t-test
BMI	Body mass index
c	Pair t-test
CAD	Coronary artery disease
cAMP	Cyclic adenosine monophosphate
CCBs	Calcium channel blockers
Cl_{Cr}	Creatinine clearance
CNR	Clopidogrel nonresponder
COX-1	Cyclooxygenase 1
CPA	Cone and platelet analyzer
CR	Clopidogrel responder
CV	Cardiovascular, Coefficient of variation
CYP	Cytochrome P-450
d	ANCOVA test
DAG	Diacylglycerol
DM	Diabetes mellitus
e	Mann-Whitney *U* test
et al	et alii (and others)
f	Pearson correlation coefficient test
g	Spearman correlation coefficient test
GERD	Gastro-esophageal reflux disease
GP	Glycoprotein
h	ANOVA test

Hct	Hematocrit
i	Kruskal-Wallis test
IPA	Inhibition of platelet aggregation
LD	Loading dose
LTA	Light transmission aggregometry
m	Meter
MD	Maintenance dose
mg	Milligrams
MI	Myocardial infarction
min	Minute
ml	Milliliters
mm	Millimeter
MPA	Maximal platelet aggregation
n	Number of patients
NSTEMI	Non ST-elevated myocardial infarction
p	p value
PCI	Percutaneous coronary intervention
PKA	Protein kinase A
POC	point of care
PPIs	Proton pump inhibitors
PPP	Platelet poor plasma
PRI	Platelet reactivity index
PRP	Platelet rich plasma
r	Correlation coefficient
SD	Standard deviation
STEMI	ST-elevated myocardial infarction
TEG	Thrombelastography
TXA_2	Thromboxane A_2
UA	Unstable angina
VASP	Vasodilator-stimulated phosphoprotein
VASP-P	Vasodilator-stimulated phosphoprotein phosphorylation
Vs	Versus
vWF	von Willebrand factor
μM	Micro molar

CHAPTER I

INTRODUCTION

1.1 Rationale and background

There is a large and increasing global burden of cardiovascular disease. Approximately in worldwide of 14 million individuals worldwide died of cardiovascular disease in 1990, and the number is projected to rise to about 25 million by 2020.[1] Reliable data from bureau of health policy and strategy, Ministry of Public Health 2006, revealed that the absolute cardiovascular death are ranked as the third of Thailand leading cause of death.[2] In addition, Thai Acute Coronary Syndrome Registry (TACSR) showed high enrollment of the patients in August 2002. In three years, records of 9373 patients were collected from 17 hospitals. The patients were classified as ST elevation myocardial infarction (STEMI) (40.9.%), non-ST-elevation myocardial infarction (NSTEMI)(37.9%) and unstable angina (UA)(21.2%).[3]

The ACC/AHA 2007 guidelines for the management of patients with UA/NSTEMI[4] and ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention (PCI)[5] recommended to use combination of aspirin and clopidogrel to reduce rates of cardiovascular ischemic events in acute coronary syndrome patients and in patients post PCI. Clopidogrel is a thienopyridine prodrug requiring several biotransformation steps, mediated mainly by cytochrome P-450 (CYP); especially CYP3A4/5, CYP2C9, CYP2C19, CYP2B6 and CYP1A2 to generate active metabolites. The active metabolite of clopidogrel will binds irreversibly to platelet adenosine 5-diphosphate (ADP) receptor $P2Y_{12}$ which involved in platelet activation and stabilization of the platelet aggregation.[6-8] Therefore, drugs which reduce the biological action of clopidogrel, probably by competitive metabolic effects on that enzyme, may decrease the effect of clopidogrel.

Patients with acute coronary syndrome (ACS) and patients post coronary stenting, who usually receive clopidogrel and aspirin dual therapy, are commonly concomitantly treated with proton pump inhibitors (PPIs) to prevent the gastrointestinal side effect. The most commonly used PPI is omeprazole. The principle isoenzymes involved in the metabolism of

proton pump inhibitors are CYP2C19 and CYP3A4[9], therefore, interaction may occur and this may reduce the effect of clopidogrel. Recent study (Gilard et al, 2008) has revealed that omeprazole significantly decreased the effect of clopidogrel on platelet activation as tested by vasodilator-stimulated phosphoprotein (VASP) assay.[10] The contribution of CYP2C19 to the overall metabolism of rabeprazole is much less compared with that of the other PPIs[9] and therefore concomitantly use of this drug should have least effect, if any, on the platelet aggregation of clopidogrel. As Pace et al, 2005[11], Dekkers et al, 1999[12] and WHO[13] have pointed out, omeprazole 20 mg/day and rabeprazole 20 mg/day have equivalent efficacy and tolerability in the treatment of erosive gastro-esophageal reflux disease (GERD), gastric ulcer and duodenal ulcer.

1.2 Hypothesis

Concomitantly taking clopidogrel with rabeprazole should cause less effect on the antiplatelet action of clopidogrel than concomitantly taking clopidogrel with omeprazole.

1.3 Objectives

To compare the effect between omeprazole and rabeprazole on the antiplatelet action of clopidogrel plus aspirin

To determine the prevalence of clopidogrel nonresponder in coronary artery disease (CAD) and patients undergoing PCI.

To compare the rate of clopidogrel nonresponsiveness before and after receiving each PPI.

1.4 Expected outcomes

Provide information regarding the effect of different PPIs on antiplatelet action when coadministered with clopidogrel plus aspirin.

Known the prevalence of nonresponder in ACS patients and patients undergoing elective PCI in Thai population.

1.5 Operational definition

Platelet aggregation : An ability of platelet to link to another platelet to form platelet aggregates after induced by ADP *in vitro*. The method to assess platelet aggregation in this study is optical aggregometry.

Clopidogrel nonresponder : Inadequate of clopidogrel to inhibition *in vitro* with 20 μM ADP, maximal platelet aggregation is > 50%

Percent increased of maximal platelet aggregation (MPA)
: $[(MPA_{treatment} / MPA_{baseline}) -1] \times 100$

Difference MPA (Δ MPA) : $MPA_{treatment} - MPA_{baseline}$

CHAPTER II

LITERATURE REVIEW

2.1 Platelet structure and function[14-19]

Platelets are small, approximately 2-3 μm in diameter, anucleated cells that derive from megacaryocyte in the bone marrow and have a life span of approximately 8-10 days. Numbers of platelets in blood vary in range of 150-350 10^9/L. The main functions of platelets include normal hemostasis as well as vessel constriction and repair. Platelets also participate in pathophysiological processes such as thrombosis, bleeding, inflammation, tumor growth and promotion of atherosclerosis. On the platelet surface membrane, there are a large number of receptors which specifically bind agonists that stimulate the physiological platelet response, for example, ADP, epinephrine, collagen, thrombin, serotonin, and platelet activating factor.[17] The platelet membrane also contains phospholipids, where they serve as substrates for phospholipase enzymes. Platelets contain three types of granules, namely: (1) α-granules; which are most numerous and contain mainly protein such as platelet factor 4, platelet-derived growth factors, β-thromboglobulin, fibrinogen, thrombospondin, plasminogen activator inhibitor-1, fibronectin, von Willebrand factor and cytokines (2) dense granules; which are rich in ADP, serotonin and calcium (3) lysosomal granules; which contain acid proteases, acid glycosidases, acid phosphatases and aryl sulphatases.[14, 17] Among these three granules, dense granule contents are easily secreted, α-granules release requires higher agonist concentrations, while lysosomal granule secretion only occurs with potent activating agents.[17]

Under normal physiological conditions when a blood vessel is damaged and the normal endothelial-cell barrier is disrupted, platelets are quickly recruited from the circulating blood to form an occlusive plug to arrest the lost of blood. In contrast, in pathological conditions, such as atherosclerosis, arterial thrombus formation may limit the blood supply to nearby tissues, thus causing local ischemia and the progression of the atherosclerotic lesion. The typical platelet response to vascular injury can be divided into three major phases. These consist of platelet adhesion; the interaction of platelets with subendothelial matrix, platelet activation; a phase during which biochemical pathways are activated (platelet undergo shape

change and secrete granule constituents, including ADP) and platelet aggregation; the interaction of platelets with each other to form platelet aggregates.

Platelet adhesion

After vessel wall injury, which is represented by rupture of an atherosclerotic plaque, platelets are exposed to a non-endothelial surface, which include collagen (most important), fibronectin and other adhesive glycoproteins, they adhere, flatten and spread on the surface of the subendothelial matrix. The platelet surface membrane has adhesion receptors that bind specific matrix molecules. These receptors include the glycoprotein (GP) Ib/IX complex; a receptor for subendothelial von Willebrand factor (vWF) and several of the membrane glycoproteins of the integrin superfamily GP Ia/IIa, a collagen receptor, GP Ic/IIa, a fibronectin receptor and GP Ic/IIa, a laminin receptor. In addition, many components of the matrix, such as vWF, thrombosondin, fibronectin and collagen can interact with one another as well as with platelets. Platelet adhesion to extracellular matrix is mediated via interactions with vWF, which acts as a bridge between platelet surface receptor, GP Ib/IX/V and exposed collagen.

Platelet activation

Platelet activation can be induced by adhesion to proteins such as collagen within the subendothelial matrix; by soluble agonists, such as epinephrine, ADP, vasopressin, serotonin and thrombin; and possibly by cell contact during platelet aggregation. The interaction between a platelet-activating agonist and its receptor causes rapid mobilization of signaling molecules within the platelet, notably calcium, diacylglycerol (DAG), and inositol 1,4,5-trisphosphate (IP), which are sufficient to initiate and complete shape change and aggregation responses (figure 1). These two compounds mediate important mechanisms of platelet activation: the activation of protein kinase C and the release of ionized calcium from intracellular stores, respectively. Protein kinase C phosphorylates substrate proteins, whereas the increase in cytoplasmic calcium activates various calcium-dependent and calmodulin-dependent reactions. Platelet activation is accompanied by the reorganization of cytoskeletal proteins productin a dramatic morphologic change from smooth discs shape to spiny spheres with protruding pseudopodia (figure 2). These activation-induced metabolic processes act in concert to stimulate platelet aggregation and granule secretion. Platelet activation leads to the surface expression of a phospholipids complex, which provides a critical nucleation site for calcium and factor binding in the intrinsic clotting pathway.

***Platelet aggregation*[15, 16]**

The process of platelet-platelet adherence is termed aggregation. Platelet aggregation is promoted by various agonists, including collagen, thrombin, ADP and throboxaneA_2 (TXA_2), acting on specific receptors on the platelet surface: activation by agonists leads to expression of GP IIb/IIIa receptors which bind fibrinogen and this links adjacent platelets sticking them together, aggregation.

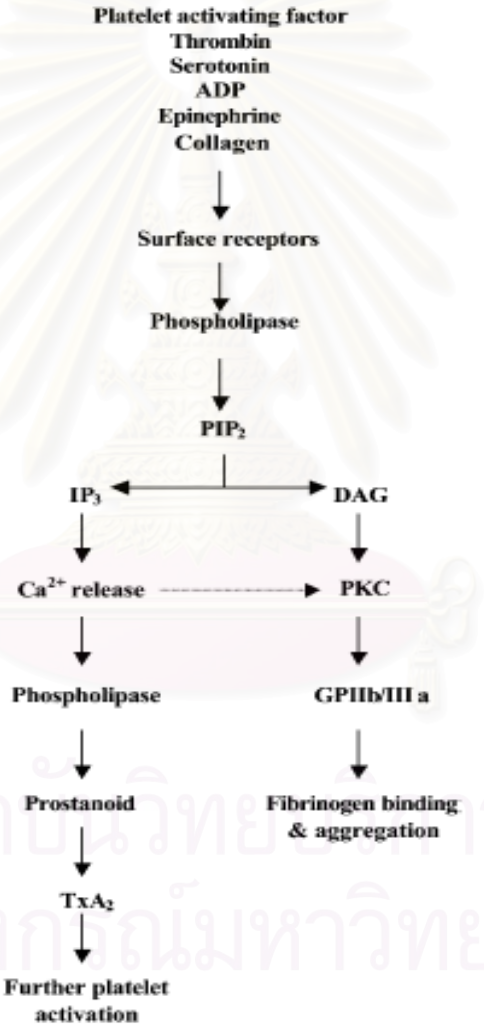

Figure 1: Mechanisms of platelet activation[17]

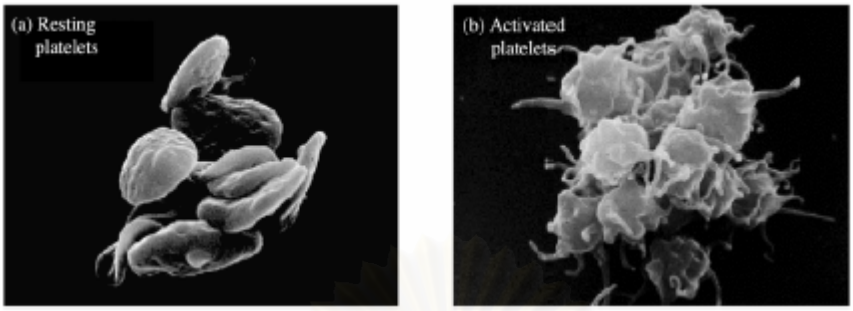

Figure 2: Resting (a) and activated platelets (b)[17]

2.2 Assessment of platelet function

Several techniques for measuring platelet function have been developed. Traditionally they have been used to assess platelet function defects and bleeding tendency prior to surgery. Recently, platelet function tests have been used in atherothrombotic disease to predict clinical outcomes and to monitor antiplatelet drugs. All techniques of measuring platelet activation and aggregation are sensitive to several variables. Different tests reflect all these variables alternatively and their sensitivity and specificity in doing so vary. The large number and variety of drugs mediated platelet defects challenge the platelet function tests. Several challenges for measuring platelet activation exist in table 1.

Table 1: An alphabetical list of currently available tests for the monitoring of antiplatelet therapy[20, 21]

Name of test	Principle	Advantages	Disadvantages	Frequency of use
AspirinWorks®	Immunoassay of urinary 11-dehydrothromboxane B_2	Measure stable thromboxane metabolite Dependent upon COX-1 activity	Indirect assay Not platelet-specific Renal function dependent	Increasing use
Bleeding time	*In vivo* cessation of blood flow	*In vivo* test Physiological POC	Insensitive Invasive Scarring High CV	Decreasing popularity
Flow cytometry	Measurement of platelet glycoproteins and activation markers by fluorescence (e.g. VASP-P to monitor $P2Y_{12}$ inhibition	Whole blood test Small blood volumes Wide variety of tests Blood samples can be mailed at room temperature to a core laboratory	Requires a flow cytometer Specialized operator Expensive Sample preparation	Widely used
HemoStatus® Device	Platelet procoagulant activity	Simple POC	Insensitive to aspirin and GPIb function	Used in surgery and cardiology
Impact® cone and platelet analyzer	Quantification of high shear platelet adhesion / aggregation onto surface	Small blood volume required High shear Rapid Simple	Instrument not yet widely available Requires pipetting	Little widespread experience

Table 1: An alphabetical list of currently available tests for the monitoring of antiplatelet therapy[20, 21] (continue)

Name of test	Principle	Advantages	Disadvantages	Frequency of use
Impedance aggregometry	Monitors changes in impedance in response to classical agonists	Whole blood test	Older instruments require electrodes to be cleaned and recycled	Widely used although less than LTA
Light transmission aggregometry (Turbidimetric)	Low shear platelet-to-platelet aggregation in response to classical agonists	Historical gold standard	Time consuming Sample preparation Expensive	Widely used in specialized labs
Platelet function analyzer (PFA-100®)	High shear platelet adhesion and aggregation during formation of a platelet plug	Whole blood test POC Small blood volume Simple and rapid	vWF and Hct dependent Does not correlate well with clopidogrel therapy	Widely used
Plateletworks®	Platelet aggregation	Minimal sample preparation Whole blood assay	Not well studied	Little widespread experience
Serum Thromboxane B_2	Activation-dependent release from platelets	Dependent upon COX-1 activity	Not platelet-specific Limited studied	Widespread use
Thromboelastography (TEG® or ROTEM®)	Monitoring or rate and quality of clot formation	Global whole blood test POC Clot information	Limited studied Requires pipetting	Used in surgery, anesthesiology
VerifyNow®	Fully automated platelet aggregometer to measure antiplatelet therapy	Simple POC 3 test cartridges (aspirin, $P2Y_{12}$ and GP IIb/IIIa)	Cartridges can only be used for single purpose	Increasing use

Abbreviation: COX-1 = cyclooxygenase 1, CV = coefficient of variation, GP = glycoprotein, Hct = hematocrit, LTA = light transmission aggregometry, POC = point of care, VASP-P = vasodilator-stimulated phosphoprotein phosphorylation, vWF = von Willebrand factor

2.3 Platelet function test for monitoring clopidogrel

2.3.1 ADP receptors as targets for antiplatelets[17]

Although the importance of ADP as a platelet stimulant *in vivo* has been long recognized, the receptors mediating ADP-induced platelet activation have, until relatively recently, been elusive. However, there are three distinct ADP receptors ($P2Y_1$, $P2Y_{12}$, $P2X_1$) on the surface of human platelets, each with distinct signaling pathways and functions. $P2Y_1$ and $P2Y_{12}$ are both serpentine, G-protein- linked receptors which are associated with Gq (stimulation of PLCβ) and G_i (inhibition of adenylyl cyclase), respectively.

The $P2Y_1$ receptor is generally seen as a mediator of ADP-induced shape change and as a "trigger" which primes the αIIbβ3 integrin. The $P2Y_{12}$ receptor is linked to G_i and thereby inhibits the activity of adenylyl cyclase and blocks the formation of cAMP (a major intracellular inhibitor of platelet function). Stimulation of $P2Y_{12}$ amplifies the platelet response to ADP and is critical for full activation of the αIIbβ3 integrin, and thus is necessary for irreversible platelet aggregation. Little is known about the function of $P2X_1$ in ADP induced platelet activation, at least partly due to the extreme sensitivity of this receptor to desensitization. However $P2X_1$ is a ligand-gated non-selective cation channel that appears to be responsible for Ca^{2+} entry in response to ADP.

2.3.2 Assessment for clopidogrel therapy[21]

There are 2 categories of available tests. First, only the VASP phosphorylation (VASP-P) assay is specific with regard to signaling through $P2Y_{12}$ and therefore to the platelet inhibitory effects of clopidogrel (figure 3). Second, ADP can be used as the stimulus. The alternative approach is to add ADP and look at one of a number of end points. However, it is important to consider that ADP binds to its platelet surface $P2Y_1$ receptor as well as to its platelet surface $P2Y_{12}$ receptor and that the active metabolite of clopidogrel only inhibits at $P2Y_{12}$, not $P2Y_1$. Therefore, the effects of ADP on platelet function reflect not only the inhibitory effects of clopidogrel on $P2Y_{12}$ but also the unblocked effect of ADP induced signaling through $P2Y_1$. In addition, concentrations of ADP to platelet aggregation have an impact of measurement of clopidogrel response. The platelet response to ADP and inhibition by clopidogrel is highly dependent on the concentration of the agonist. At low concentrations, the response to ADP is highly dependent on the generation of thromboxane and is inhibited

by aspirin. Higher concentrations of ADP induce full and irreversible platelet aggregation that is insensitive to aspirin but is inhibited by up to 90% in the presence of a $P2Y_{12}$ antagonist.[22]

With ADP as the stimulus, one of a number of end points could be chosen, including turbidimetric platelet aggregometry, impedance platelet aggregometry, the VerifyNow $P2Y_{12}$ assay, Plateletworks, platelet surface–activated GP IIb/IIIa, platelet surface P-selectin, leukocyte–platelet aggregates, the TEG Platelet Mapping system, and the Impact cone and platelet analyzer.

2.3.2.1 Light transmission aggregometry[17, 21]

Light transmission aggregometry or optical (turbidimetric) platelet aggregometry was one of the first methods developed to assess platelet function and involves quantifying the changes in light transmittance in a platelet sample suspended in plasma following induction of platelet aggregation. Briefly, platelet-rich plasma (PRP) samples are stirred in a cuvette at 37°C between a light source and a photomultiplier tube. Addition of platelet agonist performed platelets aggregate and the transmission of light increases.

The main advantage of platelet aggregometry is that it is the historical "gold standard". However, it is outweighed by its many disadvantages: platelet function *in vitro* does not necessarily reflect platelet function *in vivo*; sample aging occurs as a result of the time required preparing PRP; and the presence of substances, such as lipids, in PRP or platelet-poor plasma (PPP) can alter absorbance at the wavelength of observation. The above disadvantages may be considered as minor; however, the major problem with turbidimetric aggregometry is that centrifugation modulates platelet behavior; platelets are heterogeneous in size, density and metabolic activities, and it is likely that subpopulations of platelets are lost during the preparation of PRP that may be important determinants of haemostatic function *in vivo*.

2.3.2.2 VerifyNow®[23]

Formerly known as the Ultegra rapid platelet function analyzer, is a point of care test that is approved by the US Food and Drug Administration (FDA) to measure the aspirin or thienopyridine induced defects in platelet function. VerifyNow® uses the same principle, and therefore has the same fundamental advantage, as platelet aggregometry.

Fibrinogen-coated beads are included in the VerifyNow® system to augment the GP IIb/IIIa–dependent signal. Advantages of the VerifyNow® system include point of care use, simplicity, rapidity (results in 5 minutes), low sample volume, no sample preparation, and a whole-blood system. Three VerifyNow® assays are currently available: the VerifyNow® IIb/IIIa assay (sensitive to GP IIb/IIIa antagonists), the VerifyNow® aspirin assay (sensitive to aspirin), and the VerifyNow® $P2Y_{12}$ assay (sensitive to thienopyridines).

In the VerifyNow® $P2Y_{12}$ Assay, ADP is used as the agonist. ADP stimulates platelet aggregation via its 2 receptors: $P2Y_1$ and $P2Y_{12}$. Although the agonist used in the VerifyNow $P2Y_{12}$ Assay is ADP 20 μM, a second agent, prostaglandin E1 22 nM, is also added to suppress intracellular free calcium levels and thereby reduce the platelet activation contribution from ADP binding to its $P2Y_1$ receptor.[24]

2.3.2.3 VASP phosphorylation[20, 21]

The combination of ADP and prostaglandin E1 is also used in the flow cytometric-based VASP assay. The principle of this assay is to measure the phosphorylation of VASP, which is theoretically proportional to the level of inhibition of the $P2Y_{12}$ receptor.

Prostaglandin E1 binds to its inositol phosphate receptor on the platelet surface and signals through a G stimulatory protein and adenylyl cyclase to convert adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) and then, through protein kinase A (PKA), to convert VASP to phosphorylated VASP (VASP-P). ADP binds to its $P2Y_{12}$ receptor on the platelet surface and signals through a G inhibitory protein to inhibit prostaglandin E1–induced signaling through adenyl cyclase. $P2Y_{12}$ antagonists (for example, the active metabolite of clopidogrel) inhibit this ADP-induced effect. Therefore, in the presence of both prostaglandin E1 and ADP, VASP-P is directly proportional to the degree of $P2Y_{12}$ antagonism (figure 3). VASP-P is measured by whole blood flow cytometry, using permeabilization and a monoclonal antibody specific for the phosphorylated form of VASP.

The advantages of the VASP assay are that it is dependent on the target of clopidogrel ($P2Y_{12}$), and it involves low sample volume and whole blood assays. The disadvantages of the VASP assay are sample preparation and the requirement for a flow cytometer and an experienced technician.

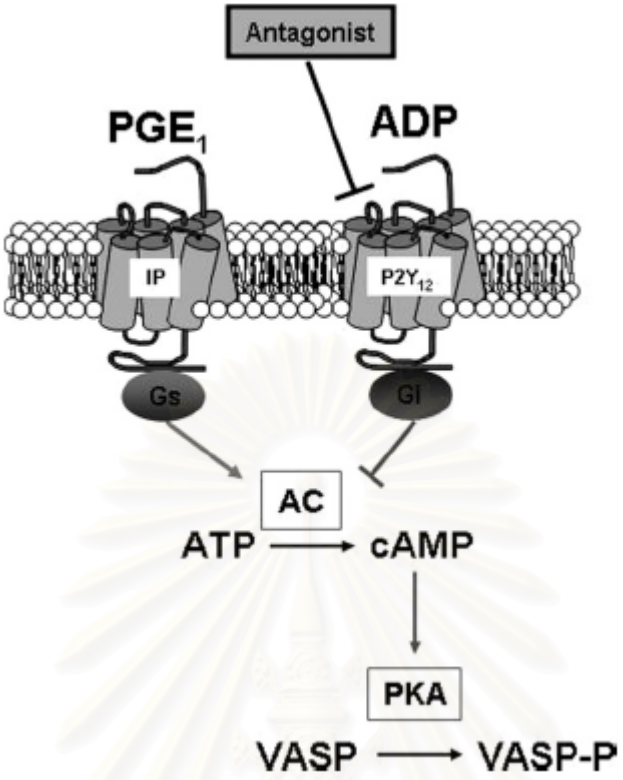

Figure 3: VASP assay for the measurement of $P2Y_{12}$ antagonism.

2.3.3 Platelet aggregation studies

In 2000, Moshfegh, et al.[25] reported a prospective study in 30 patients with CAD and a past history of MI. They found no effect of aspirin on ADP-induced platelet aggregation due to either got clopidogrel alone or in combination with aspirin markedly inhibited ADP-induced platelet aggregation compared with monotherapy with aspirin (24.6 $\pm$ 3.3% or 26.6 $\pm$ 2.7% Vs. 44.7 $\pm$ 2.9%; p , 0.001).

Another study of Farrell et al, 1999[26] revealed similar findings. Inhibition of platelet reactivity after the combination of aspirin 325 mg daily and ticlopidine 250 mg twice daily did not differ from ticlopidine alone when assessed by ADP 4 μM-induced platelet aggregation in 9 healthy subjects.

Geiger et al, 2005[27] had compared of VASP assay to ADP-only optical platelet aggregometry in 24 healthy volunteers treated 300 mg loading dose (LD) and 75 mg maintenance dose (MD) for 1 week. They found a correlation between VASP-P and ADP 5 μM-induced platelet aggregation value after 12 hour of treatment ($r = 0.87$, $P < 0.001$) and

showed a greater level of inhibition for the VASP assay than for conventional aggregometry after ingestion of clopidogrel, due to $P2Y_1$ for the ADP-only optical platelet aggregometry.

Hochholzer et al, 2007[28] conducted a study to compare the applicability of whole blood impedance aggregometry (20 μM ADP) and the point of care ULTEGRA assay with ADP-cartridges (20 μM) with optical aggregometry in PRP and determination of surface protein expression (P-Selectin and activated GP IIb/IIIa) by flow cytometry. They analyzed the correlation between the various assays revealed significant correlations only between optical aggregometry Vs p-selectin and optical aggregometry activated GP IIb/IIIa ($r = 0.515$, $r = 0.568$, $p < 0.001$, respectively).

2.4 Combination of aspirin and clopidogrel

2.4.1 Pharmacology of aspirin[16, 22]

Aspirin, (acetylsalicylic acid) is hydrolyzed more rapidly in alkaline conditions to the inactive salicylate. As it has a low pKa, it is absorbed in the stomach and appears in the blood within 10 minutes, with peak plasma concentrations seen at 30 to 40 minutes. However, when enteric coated aspirin is used the peak levels are reached between 3-4 h.

Aspirin is metabolized by esterases in blood and in the liver and has a half-life of 15 minutes. The major metabolite, salicylate has a half-life of 3 to 6 hours depending on the dose and, unlike aspirin, can be detected in plasma and urine long after the active drug has been eliminated. Despite the rapid clearance of aspirin from the circulation, the platelet-inhibitory effect lasts for the life span of platelets, which is approximately 5 to 6 days, after that the platelets function return to normal. Around 10% of platelets are replaced every day.

The target for aspirin is COX, of which there are two isoforms, COX-1 and COX-2. COX-1 is the only isoform in platelets, where it generates TXA_2. COX-1 is also expressed in vascular endothelium, where it generates prostacyclin (PGI_2), the major cyclooxygenase product of these cells. COX-2 is an inducible gene and is found at sites of inflammation and in many cancers. Aspirin is a nonselective COX inhibitor, inhibiting both isoforms. Yet, at low dose in humans, aspirin is relatively selective for platelet COX-1. The explanation lies in both the irreversible effect of aspirin on the enzyme and the slow turnover of COX-1 in platelets.

2.4.2 Pharmacology of clopidogrel[22]

Clopidogrel, thienopyridine derivative, is a prodrug and needs to be activated by hepatic cytochrome P450 isoenzymes (CYP3A4/5, CYP2C9, CYP2C19, CYP2B6 and CYP1A2) to form an active metabolite[6-8] (figure 4) that binds irreversibly and selectively to the $P2Y_{12}$ receptor via a disulfide bridge between the reactive thiol group and two cysteine residues (cys17 and cys270) presented in the extracellular domains of the $P2Y_{12}$ receptor.[29] Thus, the binding of ADP to the $P2Y_{12}$ receptor is permanently inhibited. Like aspirin, daily doses of clopidogrel have a cumulative and prolonged effect that is dissociated from the plasma half-life of the parent drug.

Clopidogrel is absorbed and metabolized relatively rapidly. The main circulating metabolite is the carboxylic acid derivative (SR26334) and it too has no effect on platelet aggregation. It represents about 85% of the circulating drug-related compounds in plasma and its half-life was 8 hours after single and repeated administration. The elimination of clopidogrel is 50% in the urine and approximately 46% in the feces. In addition, bioavailability is unaffected by food.

Ex vivo inhibition of platelet aggregation is dose- and time-dependent, and, in the absence of loading, a maximal effect (40% to 60% inhibition of ADP-induced aggregation ex vivo) occurs after 3 to 5 days. Platelet function recovers 3 to 5 days after drug withdrawal. With a LD of 300 mg clopidogrel, maximum inhibition of platelet aggregation occurs within 6 hours. However, full clinical benefit may not be achieved for 24 hours. Maximum antiplatelet response is attained approximately 2 hours after a LD of 600 mg clopidogrel, which is generally well tolerated and appears optimal.[30]

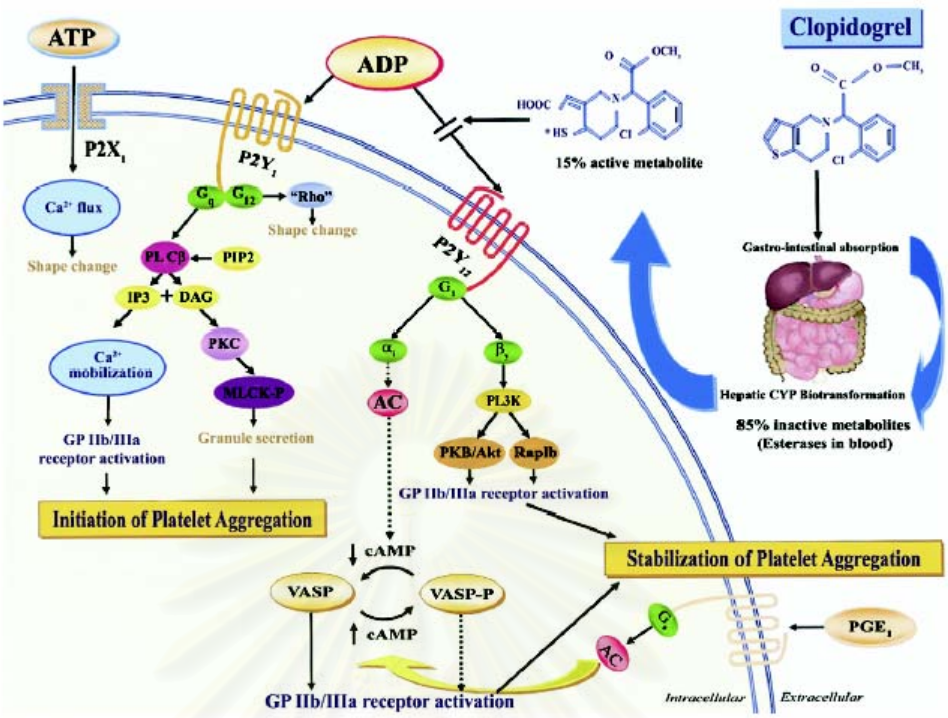

Figure 4: Mechanism of action of clopidogrel[31]

2.4.3 Aspirin and clopidogrel in cardiovascular disease

ACC/AHA 2007 guidelines for the management of patients with UA/NSTEMI[4] and ACC/AHA/SCAI 2005 guideline update for PCI[5] recommended using combination of aspirin and clopidogrel to reduce rates of cardiovascular ischemic events in acute coronary syndrome patients and in patients post PCI.

Patients undergo PCI neither take chronic aspirin nor clopidogrel should be given 300-325 mg of aspirin at least 2 hours (preferably 24 hours before the PCI procedure) and a LD of clopidogrel 300 mg should be administer before PCI at least 6 hours.[5] After PCI procedure, aspirin 325 mg/day and clopidogrel 75 mg/day should be given at least 1 month after bare-metal stent implantation, 3 months after sirolimus-eluting stent placement and 6 months after paclitaxel-eluting stent implantation (and ideally up to 12 months in patients who are not at high risk bleeding). After that chronic aspirin should be used continued indefinitely at a dose of 75-162 mg/day.[5]

For UA/NSTEMI patients who are treated with initial conservative strategy (not receiving coronary angiography and/or PCI), clopidogrel (LD followed by daily MD) should be added to aspirin and anticoagulant as soon as possible and continued for at least 1 month and ideally up to 1 year.[4]

2.4.4 Studies of combination of aspirin and clopidogrel

Use of clopidogrel in the primary prevention of atherothrombotic diseases has not been specifically studied. The CHARISMA study comprised two patient populations: those with documented atherothrombotic disease and those at high risk due to multiple risk factors. The latter group received antiplatelet medication for primary prevention of atherothrombotic events. Use of antiplatelet agents such as aspirin is beneficial in primary prevention in patients with multiple risk factors. Thus, the combination of clopidogrel and aspirin was expected to have increased beneficial effects in comparison with aspirin alone. However, in the CHARISMA study in patients with multiple risk factors the combination treatment compared to aspirin alone did not decrease the risk of adverse vascular events. In addition, in the primary prevention group the overall risk for death was increased significantly with the combination treatment compared to aspirin (5.4% vs. 3.8%)[32]

In the CHARISMA study (2006)[32] some of the patients had documented atherothrombotic disease with either cardiovascular, cerebrovascular manifestations or symptomatic peripheral arterial disease. In these patients the combination of aspirin and clopidogrel had similar efficacy and safety as aspirin treatment alone.

In the PCI-CURE trial[33] of patients with ACS 10-day pre-treatment with clopidogrel prior to PCI was associated with 30% risk reduction of the combined endpoint of cardiovascular death, MI and urgent revascularization (4.5% vs. 6.4% in clopidogrel vs. placebo in addition to aspirin groups).In addition, the combination of clopidogrel and aspirin was found to be superior in reducing cardiovascular morbidity and mortality during an 8 month treatment when compared to short-term treatment (RR 17%).

The CURE trial (2001)[34] compared the efficacy and safety of the combination of clopidogrel and aspirin to aspirin alone in patients with NSTEMI. The risk of atherothrombotic events in patients with dual antiplatelet medication was reduced by 20%

when compared with aspirin alone (9.3% vs. 11.4 %). The risk reduction rate for MI was most evident. In subgroup analyses it was found that patients with previous PCI had the most significant (40%) risk reduction.

2.5 Clopidogrel nonresponder[35]

Clopidogrel nonresponder has been used as one of the terms employed in the literature to describe different degrees of *ex vivo* low platelet inhibition after clopidogrel administration. In addition to the diverse nomenclature, the characterization of clopidogrel nonresponder has also been problematic because different authors have given different definitions. The mechanisms responsible for this decreased platelet response are not yet clearly defined, some hypotheses have been put forward but not yet demonstrated.

Although there have been no large prospective studies demonstrating that the degree of platelet inhibition is directly related to clinical outcomes, many studies and reports have shown an association between less platelet inhibition and more adverse events after PCI with clopidogrel therapy suggesting that clopidogrel nonresponder may be a marker for increased risk of recurrent cardiovascular events. Larger scale investigations are needed to support these findings.

2.5.1 Definition and prevalence of clopidogrel nonresponder

In this study the term of nonresponse to clopidogrel has been used to describe the inadequate of clopidogrel to inhibition *in vitro* with 20 μM ADP, MPA is > 50%. In the literature nonresponse and poor response to clopidogrel have been used as synonyms of clopidogrel resistance. It has been proposed that the term *clopidogrel resistance* would be used to describe the inability of clopidogrel to cause the expected platelet inhibition in laboratory measurements and the term *treatment failure* to describe failure of clopidogrel to prevent adverse clinical events.[36]

Clopidogrel non-response has been studied by several different methods. Nevertheless no uniform method has been established to determine nonresponse to clopidogrel. Platelet aggregation induced by ADP has been used widely, however its limitations are that it is labor-consuming, no uniform cut-off value has been established and

the time chosen to measure platelet aggregation, agent used to anticoagulant the blood samples as well as the concentration of the agonist used cause variation in results.

When defining clopidogrel resistance the cut-off levels of inhibition of ADP-induced platelets have different; namely: absolute difference in maximal platelet aggregation (ΔMPA) < 10%, inhibition of maximal platelet aggregation (IPA) <10%; [1 – ($MPA_{treatment}$ / $MPA_{baseline}$)] x 100 and ADP-induced MPA > 50%, MPA > 70%. In addition, clopidogrel resistance has been defined not only by inhibition of ADP-induced aggregation, but also by VASP assay.

Different studies using variable methods, definition, dosing and concentration of agonist and dosing report the prevalence of clopidogrel nonresponder to vary between 4 - 62.5% can be concluding in table 2.

Table 2: Clopidogrel response variability

Study	N	Patients	Clopidogrel LD/MD	Aspirin dose	Definition of clopidogrel nonresponder	Time	Prevalence	Clinical endpoints
Buonamici et al.[37]	804	PCI	600	325	10 μM ADP-induced aggregation, > 70% post treatment aggregation	12-18 h	13%	Incidence of stent thrombosis was 8.6% in nonresponder and 2.3% in responder
Muller et al.[38]	105	PCI	600	100	5, 20 μM ADP-induced aggregation, IPA < 10%	4 h	5-11%	-
Gurbel et al.[39]	92	PCI	300/75	325	5 μM ADP-induced aggregation, ΔMPA < 10%	24 h	31-35%	-
Mobley et al.[40]	50	PCI	300/75	Not known	1 μM ADP-induced aggregation, TEG, Plateletwork®, IPA < 10%	-	30%	-
Matetzky et al.[41]	60	PCI	300/75	200	5 μM ADP-induced aggregation, CPA, 4th quartile inhibition	5 d	25	-
Gurbel et al.[42]	190	PCI	300, 600/75	81-325	5, 20 μM ADP-induced aggregation, ΔMPA < 10%	24 h	28-32%, 8%	A 600 mg clopidogrel LD reduces the incidence of nonresponder and high platelet activity compared to a 300 mg

Table 2: Clopidogrel response variability (continue)

Study	N	Patients	Clopidogrel LD/MD	Aspirin dose	Definition of clopidogrel nonresponder	Time	Prevalence	Clinical endpoints
Geisler et al.[43]	379	PCI	600	100	20 μM ADP-induced aggregation, IPA < 30%	6 h	6%	Low responder to clopidogrel enhanced significantly of CV events and death as compared to responder of clopidogrel (22.7% Vs 5.6%, OR = 4.9, 95% CI = 1.66-14.96, p=0.004
Cuisset et al.[44]	190	PCI	600	Not known	10 μM ADP-induced aggregation, > 70% post treatment aggregation	12 h	22%	Periprocedural MI occurred significantly more frequently in patient with high post treatment platelet reactivity than in normo-responder (43% Vs 24%, p = 0.014)
Bliden et al.[45]	100	PCI	- / 75	325	5 μM ADP-induced aggregation, > 50% post treatment aggregation	-	22%	Patients with in recurrent ischemic events within 1 year displayed 70% in high platelet reactivity and 30% in normal platelet activity

Table 2: Clopidogrel response variability (continue)

Study	N	Patients	Clopidogrel LD/MD	Aspirin dose	Definition of clopidogrel nonresponder	Time	Prevalence	Clinical endpoints
Angiolillo et al.[46]	50	PCI	300, 600/ 75	250	6 μM ADP-induced aggregation, IPA < 10%	24 h	11%, 4%	-
Erlinge et al.[47]	110	CAD	600/75	Not known	5 μM ADP-induced aggregation, ΔMPA < 10%, MPA > 50%, VASP assay; PRI > 50%	24 h, 30±3 d	8, 14, 31% 11, 15,24%	-
Angiolillo et al.[48]	64	CAD& DM	- / 75	81	20 μM ADP-induced aggregation, MPA > 50%,	2-4 h	62.5	-
Cuisset et al.[49]	292	PCI	300, 600/75	160	10 μM ADP-induced aggregation, MPA > 70%,	12 h	25%, 15%	Recurrent ischemic events occurred more frequently in the 300 mg (12%) group than in the 600 mg (5%) group, p = 0.02

Table 2: Clopidogrel response variability (continue)

Study	N	Patients	Clopidogrel LD/MD	Aspirin dose	Definition of clopidogrel nonresponder	Time	Prevalence	Clinical endpoints
Price et al.[50]	380	PCI	600, 75	Not known	VerifyNow®; PRU > 235 post treatment reactivity	12 h	32	Patients with post-treatment reactivity greater than the cut-off value had significantly higher rates of CV death (2.8 Vs. 0%, P = 0.04), stent thrombosis (4.6Vs0%,P=0.004) and combined endpoint (6.5 Vs. 1.0%, P = 0.008)

ADP = adenosine diphosphate; CAD = coronary artery disease; CPA = cone and platelet analyzer; CV = cardiovascular; DM = diabetes mellitus; IPA = inhibition of platelet aggregation; LD = loading dose; MD = maintenance dose; MI = myocardial infarction; MPA = maximal platelet aggregation; PCI = percutaneous coronary intervention; TEG = thrombelastography

2.5.2 Reason for variability in clopidogrel efficacy

Several mechanisms have been suggested for non-response to clopidogrel (figure 5).

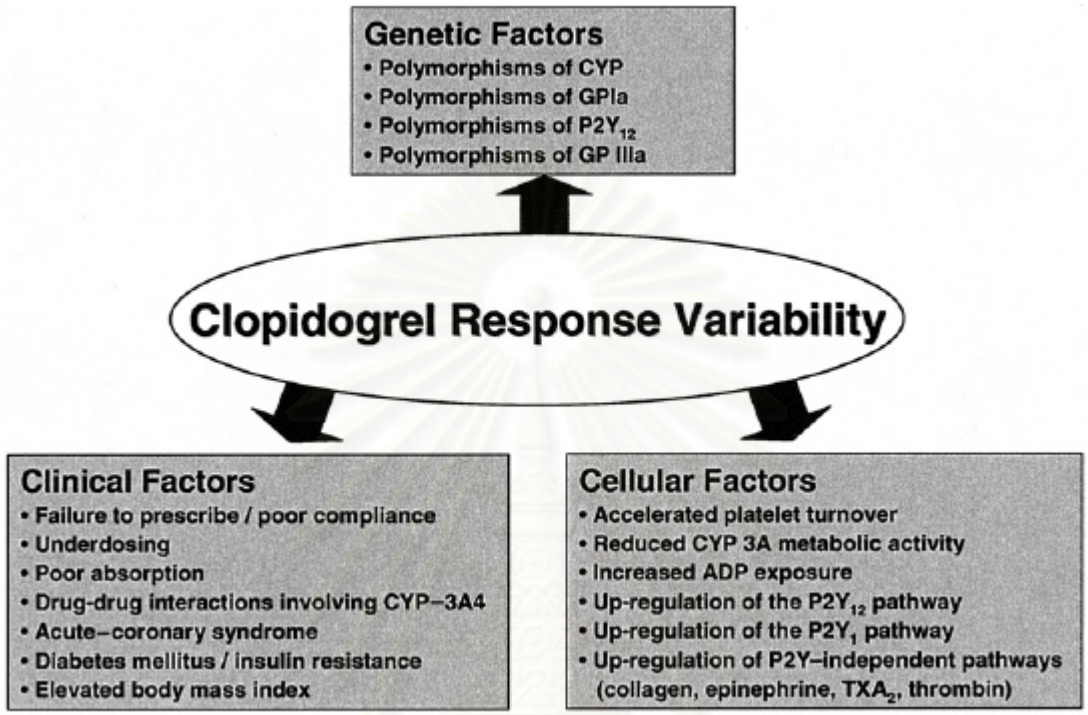

Figure 5: Clopidogrel response variability.[51]

ADP=adenosine diphosphate; CYP=cytochrome; GP=glycoprotein; TXA_2 = thromboxane A_2.

Clinical mechanisms of nonresponse are caused mostly by inefficient availability of the active metabolite. This could be caused by poor patient compliance that an important factor in resistance to any drug. Drug-drug interactions, including lipophilic statins and omeprazole, may also interfere with the pharmacodynamic effects of clopidogrel. However, to date, there is no evidence that this drug – drug interaction has any clinical impact. Other factors shown controversial to decrease the effect of clopidogrel are variability in intestinal absorption of clopidogrel.

Severity of atherothrombotic disease correlates with the clopidogrel efficacy. Patients with high treatment platelet reactivity had increased recurrent ischemic events, as described earlier. Also, diabetes mellitus (DM) and high body mass index (BMI) have been suggested to modify the clopidogrel efficacy.

Among the genetic factors, various polymorphisms have been studied. Among these, genetic polymorphisms of CYP enzymes, which are implied in generating the active metabolite of clopidogrel, appear to play a more important role than downstream targets, such as $P2Y_{12}$ receptors (target of clopidogrel).

Cellular factors may also play a role in clopidogrel response variability. These include more rapid platelet turnover, increased platelet exposure to ADP, reduced CYP activity, upregulation of purinergic signaling ($P2Y_1$ and $P2Y_{12}$), and the upregulation of nonpurinergic pathways.

2.5.2.1 Clopidogrel dosing

Higher LD of clopidogrel 600 and 900 mg have been studied. The LD most commonly compared with 300 mg has been 600 mg. The higher loading has increased efficacy in inhibiting platelet aggregation.[42, 46, 49] and had decrease the number of clopidogrel nonresponder.[46]

A 900 mg LD has not been shown to be superior to 600 mg, since limitations in clopidogrel absorption seem to block further appearance of the active metabolite in blood.[30]

A few studies comparing different clopidogrel maintenance regimens in patients with atherothrombotic disease, Angiolillo et al (2007)[48] studied the efficacy of increase maintenance dosing in a group of patients (N=20) received 75 mg daily MD and another group of patients (N=20) received 150 mg daily. The platelet aggregation induced by 20 μM ADP was measured. Significant enhanced clopidogrel-induced antiplatelet effect of 150 mg MD compared with 75 mg in high risk typed 2 DM.

2.5.2.2 Cytochrome P450 and drug-drug interactions

Clopidogrel is an inactive prodrug which requires *in vivo* conversion by liver enzymes to an active metabolite. The cytochrome P450 (CYP2C19) is responsible for the majority of clopidogrel metabolism but also CYP2B6, CYP3A4, CYP3A5, CYP2C19, CYP2C9 and CYP1A2 have been suggested to take part in converting clopidogrel to its active form.[6-8]

Lipid lowering agents

Many studies about lipophilic statins, such as atorvastatin and simvastatin which require CYP3A4 metabolization, were interfered clopidogrel-induced antiplatelet effects. However, these data are quite controversial as larger studies have shown the lack of any interaction between lipophilic statins and clopidogrel.[52, 53] In addition, most studies do not show any negative clinical interaction with coadministration of these drugs.[54]

Erythromycin[55]

The study compared platelet aggregation with the metabolic activity of CYP3A4 using the erythromycin breath test to measure CYP 3A4 activity among the healthy volunteers, and demonstrated a significant inverse correlation between CYP3A4 activity levels and platelet aggregation values after clopidogrel; the lower the CYP3A4 activity, the less clopidogrel was activated.

Rifampicin[55]

Furthermore, the inhibition of platelet aggregation after clopidogrel was found to be enhanced by rifampicin, a CYP3A4 inducer, suggesting that agents that induce the expression of CYP3A4 metabolic activity can decrease the incidence of clopidogrel resistance.[55]

Smoking[56]

Current smokers on chronic clopidogrel therapy have been shown significantly lower platelet activity compared with non-smoker within 1 year in patients undergoing elective PCI when assessed by 5, 20 μM ADP-induced platelet aggregation as $32 \pm 12\%$ Vs. $44 \pm 13\%$, $p < 0.0001$; $43 \pm 14\%$ Vs. $52 \pm 17\%$, $p < 0.008$, respectively. It has been suggested this phenomenon can be explain by activated several hepatic cytochrome P450 isoenzymes including CYP1A2. Cigarette smoking induces CYP1A2, therefore, may enhance the conversion of clopidogrel to its active metabolite.

2.5.2.3 Genetic polymorphism

Polymorphisms of both the $P2Y_{12}$ receptor and cytochrome P450 system have been proposed to explain variable clopidogrel efficacy. Three of more abundant CYP450 isoenzymes in the liver, CYP3A4, CYP3A5 and CYP2C19, appear to metabolize clopidogrel most rapidly and are therefore credited with its transformation to the active

metabolite. In principle, relative substrate concentration and binding site affinity determine competitive inhibition.

Hulot et al (2006)[7] showed the *CYP2C19* genotype is a major determinant of the pharmacodynamic response to clopidogrel in healthy volunteers and found the *CYP2C19*2* loss-of-function allele is associated with a marked decrease in platelet responsiveness to clopidogrel in young healthy male volunteers and may therefore be an important genetic contributor to clopidogrel resistance in the clinical setting but none of effect in *CYP2B6*, *CYP3A5* and *CYP1A2*.

Another study, Giusti et al (2007)[57], confirm that finding was *CYP2C19*2* but not *CYP3A4 IVS10 + 12G/A* and *P2Y12 T744C* polymorphisms, is associated with higher platelet activity treatment in 1,416 acute coronary syndrome patients.

In addition, in Lev et al (2007)[58] did not find an association between polymorphisms in the platelet receptors GP IIIa, $P2Y_{12}$ or $P2Y_1$ and response to aspirin or clopidogrel in patients undergoing PCI.

2.5.2.4 Diabetes mellitus

Angiolillo et al (2005)[59] have shown that patients with DM have a higher number of clopidogrel nonresponders and a reduced sensitivity to clopidogrel, and that high platelet reactivity in diabetic patients (MPA > 50%) on dual antiplatelet therapy is associated with a higher risk of long-term adverse cardiovascular events.

Similar result with Erlinge et al (2008)[47] in diabetes patients were over-represented in the poor-responder and had significantly lower levels of active metabolite. The reason that diabetic patients have lower levels of active metabolite is unclear. However, diabetic patients can be increased activity of esterase, which would convert more of the clopidogrel prodrug into inactive metabolite. Another possibility is that reduced gastric motility in diabetic patients could lead to slower absorption of the prodrug or that alterations at the megakaryocyte level changes platelet turnover and receptor expression.[47] Finally, explained by insulin reduces platelet aggregation by inhibition the $P2Y_{12}$ pathway, therefore, type2 DM patients have decreased sensitivity to insulin leading to lower cAMP levels and reduced $P2Y_{12}$ inhibition, which overall leads to increased platelet reactivity.[59]

The management of those were increasing MD of clopidogrel to 150 mg associated with enhanced antiplatelet effects compared with 75 mg in high risk type2 DM patients.[48]

2.5.2.5 Body mass index (BMI)

In patients with high BMI (BMI > 25 kg/m^2) was a trend to be higher platelet aggregation.[60]

2.6 Proton pump inhibitors and clopidogrel

2.6.1 Proton pump inhibitors[9, 61]

The proton pump inhibitors, which are specific for $H^+.K^+$-ATPase, inhibit the function of the proton pump responsible for the terminal step in gastric acid secretion. PPIs are considered to be the most effective medical treatment for the management of patients with acid-related disease (e.g. peptic ulcer, GERD, Zollinger-Ellison syndrome). Comparative studies have demonstrated that PPIs provide superior acid suppression, pain relief and peptic ulcer healing compared with histamine type 2 (H_2)-receptor antagonists.

The substituted benzimidazole PPIs consisted of rabeprazole, omeprazole, esomeprazole, lansoprazole and pantoprazole underwent an extensive hepatic biotransformation. They are metabolized to varying degree by several CYP isoenzymes especially CYP2C19 and CYP3A4 (figure 6). Of these two, mutations in *CYP2C19* affect its activity in the liver. Moreover, PPIs have a relatively short plasma half-life of the order of one hour and this is due to rapid hepatic metabolism to inactive metabolite. However, despite this, they can inhibit acid secretion for 24 hours or longer and due to the irreversible denaturation of the proton pump.

The major metabolic of omeprazole is the formation of 5-hydroxyomeprazole by CYP2C19 and also metabolized by CYP3A4 to omeprazole sulfone (figure 6). The affinity of omeprazole for CYP3A4 is approximately 10 folds less than CYP2C19, therefore, omeprazole has a greater potential to interact and interfere with the metabolism of substrates for CYP2C19 than with that of the substrates of CYP3A4.

The metabolism of rabeprazole, the newest benzimidazole, is metabolized mainly via a nonenzymatic reduction to a thioether compound with minor CYP2C19 and CYP3A4 involvement in formation of rabeprazole sulfone (figure 6). However, the contribution of CYP2C19 to the overall metabolism of rabeprazole is much less compared with that of the other PPIs and approximately half that of omeprazole. Thus, CYP2C19 makes a major contribution to the pharmacokinetics parameters of all PPIs but not rabeprazole. Moreover, rabeprazole is shown to be the least affected by CYP2C19 function due to genetic polymorphism.

Both rabeprazole and omeprazole have a similarly low activity in inhibiting the metabolism of CYP3A4 or CYP2D6.

The most frequent adverse reactions to these drugs are episodes of diarrhea, nausea, abdominal pain, dizziness, headache, and skin rashes.

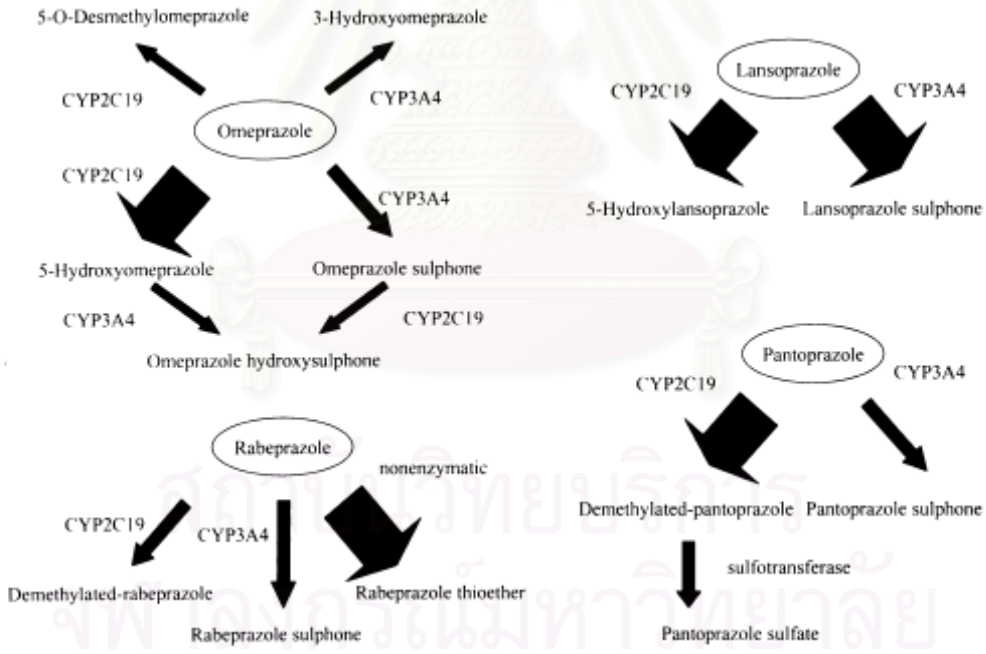

Figure 6: Metabolic pathways of omeprazole, lansoprazole, pantoprazole, rabeprazole and their cytochrome P450 isoforms. The thickness of arrows indicates an approximate contribution of CYP isoforms to each of the metabolic pathways and thin arrows a less dominant biotransformation pathway mediated via each CYP isoform.[9]

Rabeprazole has demonstrated a greater efficacy than H_2-receptor antagonists in the treatment of duodenal ulcer and GERD and has been as effective as omeprazole in these acid-related diseases.[11, 12] In addition, from WHO, showed equivalent efficacy between have omeprazole 20 mg/day and rabeprazole 20 mg/day in the treatment of GERD and gastric ulcer.[13]

2.6.2 Drug interaction between PPIs and clopidogrel

Several studies alerted the scientific community that concomitant treatment with clopidogrel and PPIs may have an effect to response of antiplatelet effect of clopidogrel but some was not.

Study of Siller-Mtula et al (2009)[62] was conducted to investigate the platelet inhibition when concomitant clopidogrel with pantoprazole, esomeprazole and without PPIs in 300 patients with CAD undergoing PCI. There was no difference between platelet reactivity index (PRI assessed by the VASP assay) and ADP-induced platelet aggregation (assessed by impedance aggregometry; Multiple platelet function analyzer) in patients taking clopidogrel with pantoprazole (n = 152; PRI = 50%; aggregation = 47 U), esomeprazole (n = 74; PRI = 54%; aggregation = 42 U), or without PPI (n = 74; PRI = 49%; aggregation = 41 U; P = .382). This may be concluded the intake of pantoprazole or esomeprazole is not associated with impaired response to clopidogrel.

Another study in 124 patients undergoing stenting, Gilard et al (2008),[10] showed omeprazole significantly decreased clopidogrel inhibitory effect on platelet $P2Y_{12}$ as assessed by VASP assay compared without omeprazole. PRI decrease was -43.3% in the placebo group and -32.6% in the omeprazole group after 7 days of treatment ($p < 0.0001$).

Small et al (2008)[63] confirmed the decreasing of antiplatelet effect when receiving clopidogrel with lansoprazole in healthy population. Lansoprazole decreased inhibition of platelet aggregation by turbidimetric aggregometry 4-24 hour after treatment from 49% to 39%, but did not affect IPA after the prasugrel dose.

In addition, Ho PM et al (2009),[64] assessed outcomes of patients taking clopidogrel with or without a PPIs after hospitalization for ACS. Of 8,205 patients taking clopidogrel after discharge, 63.9% (n=5,244) were prescribed PPIs at discharge, during

follow-up, or both and 36.1% (n=2,961) were not prescribed PPI. Death or rehospitalization for ACS occurred in 20.8% (n=615) of patients taking clopidogrel without PPI and 29.8% (n=1561) of patients taking clopidogrel plus PPIs (adjusted OR 1.25, 95%CI 1.11-1.41). In analyses of secondary outcomes, patients taking clopidogrel plus PPIs had a higher risk of hospitalizations for recurrent ACS compared with patients taking clopidogrel without PPI (14.6% Vs 6.9%; adjusted OR, 1.86 [95% CI, 1.57-2.20]) and revascularization procedures (15.5% Vs 11.9%; Adjusted OR, 1.49 [95% CI, 1.30-1.71]), but not for all-cause mortality (19.9% Vs 16.6%; adjusted OR, 0.91 [95% CI, 0.80-1.05]). The association between use of clopidogrel plus PPIs and increased risk of adverse outcomes also was consistent using a nested case-control study design (adjusted OR, 1.32; 95% CI, 1.14-1.54).

CHAPTER III

RESEARCH METHODOLOGY

3.1 Patients

This prospective study was conducted in adult CAD patients recruited from the outpatient and inpatient clinic at Cardiology Department, Phramongkutklao Hospital. Patients included were either stable CAD patients who had taken clopidogrel 75 mg/day for at least 5 days or patients scheduled for elective PCI who received LD of clopidogrel 300 mg with aspirin 300-325 mg before procedure. All patients received aspirin therapy with a daily dose of 81-325 mg at least 7 days prior to the study.

In patients scheduled for elective PCI, platelet function was assessed as a baseline 24 hour after receiving LD of clopidogrel 300 mg and aspirin 300-325 mg (after an overnight fasting) followed by clopidogrel 75 mg as a once daily MD. For stable CAD group the platelet function was measured before taking clopidogrel 75 mg/day (after an overnight fasting). After measured baseline platelet aggregation, the patients were randomized to 2 treatment groups: 20 mg/day omeprazole or 20 mg/day rabeprazole concomitant with clopidogrel 75 mg/day OD for at least 2 weeks prior to a sample blood at follow up as shown in figure 7.

Patients were reevaluated for platelet aggregation test at follow up after receiving omeprazole or rabeprazole for at least 2 weeks as. Major adverse coronary events defined as death due to cardiovascular or unknown causes, recurrent MI, rehospitalization for angina pectoris, rehospitalization for myocardial infarction, or need for target vessel revascularization, if any, were recorded. Patient compliance with antiplatelet treatment was assessed by interview.

The study was performed according to the Declaration of Helsinki and after approval by the Institutional Ethics Committee of the Phramongkutklao hospital. All patients were extensively informed and provided written consent before including into the study.

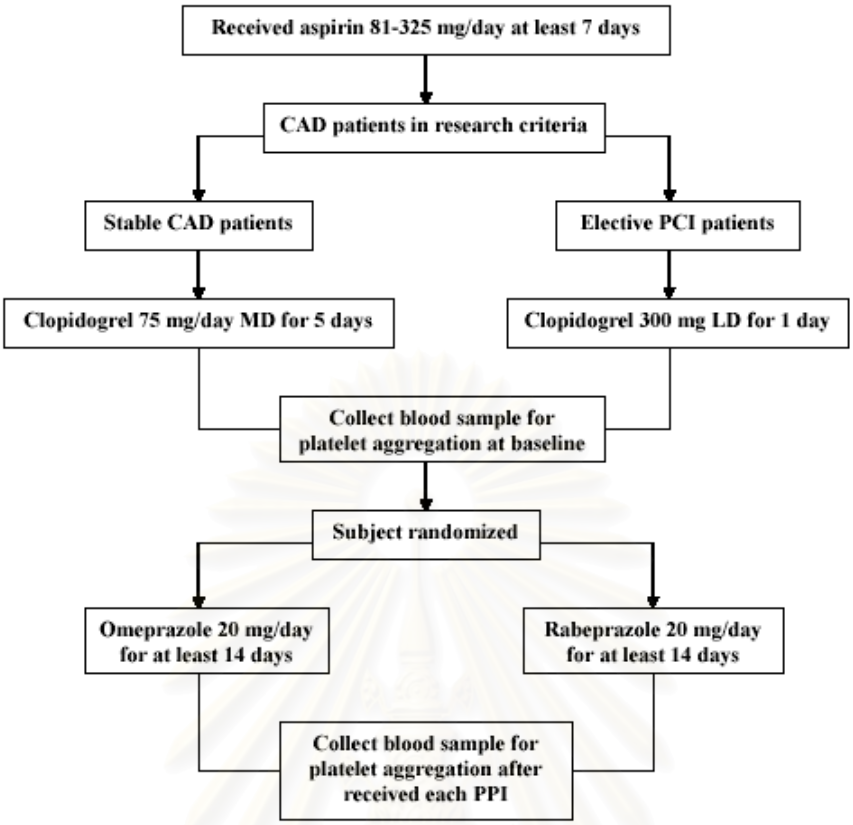

Figure 7: Flow chart of this study

Exclusion criteria included the following:

- Pregnancy
- Taking warfarin within the previous 1 month
- Using CYP2C19 inhibitors such as phenytoin, escitalopram within the previous 1 month
- Receiving proton pump inhibitors (PPIs), CYP3A4 inhibitors or inducers such as ketoconazole, erythromycin, rifampicin within the previous 2 weeks
- Using Glycoprotein (GP) IIb/IIIa and thrombolytic drugs before PCI procedure
- Use of nasogastric tube feeding
- High risk of bleeding
- AST/ALT > 3 fold of upper limit
- Platelet count < 100,000/mm^3
- Serum creatinine > 1.5 mg/dl
- Active gastrointestinal ulcer

3.1.1 Sample size calculation

As formula followed

$$n = \frac{(Z_\alpha + Z_\beta)^2 \, 2 \, S_P^2}{d^2}$$

$$S_P^2 = \frac{(n_1 - 1) S_1^2 + (n_2 - 1) S_2^2}{n_1 + n_2 - 2}$$

As Gilard et al (2008)[10] has shown the mean platelet reactivity index variation in the placebo and omeprazole groups were 43.3% (SD 15.9) and 32.6% (SD 16.4) respectively ($p < 0.0001$).

To assign α = 0.05 (two-sided); $Z_\alpha = 1.96$

β = 0.2 (one-sided); $Z_\beta = 0.84$

S_1 = SD of omeprazole group (n = 64) = 16.4

S_2 = SD of placebo group (n = 60) = 15.9

d = difference in platelet reactivity index between group = 10

$$S_P^2 = \frac{(64 - 1)(16.4)^2 + (60 - 1)(15.9)^2}{64 + 60 - 2}$$

$$S_P^2 = 261.15$$

$$n = \frac{(1.96 + 0.84)^2 \, 2 \, (261.15)}{100} = 41$$

Approximately of 10% drop out from the research

$$n = \frac{41}{(1 - 0.1)} = 45$$

Therefore, the estimated sample size was at least 45 patients for each group or the total was 90 patients.

3.2 Platelet aggregation analysis

Blood was collected in tubes containing 3.2% trisodium citrate for assessment of platelet aggregation. Aggregation induced by 20 μM ADP (Helena laboratory, beaumont, Texas) was assessed in PRP using the light transmission aggregometry method in a 4-channel aggregometer (Aggrecoder PA-3210 model, Kyoto Daichi, Kagaku Co., Ltd, Kyoto, Japan). PRP was obtained as a supernatant after centrifugation of citrated blood at 1000 rpm for 10 minutes. The isolated PRP was kept at room temperature before use. Platelet poor plasma was

obtained by a second centrifugation of the blood fraction at 3500 rpm for 10 minutes. The platelet count in PRP was adjusted to the range of 200,000–300,000/mm^3 by dilution with PPP when out of range. Light transmission was adjusted to 0% with PRP and to 100% for PPP in each measurement. Platelet aggregation was assessed within 4 hour from blood sampling. Curves were recorded for 10 minute and platelet aggregation was determined as the percent of maximal platelet aggregation in light transmittance from baseline using PPP as reference. Clopidogrel nonresponsiveness was defined as maximal platelet aggregation by ADP 20μM > 50 % (MPA > 50%).

3.3 Definition of clopidogrel nonresponder

Clopidogrel nonresponsiveness was defined as maximal platelet aggregation by induction with ADP 20μM > 50 % (MPA > 50%).

3.4 Statistical analysis

The Kolmogorov–Smirnov test was used to test for normality. Normally continuous variables are reported as mean ± standard deviation (SD). Variable, percent of increase maximal platelet aggregation, do not follow a normal distribution is represented as median and interquartile range. Categorical variables are presented as frequencies and percentages. Comparisons of gender, health behavior, groups of patients, clinical risk factors and co-medication between the 2 groups were done by Chi-square test or Fisher's exact test. The independent t-test and analysis of variance (ANOVA) were used to compare characteristics and clinical data between clopidogrel nonresponders and responders and to compare between patients who intake omeprazole and rabeprazole when these were normally distributed and the Mann-Whitney *U* test or Kruskal-Wallis test if not normally distributed. Pair t-test was used for evaluating changes in platelet aggregation within the groups before and after taking PPIs. The Scheffe post hoc comparison was used to compare increasing of platelet aggregation with diuretics medication. A univariate analysis of covariance (ANCOVA) was used to compare platelet aggregation and a change of platelet aggregation with clinical data between PPIs groups by used baseline platelet aggregation as a covariate factor. Correlations analyzes were performed according to the Pearson or the Spearman correlation coefficient if normally or not normally distribution, respectively. A p value < 0.05 was considered statistically significant. Statistical analysis was performed using a SPSS v 13.0.

CHAPTER IV

RESULTS

Results of the study are presented in five parts which are (1) Patient characteristics; (2) Baseline platelet aggregation; (3) Effect of PPIs on platelet aggregation; (4) Factors influencing platelet response to clopidogrel at baseline and after taking PPIs; and (5) Prevalence of clopidogrel nonresponder before and after taking PPIs.

4.1 Patient characteristics

Ninety consecutive patients were eligible and agreed to participate in this study during August 2008 to March 2009. Finally, the data of 87 patients were analyzed which 43 patients received omeprazole while 44 patients received rabeprazole and 3 patients were lost of follow up due to lack of blood samples. No serious adverse drug events had been found or recorded in this study.

Demographic and clinical data of CAD patients in omeprazole and rabeprazole group were shown in table 3. Mean age of the subjects in omeprazole group was 62.42 $\pm$ 10.63 years whereas in rabeprazole group was 63.50 $\pm$ 10.49 years, p = 0.634. There were 11 women (25.6%) and 32 men (74.4%) in omeprazole group, whereas in rabeprazole, 9 (20.5%) were women and 35 (79.5) were men. Patients in rabeprazole group had significantly higher BMI than in omeprazole group (p = 0.038). There were 18.4% and 81.6% in elective PCI group and stable CAD group, respectively. Co-morbidities, platelet count and creatinine clearance of both groups were comparable with no statistically significantly difference.

Baseline medications used were shown in table 4. Dose of aspirin varied between 81-325 mg. Use of beta blockers, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, lipid lowering agents, calcium channel blockers, nitrates, alpha-blockers and diuretics were similar in both groups.

Table 3: Demographic and clinical characteristics at baseline

	Overall (n = 87)	**Omeprazole (n = 43)**	**Rabeprazole (n = 44)**	**p Value**
Age (yrs)	62.97 ± 10.51	62.42 ± 10.63	63.50 ± 10.49	0.634 [b]
Male, n (%)	67 (77)	32 (74.4)	35 (79.5)	0.570 [a]
Body mass index (Kg/m^2)	25.13 ± 3.29	24.39 ± 3.52	25.86 ± 2.92	0.038 [b*]
Groups of patients with, n (%)				
Elective PCI	16 (18.4)	8 (18.6)	8 (18.2)	0.959 [a]
Stable CAD	71 (81.6)	35 (81.4)	36 (81.8)	
Current Smoking, n (%)	2 (2.3)	2 (4.7)	0 (0)	0.241 [a]
Family history, n (%)	6 (6.9)	5 (11.6)	1 (2.3)	0.110 [a]
Co-morbidities, n (%)				
Diabetes mellitus	37 (42.5)	19 (44.2)	18 (40.9)	0.757 [a]
Hypertension	72 (82.8)	36 (83.7)	36 (81.8)	0.814 [a]
Dyslipidemia	81 (93.1)	39 (90.7)	42 (95.5)	0.434 [a]
Single vessel disease	13 (14.9)	4 (9.3)	9 (20.5)	0.145 [a]
Double vessel disease	20 (23.0)	10 (23.3)	10 (22.7)	0.953 [a]
Triple vessel disease	18 (20.7)	9 (20.9)	9 (20.5)	0.956 [a]
Non-STEMI	9 (10.3)	6 (14.0)	3 (6.8)	0.314 [a]
STEMI	8 (9.2)	4 (9.3)	4 (9.1)	1.000 [a]
Creatinine clearance (ml/min)	69.01 ± 26.20	66.78 ± 23.88	71.20 ± 28.38	0.435 [b]
Platelet count (x10^3 / mm^3)	252.21 ± 67.49	254.60 ± 74.89	249.86 ± 60.17	0.745 [b]

PCI = percutaneous coronary intervention, CAD = coronary artery disease, PPIs = proton pump inhibitors, STEMI = ST-elevated myocardial infarction

[a] Chi-square or Fisher's exact test, [b] Independent t-test

* Significant; $p < 0.05$

Table 4: Co-medication at baseline

	Overall (n = 87)	Omeprazole (n = 43)	Rabeprazole (n = 44)	p Value
Aspirin				
81 mg/day	21 (24.1)	11 (25.6)	10 (22.7)	0.756 [a]
100-162 mg/day	6 (6.9)	4 (9.3)	2 (4.5)	0.434 [a]
300-325 mg/day	60 (69.0)	28 (65.1)	32 (72.7)	0.443 [a]
Beta-blockers	69 (79.3)	34 (79.1)	35 (79.5)	0.956 [a]
ACEIs	49 (56.3)	28 (65.1)	21 (47.7)	0.102 [a]
Angiotensin receptor blockers	17 (19.5)	6 (14.0)	11 (25.0)	0.194 [a]
Lipid lowering agents				
None	6 (6.9)	4 (9.3)	2 (4.5)	0.434 [a]
Simvastatin	25 (28.7)	12 (27.9)	13 (29.5)	0.866 [a]
Atorvastatin	37 (42.5)	15 (34.9)	22 (50.0)	0.154 [a]
Rosuvastatin	16 (18.4)	11 (25.6)	5 (11.4)	0.087 [a]
Pravastatin	3 (3.4)	1 (2.3)	2 (4.5)	1.000 [a]
Calcium channel blockers	24 (27.6)	10 (23.3)	14 (31.8)	0.372 [a]
Nitrates	58 (66.7)	28 (65.1)	30 (68.2)	0.762 [a]
α-blockers	8 (9.2)	3 (7.0)	5 (11.4)	0.713 [a]
Diuretics	31 (35.6)	12 (27.9)	19 (43.2)	0.628 [a]

ACEIs = angiotensin converting enzyme inhibitors

[a] Chi-square or Fisher's exact test

4.2 Baseline platelet aggregation

The mean MPA after ADP 20 μM stimuli at baseline was 38.53 $\pm$ 20.16%, which was highly variable and followed a normal bell-shaped distribution tested by one-sample Kolmogorov-Smirnov test; p = 0.485 (figure 8). Platelet activities were further categorized into two groups; elective PCI group and stable CAD group as shown in table 5. The duration of intake clopidogrel in stable CAD group was in range of 5 -1,823 days. The median duration of taking clopidogrel was 88 days. The correlation coefficient indicated that longer duration of intake clopidogrel in stable CAD patients was not significantly associated with higher

baseline platelet aggregation (r = 0.156, p = 0.194). The platelet activities at baseline were also not significantly different between the omeprazole and rabeprazole groups.

Table 5: Platelet activities at baseline

Platelet activity (mean ± SD)	Overall (n = 87)		Omeprazole (n = 43)		Rabeprazole(n=44)		p Value
	% MPA	n	% MPA	n	% MPA	n	
Elective PCI patients	38.24 ± 16.11	16	33.36 ± 18.10	8	43.13 ± 13.20	8	0.238 [b]
Stable CAD patients	38.59 ± 21.06	71	42.36 ± 18.84	35	34.93 ± 22.68	36	0.138 [b]
Overall	38.53 ± 20.16	87	40.68 ± 18.82	43	36.42 ± 21.39	44	0.326 [b]
p Value	0.951 [b]		0.227 [b]		0.332 [b]		

MPA = maximal platelet aggregation, PCI = percutaneous coronary intervention, CAD = coronary artery disease

[B] Independent t-test

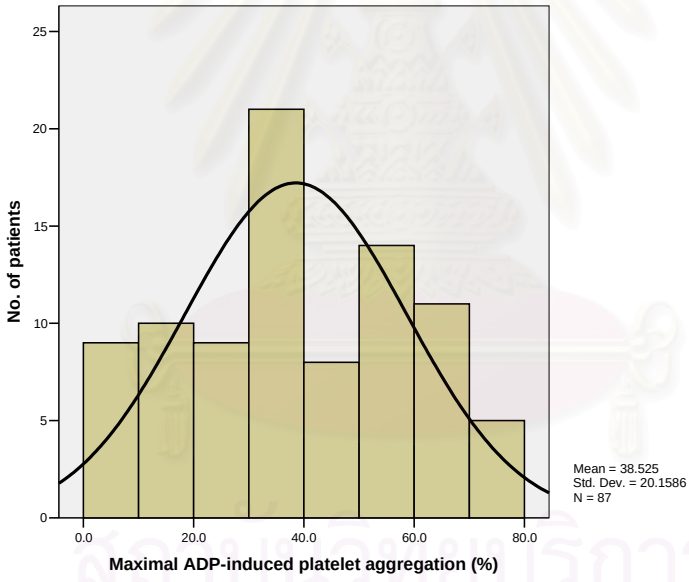

Figure 8: Interindividual distribution of ADP 20 μM-induced platelet aggregation at baseline in patients with elective PCI and stable CAD.

4.3 Effect of proton pump inhibitors on platelet aggregation

The mean MPA after ADP 20 μM stimuli after receiving PPIs of the total patients included was 52.05 ± 19.46% whereas divided into omeprazole group and rabeprazole group, the mean MPA were 55.73 ± 19.66% and 48.46 ± 18.80%, respectively as described in table 6

and depicted in figure 9. The MPA after receiving either omeprazole or rabeprazole was significantly increased from the MPA at baseline. Even though the increase in MPA seem to be higher in the omeprazole group when compared to the rabeprazole group, this difference showed no statistically significant at $p < 0.05$.

Table 6: Platelet aggregation after receiving PPIs

Platelet activity (mean ± SD)	**Overall (n =87)**	**Omeprazole (n = 43)**	**Rabeprazole (n = 44)**	**p Value**
MPA baseline	38.53 ± 20.16%	40.68 ± 18.82%	36.42 ± 21.39%	0.326 [b]
MPA after receiving PPIs	52.05 ± 19.46%	55.73 ± 19.66%	48.46 ± 18.80%	0.141 [d]
p Value	< 0.0001 [c**]	< 0.0001 [c**]	0.002 [c**]	
Δ MPA	13.52 ± 21.98%	15.04 ± 19.61%	12.04 ± 24.22%	0.141 [d]
Percent increased of MPA	20.0 [-5.93-111.76]	32.5 [1.16 -102.48]	14.0 [-9.66-171.36]	0.519 [e]

MPA = maximal platelet aggregation, PPIs = proton pump inhibitors

[b] Independent t-test, [c] Pair t-test, [d] ANCOVA test, [e] Mann-Whitney *U* test, [**] Significant; $p < 0.01$

Data of percent increased of MPA presented as median and interquartile range

Table 7 showed the platelet aggregation after receiving PPIs by further categorized into elective PCI and stable CAD patients. No statistically significant differences between the two groups were observed even though there was tendency for omeprazole to cause higher increase in platelet aggregation in the elective PCI patients as compared to the stable CAD patients. This same result could not be observed in the rabeprazole group.

Table 7: Comparison of platelet aggregation after receiving PPIs between elective PCI and stable CAD patients

Platelet activity (mean ± SD)	Overall (n =87)	Omeprazole (n = 43)	Rabeprazole (n = 44)	P Value
MPA baseline				
Elective PCI patients	38.24 ± 16.11%	33.36 ± 18.10%	43.13 ± 13.20%	0.238 [b]
Stable CAD patients	38.59 ± 21.06%	42.36 ± 18.84%	34.93 ± 22.68%	0.138 [b]
p Value	0.951 [b]	0.227 [b]	0.332 [b]	
MPA after receiving PPIs				
Elective PCI patients	55.01 ± 20.92%	61.06 ± 21.56%	48.96 ± 19.72%	0.246 [d]
Stable CAD patients	51.38 ± 19.22%	54.51 ± 19.33%	48.34 ± 18.87%	0.450 [d]
p Value	0.456 [d]	0.099 [d]	0.846 [d]	
Δ MPA				
Elective PCI patients	16.77 ± 26.22%	27.70 ± 28.17%	5.84 ± 20.15%	0.246 [d]
Stable CAD patients	12.79 ± 21.06%	12.15 ± 16.29%	13.42 ± 25.07%	0.450 [d]
p Value	0.456 [d]	0.099 [d]	0.846 [d]	
Percent increased of MPA				
Elective PCI patients	16.7 [-8.03-196.54]	118.3 [5.45-321.88]	-1.7 [-24.00-42.23]	0.074 [e]
Stable CAD patients	20.1 [-4.26-110.84]	29.50 [1.00-79.08]	17.2 [-9.43-202.76]	0.783 [e]
p Value	0.991 [e]	0.151 [e]	0.301 [e]	

MPA = maximal platelet aggregation, the frequency of each group equal in table 5

[b] Independent t-test, [d] ANCOVA test, [e] Mann-Whitney *U* test

Data of percent increased of MPA presented as median and interquartile range

Platelet activity quartile cut points for the 25th, 50th, and 75th percentiles of the study population were 23.00%, 36.90%, and 56.20% ($p < 0.0001$). Details of the result were shown in table 8.

Using pair-t test to compare platelet aggregation before and after taking PPIs when categorized into quartiles revealed that only quartile 1 and quartile 2 showed significantly increased; $p < 0.0001$, $p < 0.0001$, respectively. In the 3rd quartile, the MPA of patients receiving omeprazole only was significantly increased but not in patients receiving

rabeprazole. Percent increase of MPA in the 1st quartile was highest, there; the percent increases were lower in the 2nd, 3rd and 4th quartiles, respectively.

Table 8: Platelet activity before and after taking PPIs divided into quartiles

Quartiles	Timing	N	Platelet activity (%) mean ± SD	p Value
MPA ≤ 23.00	Baseline	22	12.56 ± 6.95	
	After taking PPIs	22	44.46 ± 22.45	
	Δ MPA	22	31.90 ± 19.82	< 0.0001 [c**]
	Omeprazole	8	32.61 ± 18.67	0.002 [c**]
	Rabeprazole	14	31.50 ± 21.12	< 0.0001 [c**]
	% increased of MPA	22	275.5 [150.11 - 355.43]	
23 < MPA ≤ 36.90	Baseline	22	31.52 ± 4.02	
	After taking PPIs	22	50.46 ± 20.61	
	Δ MPA	22	18.94 ± 21.11	< 0.0001 [c**]
	Omeprazole	12	18.88 ± 21.31	0.011 [c*]
	Rabeprazole	10	19.01 ± 22.02	0.023 [c*]
	% increased of MPA	22	68.5[1.94 -110.40]	
36.9 < MPA ≤ 56.20	Baseline	22	46.30 ± 6.90	
	After taking PPIs	22	51.62 ± 15.54	
	Δ MPA	22	5.33 ± 15.22	0.116 [c]
	Omeprazole	14	9.26 ± 15.75	0.047 [c*]
	Rabeprazole	8	-1.55 ± 12.25	0.731 [c]
	% increased of MPA	22	3.4 [-10.16 - 46.04]	
MPA > 56.20	Baseline	21	64.93 ± 5.97	
	After taking PPIs	21	62.11 ± 15.10	
	Δ MPA	21	-2.82 ± 14.01	0.367 [c]
	Omeprazole	9	3.30 ± 11.92	0.430 [c]
	Rabeprazole	12	-7.41 ± 14.14	0.097 [c]
	% decreased of MPA	21	-4.3 [-15.01 - 10.54]	

MPA = maximal platelet aggregation

[c] Pair t-test, [*] Significant; $p < 0.05$, [**] Significant; $p < 0.01$

Data of percent increased of MPA presented as median and interquartile range

Table 9: Comparison of platelet aggregation after taking omeprazole and rabeprazole categorized into quartiles

Quartiles	Timing	n	Platelet activity (%) mean ± SD	p Value
MPA ≤ 23.00				
Baseline	Omeprazole	8	13.30 ± 6.47	0.715 [b]
	Rabeprazole	14	12.14 ± 7.42	
After taking PPIs	Omeprazole	8	45.91 ± 24.18	0.969 [d]
	Rabeprazole	14	43.64 ± 22.31	
Δ MPA	Omeprazole	8	32.61 ± 18.67	0.969 [d]
	Rabeprazole	14	31.50 ± 21.12	
% increased of MPA	Omeprazole	8	256.2 [172.55-328.91]	0.733 [e]
	Rabeprazole	14	292.8 [95.62-507.98]	
23 < MPA ≤ 36.90				
Baseline	Omeprazole	12	31.33 ± 4.10	0.820 [b]
	Rabeprazole	10	31.74 ± 4.13	
After taking PPIs	Omeprazole	12	50.22 ± 21.63	0.950 [d]
	Rabeprazole	10	50.75 ± 20.46	
Δ MPA	Omeprazole	12	18.88 ± 21.31	0.950 [d]
	Rabeprazole	10	19.01 ± 22.02	
% increased of MPA	Omeprazole	12	54.8 [16.47- 102.52]	0.843 [e]
	Rabeprazole	10	91.2 [-7.27 - 111.07]	
36.9 <MPA ≤ 56.20				
Baseline	Omeprazole	14	48.30 ± 6.45	0.070 [b]
	Rabeprazole	8	42.79 ± 6.59	
After taking PPIs	Omeprazole	14	57.56 ± 14.36	0.034 [d*]
	Rabeprazole	8	41.24 ± 12.13	
Δ MPA	Omeprazole	14	9.26 ± 15.75	0.034 [d*]
	Rabeprazole	8	-1.55 ± 12.25	
% increased of MPA	Omeprazole	14	8.8 [-2.41 - 54.55]	0.133 [e]
	Rabeprazole	8	-7.3 [-30.33 -14.68]	

MPA = maximal platelet aggregation, PPIs = proton pump inhibitors

[b] Independent t-test, [d] ANCOVA test, [e] Mann-Whitney *U* test, [*]Significant; $p < 0.05$

Data of percent increased/decreased of MPA presented as median and interquartile range

Table 9: Comparison of platelet aggregation after taking omeprazole and rabeprazole categorized into quartiles (continue)

Quartiles	Timing	n	Platelet activity (%) mean ± SD	p Value
MPA > 56.20				
Baseline	Omeprazole	9	65.64 ± 7.04	0.646 [b]
	Rabeprazole	12	64.39 ± 5.29	
After taking PPIs	Omeprazole	9	68.94 ± 13.99	0.087 [d]
	Rabeprazole	12	56.98 ± 14.33	
Δ MPA	Omeprazole	9	3.30 ± 11.92	0.087 [d]
	Rabeprazole	12	-7.41 ± 14.14	
% decreased of MPA	Omeprazole	9	1.0 [-8.17 - 22.27]	0.136 [e]
	Rabeprazole	12	-7.0 [-24.55 -1.07]	

MPA = maximal platelet aggregation, PPIs = proton pump inhibitors

[b] Independent t-test, [d] ANCOVA test, [e] Mann-Whitney *U* test, [*]Significant; $p < 0.05$

Data of percent increased/decreased of MPA presented as median and interquartile range

From table 9, there were no significant differences in platelet aggregation either before or after taking PPIs between omeprazole and rabeprazole groups in the 1st, the 2nd and the 4th quartiles of platelet aggregation. However, in the 3rd quartile, omeprazole showed significantly higher effect on platelet aggregation as compared to rabeprazole.

The maximal platelet aggregation after receiving PPIs showed highly variable and followed a normal bell-shaped using one-sample Kolmogorov-Smirnov test as depicted in figure 9, 10, 11 for total patients, patients who were taking omeprazole only and patients who were taking rabeprazole only; (p = 0.835, 0.887, 0.533), respectively. In addition, figure 12 show maximal platelet aggregation after receiving PPI when categorized based on different PPI received and different groups of patients either elective PCI or stable CAD. Also, change of platelet aggregation after taking each PPI demonstrated normal distribution for total patients, patients who were taking omeprazole only and patients who were taking rabeprazole only; p = 0.224, 0.728, 0.309, respectively (figure 13-14); while percent increased of platelet aggregation for total patients and when divided into omeprazole and rabeprazole groups were not normally distribution (p = 0.0001, 0.031, 0.0001, respectively) as depicted in figure 15-16.

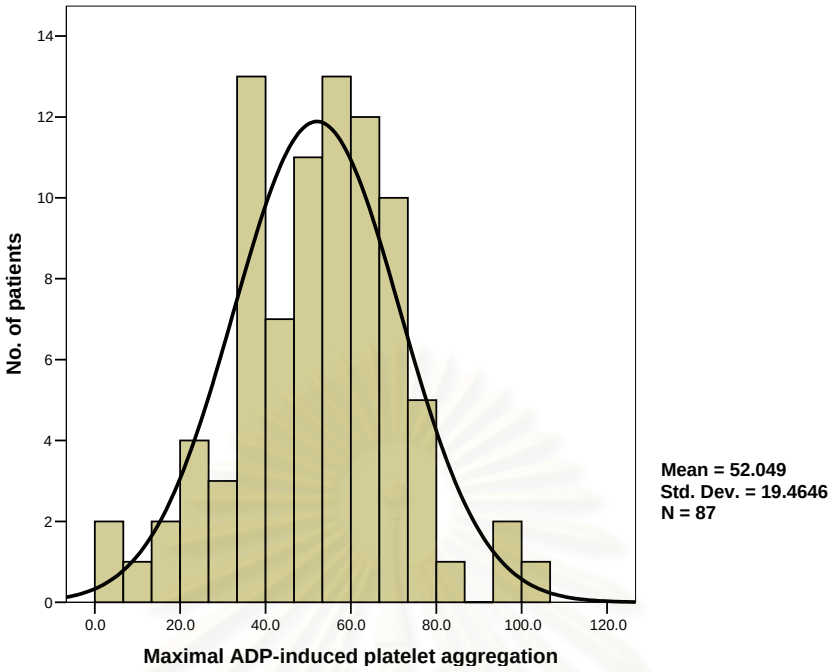

Figure 9: Interindividual distribution of ADP 20 μM-induced platelet aggregation after receiving PPIs in ***all patients***.

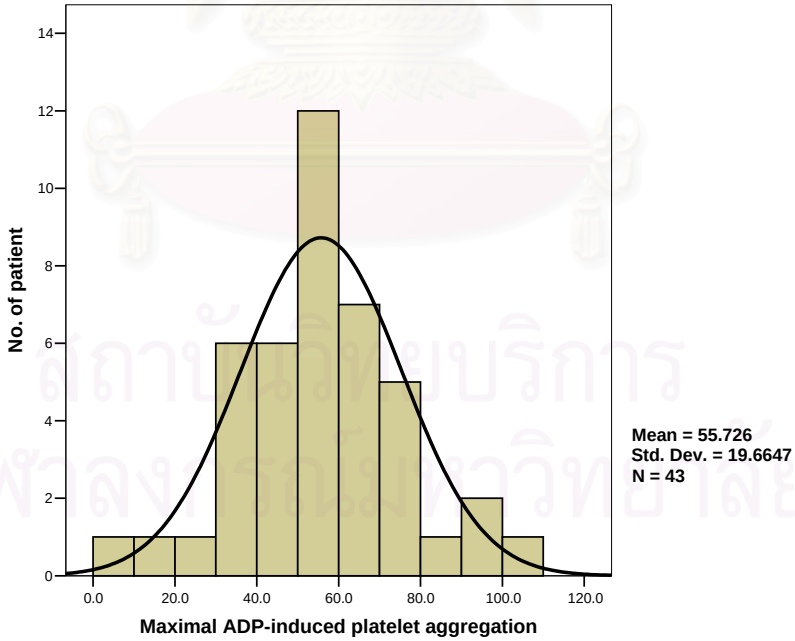

Figure 10: Interindividual distribution of ADP 20 μM-induced platelet aggregation after receiving ***omeprazole*.**

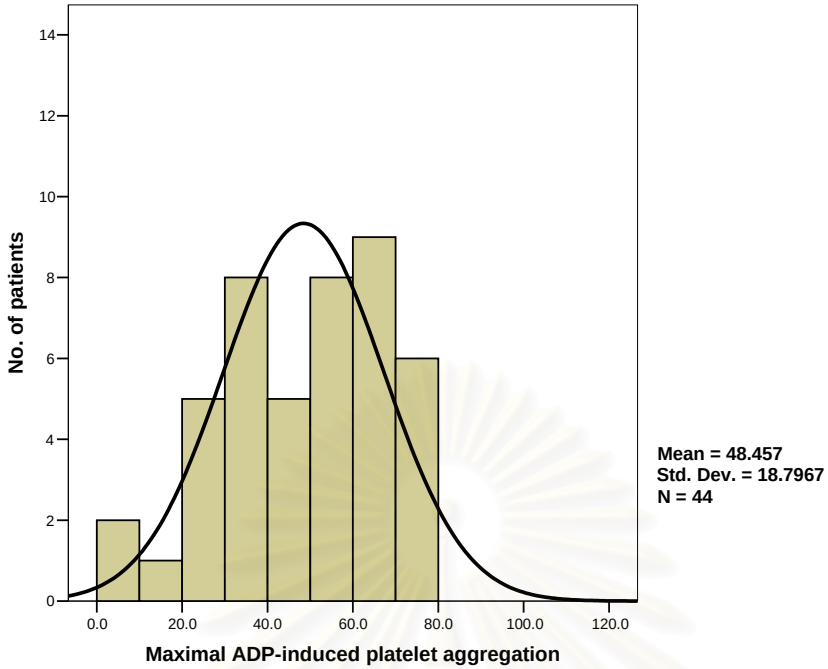

Figure 11: Interindividual distribution of ADP 20 μM-induced platelet aggregation after receiving ***rabeprazole***.

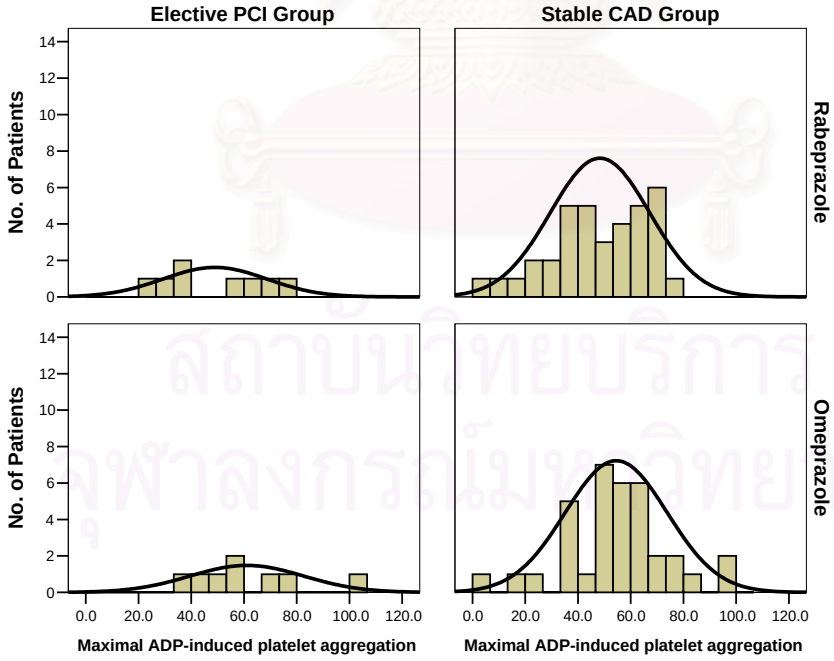

Figure 12: Interindividual distribution of ADP 20 μM-induced platelet aggregation after receiving PPIs when categorized based on different PPI received and different patients groups either elective PCI or stable CAD.

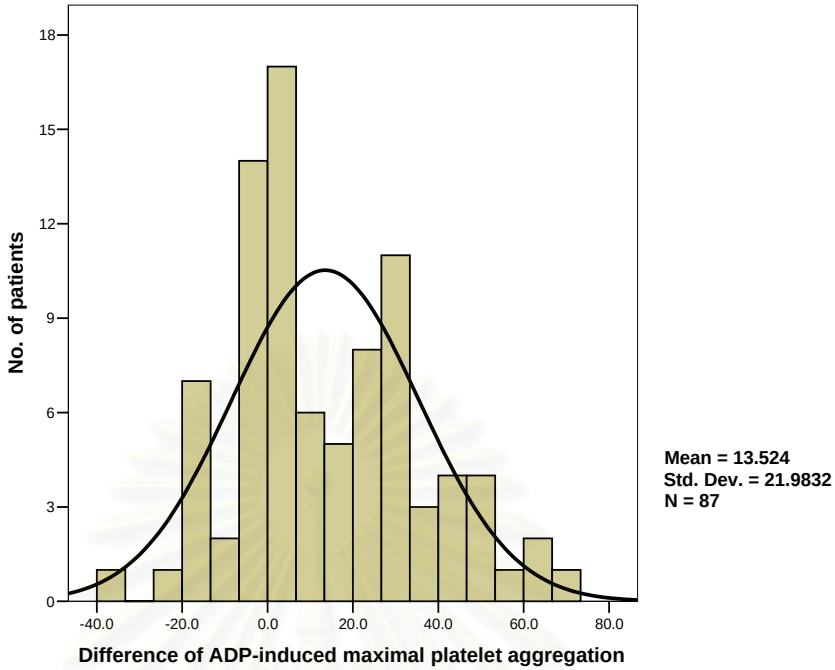

Figure 13: Interindividual distribution of ***change in ADP20 μM-induced platelet aggregation*** after receiving PPIs in all patients.

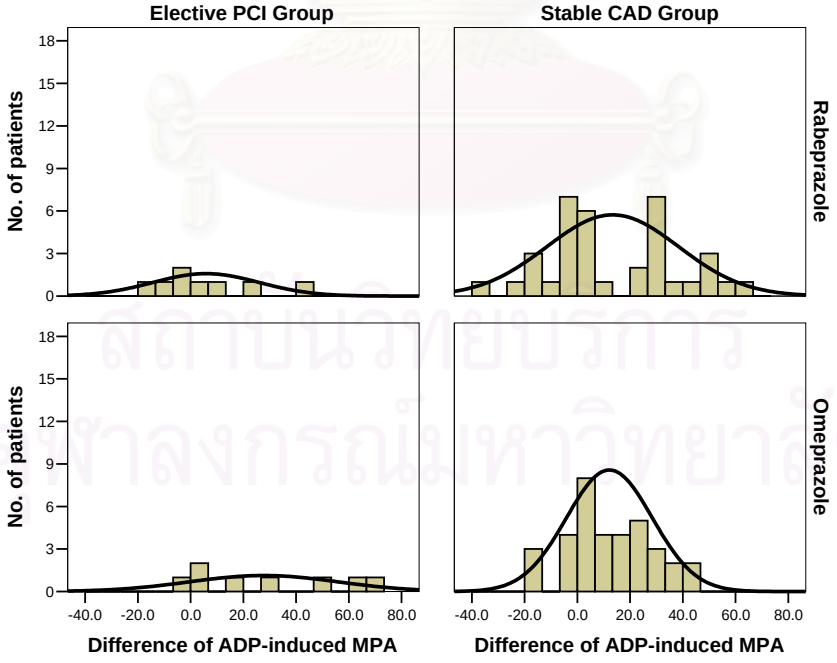

Figure 14: Interindividual distribution of ***change in ADP20 μM-induced platelet aggregation*** after receiving PPIs when categorized based on different PPI received and different patients groups either elective PCI or stable CAD.

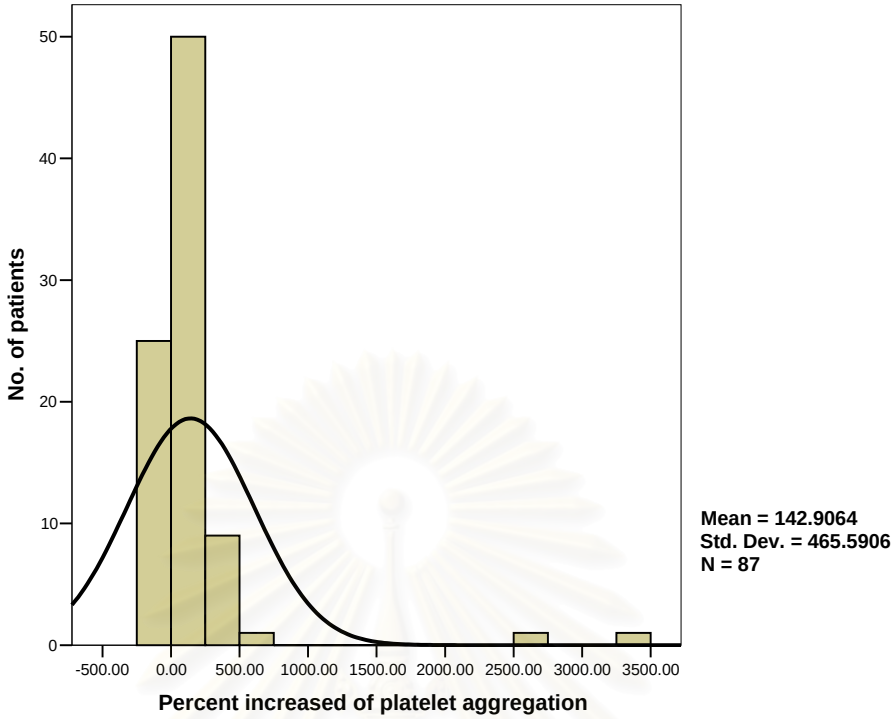

Figure 15: Interindividual distribution of ***percent increased of platelet aggregation*** after receiving PPIs in all patients

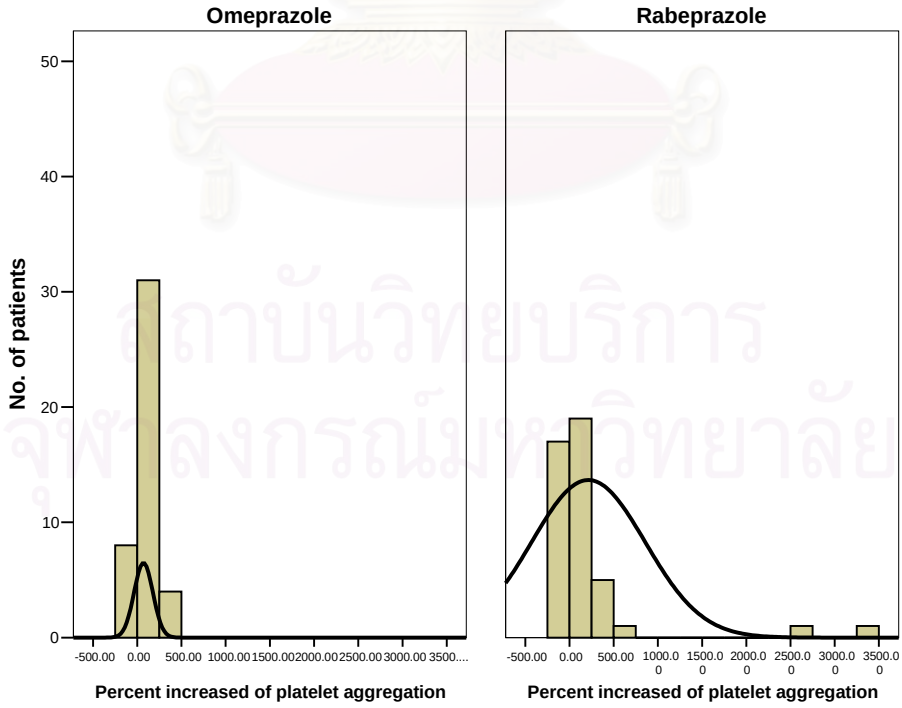

Figure 16: Interindividual distribution of ***percent increase of platelet aggregation*** after receiving PPIs when categorized based on different PPI received

The duration of taking PPIs in this study was varied widely from 14 days until 90 days. The average duration of taking PPIs was 35 days. The correlation coefficient indicated that longer duration of taking PPIs was not significantly associated with higher platelet aggregation (r = 0.061, p = 0.576) as described in table 10.

Table 10: Association between duration of taking PPIs and platelet activity

Duration of taking PPIs (average; days)	**n (%)**	**Platelet activity (%) (Mean ± SD)**	**Correlation coefficient** [f]	**p Value**
14 – 30 (21)	42 (48)	51.78 ± 23.16	0.252	0.108
31 – 60 (40)	34 (39)	52.15 ± 16.15	-0.031	0.861
61 – 90 (73)	11 (13)	52.76 ± 14.02	0.118	0.729
14 – 90 (35)	87 (100)	52.05 ± 19.46	0.061	0.576

PPIs = Proton pump inhibitors

[f] Pearson correlation coefficient

4.4 Factors influencing platelet response to clopidogrel at baseline and after taking PPIs

4.4.1 General demographic data

As shown in table 11, there was a significantly higher percentage of platelet aggregation induced by ADP 20 μM in female at baseline. However, change in platelet aggregation and percent increased of MPA after receiving PPIs were not significantly different between genders.

Table 11: Baseline platelet aggregations and change of platelet aggregation after taking PPIs in different genders

Gender	**N**	**Baseline MPA (Mean ± SD)**	**p Value**	**Δ MPA (Mean ± SD)**	**p Value**	**Increase of MPA (%)**	**p Value**
Male	67	36.17 ± 20.68%	0.045 [b*]	14.29 ± 20.42%	0.493 [d]	32.5 [-4.26 -118.57]	0.242 [e]
Female	20	46.42 ± 16.37%		10.96 ± 27.01%		10.9 [-9.75 -73.88]	

MPA = maximal platelet aggregation

[b] Independent t-test, [d] ANCOVA test, [e] Mann-Whitney *U* test, [*] Significant; $p < 0.05$

Data of percent increased of MPA presented as median and interquartile range

Table 12 demonstrates that BMI and creatinine clearance were not correlated significantly with platelet aggregation. Age, namely, older patient associated with higher platelet aggregation; and platelet count, namely, patients with lower platelet count correlate with increase in platelet aggregation after receiving PPIs. There were no correlation between age, BMI, creatinine clearance and platelet count with percent increased of platelet aggregation after receiving PPIs.

Table 12: Correlation coefficient of age, BMI, Cl_{Cr} and platelet correlation on platelet aggregations

	Baseline MPA		**Δ MPA**		**Increase of MPA (%)**	
	r [g]	**p value**	**r** [f]	**p value**	**r** [g]	**p value**
Age (years)	0.243	0.024*	-0.06	0.579	-0.106	0.328
BMI (Kg/m^2)	-0.056	0.605	-0.112	0.303	-0.019	0.864
Cl_{Cr}(ml/min)	-0.206	0.056	0.070	0.518	0.110	0.310
Platelet count ($x10^3/mm^3$)	0.168	0.120	-0.217	0.044*	-0.148	0.172

MPA = maximal platelet aggregation, Cl_{Cr} = creatinine clearance, BMI = body mass index

[f] Pearson correlation coefficient, [g] Spearman correlation coefficient, * Significant; $p < 0.05$

4.4.2 Co-morbidities

As shown in table 13, there were no significant influences of co-morbidities including diabetes mellitus, hypertension, single vessel disease, double vessel disease, triple vessel disease and STEMI on platelet aggregation. However, NSTEMI showed significant influence on Δ MPA after taking PPIs. Patients who had NSTEMI showed higher Δ MPA than those who did not have this condition. There were no statistically significant association between co-morbidities and percent increase of platelet aggregation after receiving PPIs.

Table 13: Univariate analysis of the influence of co-morbidities on platelet aggregations

		N	Baseline MPA (mean ± SD)	p Value	Δ MPA (mean ± SD)	P Value	% Increase Of MPA	P Value
DM	Yes	37	41.58 ± 17.81	0.226 [b]	14.51 ± 23.71	0.194 [d]	19.7[-3.91-106.88]	0.643 [e]
	No	50	36.26 ± 21.63		12.79 ± 20.83		20.1[-6.11-191.39]	
Hypertension	Yes	72	39.70 ± 19.35	0.237 [b]	13.11 ± 21.76	0.713 [d]	20.1[-5.51-110.69]	0.613 [e]
	No	15	32.90 ± 23.58		15.50 ± 23.69		13.7 [-8.73-271.89]	
Dyslipidemia	Yes	81	38.43 ± 20.65	0.874 [b]	12.61 ± 21.60	0.065 [d]	19.7 [-6.16-111.30]	0.412 [e]
	No	6	39.80 ± 12.91		25.85 ± 25.46		48.1[10.51-192.22]	
Single vessel	Yes	13	42.53 ± 24.24	0.440 [b]	12.75 ± 20.66	0.708 [d]	20.1[-6.16 - 214.76]	0.905 [e]
disease	No	74	37.82 ± 19.46		13.66 ± 22.34		19.8 [-4.67 - 111.07]	
Double vessel	Yes	20	38.76 ± 18.67	0.953 [b]	15.39 ± 19.52	0.573 [d]	17.2 [0.09 - 148.84]	0.650 [e]
disease	No	67	38.46 ± 20.72		12.97 ± 22.78		20.1[-6.25 -110.84]	
Triple vessel	Yes	18	32.19 ± 18.24	0.135 [b]	10.90 ± 21.32	0.077 [d]	16.1[-0.20 -102.78]	0.706 [e]
disease	No	69	40.18 ± 20.43		14.21 ± 22.25		20.1 [-6.16 -137.07]	
Non-STEMI	Yes	9	47.26 ± 18.41	0.171 [b]	20.37 ± 25.66	0.028 [d*]	32.5 [-11.16-141.83]	0.933 [e]
	No	78	37.52 ± 20.22		12.74 ± 21.57		19.1[-4.67 -112.20]	
STEMI	Yes	8	43.38 ± 22.77	0.478 [b]	10.90 ± 18.61	0.945 [d]	8.1 [-4.12 -104.91]	0.780 [e]
	No	79	38.03 ± 19.97		13.79 ± 22.38		20.1[-6.07 -111.76]	

DM = diabetes mellitus, MPA = maximal platelet aggregation, STEMI = ST-elevated myocardial infarction

[b] Independent t-test, [d] ANCOVA test, [e] Mann-Whitney *U* test, [*] Significant; $p < 0.05$

Data of percent increased of MPA was presented as median and interquartile range

4.4.3 Co-medications

Univariate analysis found that patients receiving medication of angiotensin converting enzyme inhibitors and did not receive diuretic were significant associated with increase of MPA and if use Scheffe post hoc comparison found that loop diuretic was significantly associated with decreasing of platelet aggregation compared with patients who did not receive any diuretic medication (p = 0.028). There was no statistical significant associated between co-medication and percent increased of platelet aggregation as described in table 14.

Table 14: Univariate analysis of the influence of co-medication on platelet aggregations

		N	Baseline %MPA (Mean ± SD)	p value	Δ %MPA (Mean ± SD)	p value	% Increase of MPA	p value
Aspirin (mg/day)								
81		21	36.91 ± 19.68	0.812 [h]	18.21 ± 26.53	0.509 [d]	49.8 [1.51-249.35]	0.564 [i]
100-162		6	42.95 ± 20.14		6.23 ± 17.03		20.9 [-8.96 -58.51]	
300-325		60	38.65 ± 20.59		12.62 ± 20.67		17.9 [-6.20 -111.53]	
Beta-blockers	Yes	69	39.78 ± 20.98	0.257 [b]	12.03 ± 22.06	0.476 [d]	18.6 [-7.05 -110.54]	0.379 [e]
	No	18	33.70 ± 16.24		19.26 ± 21.32		50.6 [0.84 -189.24]	
ACEIs	Yes	49	41.39 ± 19.30	0.133 [b]	15.53 ± 21.19	0.023 [d*]	20.1[-1.78 - 110.54]	0.745 [e]
	No	38	34.83 ± 20.88		10.94 ± 22.99		17.8[-14.08-136.17]	
ARBs	Yes	17	31.15 ± 17.75	0.093 [b]	12.77 ± 21.04	0.164 [d]	26.8 [-17.67 - 153.77]	1.000 [e]
	No	70	40.32 ± 20.42		13.71 ± 22.35		19.8 [-4.25 - 110.40]	
Statins								
None		6	39.80 ± 12.91	0.798 [h]	25.85 ± 25.46	0.204 [d]	48.1 [10.51- 192.22]	0.708 [i]
Simvastatin		25	40.85 ± 18.12		15.32 ± 20.95		20.0 [-8.28 -106.88]	
Atorvastatin		37	35.41 ± 23.01		12.95 ± 24.42		29.5 [-14.26 -207.85]	
Rosuvastatin		16	41.90 ± 19.56		6.61 ± 14.63		6.6 [-6.03 - 55.91]	
Pravastatin		3	37.03 ± 19.28		17.77 ± 25.41		15.9 [0.36 -279.17]	
CCBs	Yes	24	39.97 ± 19.15	0.683 [b]	16.09 ± 26.62	0.271 [d]	17.4 [-8.51 - 150.11]	0.849 [e]
	No	63	37.98 ± 20.65		12.55 ± 20.10		20.1 [-4.26 -102.54]	
Nitrates	Yes	58	37.34 ± 21.57	0.442 [b]	13.03 ± 22.57	0.369 [d]	19.8 [-9.04 - 114.78]	0.864 [e]
	No	29	40.89 ± 17.11		14.50 ± 21.11		20.1[-1.28 -106.66]	
α-blockers	Yes	8	38.46 ± 19.12	0.993 [b]	16.44 ± 22.57	0.640 [d]	47.5 [-2.17 - 171.36]	0.826 [e]
	No	79	38.53 ± 20.38		13.23 ± 22.05		20.0[-6.07 - 110.84]	
Diuretics								
None		56	35.45 ± 20.44	0.221 [h]	19.56 ± 22.03	0.028 [d*]	52.6 [3.53- 208.09]	0.003 [i**]
Thiazides		14	38.84 ± 16.80		10.30 ± 17.67		15.5 [-4.86 - 111.07]	
Loop		11	48.16 ± 19.05		-3.17 ± 17.69		-6.1 [-24.08 - 1.68]	
K^+-sparing		4	45.35 ± 27.94		-5.73 ± 11.62		-0.1[-27.94 -10.15]	
Thiazides + K^+-sparing		2	55.70 ± 0.71		-2.70 ± 4.10		-4.8 [-7.47 - 0.27]	

[b] Independent t-test, [d] ANCOVA test, [e] Mann-Whitney *U* test, [h] ANOVA test, [i] Kruskal-Wallis test

[*] Significant; $p < 0.05$, [**] Significant; $p < 0.01$

Data of percent increased of MPA was presented as median and interquartile range

4.5 Prevalence of clopidogrel nonresponder

In this study, clopidogrel nonresponder was defined as maximal platelet aggregation by induction with ADP 20μM > 50 % (MPA > 50%).

4.5.1 Frequency of clopidogrel nonresponder

Rate of clopidogrel nonresponder prior to receiving PPIs was 34% and increased to 59% after taking PPIs as shown in figure 17. The prevalence of clopidogrel nonresponsiveness in elective PCI group before receiving PPIs was 25% and increased to 56% after taking PPIs and in stable CAD patients, the incidence of clopidogrel nonresponder was increased from 37% to 59% after receiving PPIs.

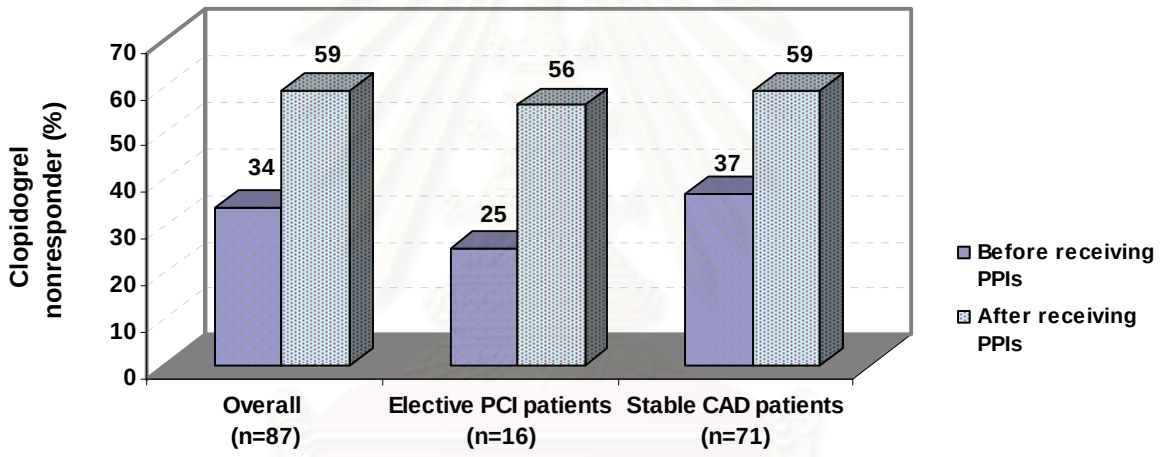

Figure 17: Frequency of clopidogrel nonresponder before and after receiving PPIs

Frequency of clopidogrel nonresponders in elective PCI group who were receiving omeprazole and rabeprazole were not significantly different (p = 1.000). Likewise, the percentage of clopidogrel nonresponder in the patients with stable CAD who were taking omeprazole or rabeprazole was not significantly different (p = 0.268) as describe in table 15.

Table 15: Prevalence of clopidogrel nonresponder after receiving omeprazole and rabeprazole

Patients groups	PPIs	Overall; n (%)	CNR; n (%)	
Elective PCI	Omeprazole	8 (50.0)	5 (62.5)	
(n = 16)	Rabeprazole	8 (50.0)	4 (50.0)	0.863 [a]
	p Value		1.000 [a]	
Stable CAD	Omeprazole	35 (49.3)	23 (65.7)	
(n = 71)	Rabeprazole	36 (50.7)	19 (52.8)	1.000 [a]
	p Value		0.268 [a]	

PPIs = proton pump inhibitors, CNR = clopidogrel nonresponder, PCI = percutaneous coronary intervention, CAD = coronary artery disease

[a] Chi-square or Fisher's exact test

In addition, figure 18 depicted the percentage of patients with nonresponsiveness to clopidogrel. The result showed higher frequency of clopidogrel nonresponder in patients who were taking omeprazole than patient who were taking rabeprazole, however, this difference was not statistically significant (65.1% Vs 52.3%, p = 0.224)

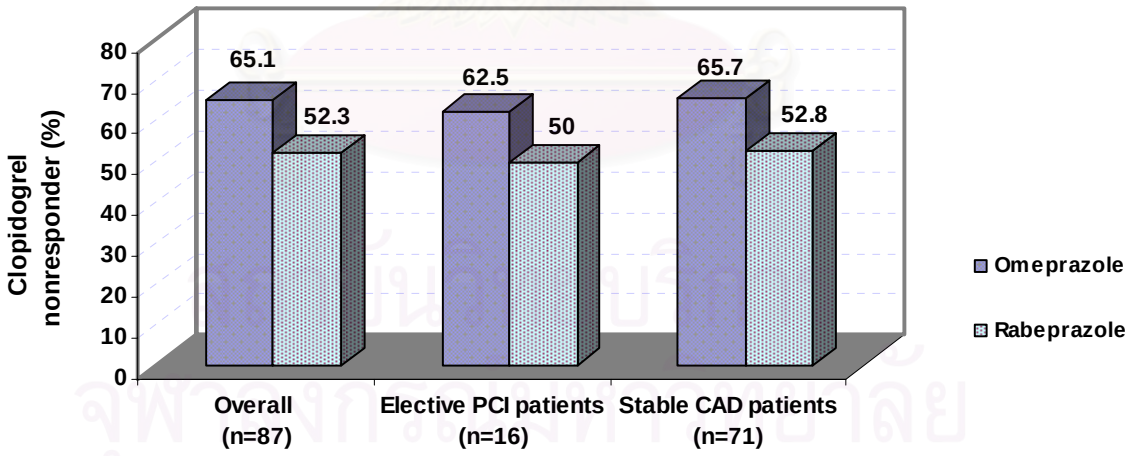

Figure 18: Rate of clopidogrel nonresponder after receiving omeprazole and rabeprazole

4.5.2 Characteristics of clopidogrel nonresponder

Comparisons of the demographic and clinical characteristics between clopidogrel nonresponders and clopidogrel responders were provided in table 16. Most of the characteristics showed no significant association to clopidogrel nonresponders except for those patients with diabetes mellitus who showed higher percentage in the clopidogrel nonresponder group as compared to the clopidogrel responder group after taking PPIs, p = 0.019. In addition, nonresponders were somewhat more likely to have a risk factor of NSTEMI (20.0% Vs 5.3%, p = 0.058) as compared with responders. The other demographic data showed similar percentage in the responder and nonresponder groups.

Table 16: Influence of demographic and clinical Characteristics on platelet activity before and after taking PPIs

	Overall	**Before taking PPIs**			**After taking PPIs**		
	(n=87)	**CNR (n = 30)**	**CR (n = 57)**	**p value**	**CNR (n = 51)**	**CR (n = 36)**	**P value**
Age (yrs)	63 ± 11	66 ± 11	62 ± 10	0.094 [b]	64 ± 11	62 ± 10	0.325 [b]
Gender							
Male, n (%)	67 (77)	20 (66.7)	47 (82.5)	0.096 [a]	37 (72.5)	30 (83.3)	0.239 [a]
Female, n (%)	20 (23)	10 (33.3)	10 (17.5)		14 (27.5)	6 (16.7)	
BMI (Kg/m^2)	25 ± 3.3	24.9 ± 2.8	25.3 ± 3.5	0.644 [b]	25.0 ± 3.3	25.4 ± 3.3	0.537 [b]
Patients groups, n (%)							
Elective PCI	16 (18.4)	4 (13.3)	12 (21.1)	0.377 [a]	9 (17.6)	7 (19.4)	0.831 [a]
Stable CAD	71 (81.6)	26 (86.7)	45 (78.9)		42 (82.4)	29 (80.6)	
Current Smoking, n (%)	2 (2.3)	0 (0)	2 (3.5)	0.543 [a]	1 (2.0)	1 (2.8)	1.000 [a]
Co-morbidities, n (%)							
Diabetes mellitus	37 (42.5)	15 (50.0)	22 (38.6)	0.306 [a]	27 (52.9)	10 (27.8)	0.019 [a*]
Hypertension	72 (82.8)	26 (86.7)	46 (80.7)	0.484 [a]	41 (80.4)	31 (86.1)	0.487 [a]
Dyslipidemia	81 (93.1)	29 (96.7)	52 (91.2)	0.660 [a]	46 (90.2)	35 (97.2)	0.394 [a]
Single vessel disease	13 (14.9)	6 (20.0)	7 (12.3)	0.358 [a]	9 (17.6)	4 (11.1)	0.400 [a]
Double vessel disease	20 (23.0)	6 (20.0)	14 (24.6)	0.631 [a]	12 (23.5)	8 (22.2)	0.887 [a]
Triple vessel disease	18 (20.7)	4 (13.3)	14 (24.6)	0.219 [a]	8 (15.7)	10 (27.8)	0.170 [a]
Non-STEMI	9 (10.3)	6 (20.0)	3 (5.3)	0.058 [a]	7 (13.7)	2 (5.6)	0.296 [a]
STEMI	8 (9.2)	3 (10.0)	5 (8.8)	1.000 [a]	6 (11.8)	2 (5.6)	0.461 [a]
Family history, n (%)	6 (6.9)	2 (6.7)	4 (7.0)	1.000 [a]	4 (7.8)	2 (5.6)	1.000 [a]

Data are expressed as mean ± SD or number of patients (%)

BMI = body mass index, CAD = coronary artery disease, CNR = clopidogrel nonresponder, CR = clopidogrel responder, PCI = percutaneous coronary intervention, PPIs = proton pump inhibitors, STEMI = ST-elevated myocardial infarction

[a] Chi-square or Fisher's exact test, [b] Independent t-test, * Significant; $p < 0.05$

Table 16: Influence of demographic and clinical Characteristics on platelet activity before and after taking PPIs (continue)

Characteristics	Overall (n=87)	Before taking PPIs			After taking PPIs		
		CNR (n = 30)	CR (n = 57)	p value	CNR (n = 51)	CR (n = 36)	P value
Co-medications, n (%)							
PPIs							
Omeprazole	43 (49.4)	17 (56.7)	26 (45.6)	0.327 [a]	28 (54.9)	15 (41.7)	0.224 [a]
Rabeprazole	44 (50.6)	13 (43.3)	31 (54.4)		23 (45.1)	21 (58.3)	
Aspirin							
81 mg/day	21 (24.1)	8 (26.7)	13 (22.8)	0.689 [a]	2 (3.9)	2 (5.6)	1.000 [a]
100-162 mg/day	6 (6.9)	2 (6.7)	4 (7.0)	1.000 [a]	2 (3.9)	4 (11.1)	0.226 [a]
300-325 mg/day	60 (69.0)	20 (66.7)	40 (70.2)	0.737 [a]	47 (92.2)	30 (83.3)	0.307 [a]
Beta-blockers	69 (79.3)	26 (86.7)	43 (75.4)	0.219 [a]	42 (82.4)	31 (86.1)	0.638 [a]
ACEIs	49 (56.3)	18 (60.0)	31 (54.4)	0.616 [a]	31 (60.8)	18 (50.0)	0.318 [a]
ARBs	17 (19.5)	3 (10.0)	14 (24.6)	0.103 [a]	8 (15.7)	11 (30.6)	0.098 [a]
Lipid lowering agents							
None	6 (6.9)	1 (3.3)	5 (8.8)	0.660 [a]	2 (3.9)	1 (2.8)	1.000 [a]
Simvastatin	25 (28.7)	9 (30.0)	16 (28.1)	0.850 [a]	17 (33.3)	6 (16.7)	0.083 [a]
Atorvastatin	37 (42.5)	12 (40.0)	25 (43.9)	0.729 [a]	20 (39.2)	19 (52.8)	0.210 [a]
Rosuvastatin	16 (18.4)	7 (23.3)	9 (15.8)	0.388 [a]	10 (19.6)	9 (25.0)	0.549 [a]
Pravastatin	3 (3.4)	1 (3.3)	2 (3.5)	1.000 [a]	2 (3.9)	1 (2.8)	1.000 [a]
CCBs	24 (27.6)	9 (30.0)	15 (26.3)	0.715 [a]	15 (29.4)	5 (13.9)	0.090 [a]
Nitrates	58 (66.7)	19 (63.3)	39 (68.4)	0.632 [a]	31 (60.8)	24 (66.7)	0.575 [a]
α-blockers	8 (9.2)	3 (10.0)	5 (8.8)	1.000 [a]	6 (11.8)	4 (11.1)	1.000 [a]
Diuretics	31 (35.6)	13 (43.3)	18 (31.6)	0.067 [a]	12 (23.5)	16 (44.4)	0.189 [a]
Cl_{Cr} (ml/min)	69 ± 26	64 ± 25	72 ± 26	0.157 [b]	68 ± 26	71 ± 27	0.605 [b]
Platelet count ($x10^3/mm^3$)	252 ± 67	265 ± 83	245 ± 58	0.198 [b]	248 ± 70	258 ± 64	0.474 [b]

Data are expressed as mean ± SD or number of patients (%)

ACEI = angiotensin converting enzyme inhibitors, ARBs = angiotensin receptor blockers, CCBs = calcium channel blockers, Cl_{Cr} = creatinine clearance, CNR = clopidogrel nonresponder, CR = clopidogrel responder, PCI = percutaneous coronary intervention, PPIs = proton pump inhibitors,

[a] Chi-square or Fisher's exact test, [b] Independent t-test, * Significant; $p < 0.05$

The results from table 16 identified that DM might be one of the risk factors for nonresponsiveness of clopidogrel in patients taking clopidogrel plus PPIs. The prevalence of clopidogrel nonresponder in diabetic patients who were taking with PPIs was 73% while in non diabetic patients the prevalence was 48% (OR = 2.93, 95%CI = 1.17-7.29, p = 0.019). The prevalence of clopidogrel nonresponder were 41% and 30% in DM and non-DM patients who were taking clopidogrel without PPIs, respectively; this was not statistically significant (OR = 1.59, 95%CI = 0.652-3.884, p = 0.306) as described in table 17.

Table 17: Prevalence of clopidogrel nonresponder in diabetic and non-diabetic patients

	Overall	**CNR**	
	(n = 87)	**Before receiving PPIs n = 30, (%)**	**After receiving PPIs n = 51, (%)**
Diabetic patients	37	15 (41)	27 (73)
Non-diabetic patients	50	15 (30)	24 (48)
p Value		0.306 [a]	0.019 [a*]

CNR = clopidogrel nonresponder, PPIs = proton pump inhibitors

[a] Chi-square or Fisher's exact test, * Significant; $p < 0.05$

In this study, prevalence of clopidogrel nonresponder in patients receiving omeprazole and rabeprazole were 65.1% (28/43) and 52.3% (23/44), respectively as shown in table 18. We found that the incidence of clopidogrel nonresponder after patients receiving omeprazole was higher than after patients taking rabeprazole but this different was not statistically significant (OR = 1.70, 95%CI = 0.720 – 4.036, p = 0.224).

Table 18: Prevalence of clopidogrel nonresponder in patients receiving omeprazole or rabeprazole

PPIs	**Overall**	**CNR, (%)**	
	(n = 87)	**Before receiving PPIs (n = 30)**	**After receiving PPIs (n = 51)**
Omeprazole	43	17 (39.5)	28 (65.1)
Rabeprazole	44	13 (29.5)	23 (52.3)
p value		0.327 [a]	0.224 [a]

PPIs = proton pump inhibitors, CNR = clopidogrel nonresponder

[a] Chi-square or Fisher's exact test

Platelet aggregation in elective PCI and stable CAD patients showed no significant difference either when the patients were taking clopidogrel without PPIs (p = 0.252) or when the patients were taking clopidogrel with PPIs (p = 0.198), as described in table 19. Moreover, frequency of clopidogrel nonresponder was not significantly different between elective PCI group and stable CAD group either the comparisons were performed while the patients were taking clopidogrel without PPIs (p = 0.377) or while the patients were taking clopidogrel with PPIs (p = 0.831).

Table 19: Comparison of platelet activity between elective PCI and stable CAD patients

Patient's group	**Platelet activity before taking PPIs**				**Platelet activity after taking PPIs**			
	CNR (n = 30)	**n (%)**	**CR (n = 57)**	**n (%)**	**CNR (n = 51)**	**n (%)**	**CR (n = 36)**	**n (%)**
Elective PCI (n = 16)	57.63 ± 4.24	4 (25)	31.78 ± 12.92	12 (75)	69.57 ± 14.81	9 (56)	36.30 ± 8.69	7 (44)
Stable CAD (n = 71)	62.16 ± 7.50	26 (37)	24.97 ± 12.48	45 (63)	64.06 ± 10.72	42 (59)	33.02 ± 12.84	29 (41)
p Value	0.252 [a]	0.377 [a]	0.101 [a]		0.198 [a]	0.831 [a]	0.528 [a]	
Total (n = 87)	61.55 ± 7.26		26.41 ± 12.77		65.03 ± 11.57		33.66 ± 12.11	

CNR = clopidogrel nonresponder, CR = clopidogrel responder, LD = loading dose, MD = maintenance dose, PPIs = proton pump inhibitors

[a] Chi-square test or Fisher's exact test

CHAPTER V

DISCUSSION

5.1 Patients characteristics

About three-fourth of the patients with CAD found in this study were male, this may due to customers of Phramongkutklao hospital were mostly soldier families. However, gender was not significantly different between omeprazole and rabeprazole groups ($p = 0.570$). Previous study in Thai ACS patients demonstrated that about 60% of patients were male.[3] The average age of patients included into this study were 63 ± 11 years, this was consistent with previous study which showed the average age to be 65 ± 12 years.[3] Nevertheless, there were 2 patients whose ages were less than 50 years, namely 33 years and 41 years. The BMI showed that patients included were mostly obese ($BMI \geq 25.0$) which accounted for 53%, overweight ($23.0 \leq BMI \leq 24.9$) accounted for 17% and within the range ($18.5 \leq BMI \leq 22.9$) accounted for 23% according to Asian definition. The dosage of aspirin consuming were mostly 300-325 mg/day (69%) according to the guideline which recommend this dosage for 12 months in patients who are not at high risk of bleeding, then continue indefinitely at a dose of 75-162 mg/day.[5] In this study, there were two patients who were receiving diltiazem, non-dihydropyridine calcium channel blocker, which might have interactions with clopidogrel through CYP3A4. However, baseline platelet aggregations of these patients were 35.2% and 35.0%, respectively.

5.2 Baseline platelet aggregation

Table 5 showed that the average values of MPA at baseline were not significantly different between patients who were receiving omeprazole or rabeprazole ($40.68 \pm 18.82\%$ Vs $36.42 \pm 21.39\%$, $p = 0.326$), and between elective PCI patients and stable CAD patients ($38.24 \pm 16.11\%$ Vs $38.59 \pm 21.06\%$, $p = 0.951$). At baseline, patients in elective PCI group who were planed to receive omeprazole seem to show lower platelet activity than patients who were planed to receive rabeprazole, but this difference was not statistically significant ($33.36 \pm 18.10\%$ Vs $43.13 \pm 13.20\%$, $p = 0.238$). In contrary, the platelet activity of patients in stable CAD group who were planed to omeprazole seem to be higher than patients who

were planed to receive rabeprazole, but was not statistically significant (42.36 $\pm$ 18.84% Vs 34.93 $\pm$ 22.68%, respectively, p = 0.138).

5.3 Effect of proton pump inhibitors on platelet aggregation

Clopidogrel is an inactive prodrug which requires *in vivo* conversion by liver enzymes to an active metabolite. The cytochrome P450 (CYP2C19) is responsible for the majority of clopidogrel for converting, therefore, interaction with this may reduce the effect of clopidogrel. PPIs were commonly prescribed for patients receiving combination of clopidogrel and aspirin to prevent the gastrointestinal side effect. From previous study in patients receiving dual antiplatelet clopidogrel and aspirin, 63.9% were prescribed PPIs at discharge, during follow up or both.[64] Moreover, PPIs are one of the most frequently prescribed classes of drug in the world[65], 12.4 million prescriptions had been dispensed in Canada in 2004.[66]

Our study demonstrated that both PPIs significantly increased ADP-induced platelet aggregation ($p < 0.0001$, $p = 0.002$) but not significantly different between omeprazole and rabeprazole ($p = 0.141$). In addition, we found that lower baseline MPA showed trend of higher increase in platelet aggregation after treatment with PPIs especially when treated with omeprazole. If divided into quartiles, the results indicated that only the first and the second quartiles showed significant increase in ADP-induced platelet aggregation. In the 3rd quartile, we found that the increase in platelet activity was significantly higher in patients who were receiving omeprazole than patients who were receiving rabeprazole ($p = 0.047$). In present study, percentage of increasing in MPA did not show normally distribution because patients who had lower MPA at baseline (MPA < 5%), the increase in MPA after treated with PPIs (MPA >30%) when calculated as percentage well become very high. The average percent increase of MPA in the 1st quartile was the highest, 275.5%, due to similar reason as above, lower platelet aggregation at baseline resulted in higher percentage of increasing in platelet aggregation after treated with PPIs. Several values of percent increase in MPA were extremely over the normal range, namely: 730%, 2536% and 3463% among patients who were receiving rabeprazole. If these values were treated as outliers, the result would be slightly different. The average percent increased of MPA would be 19% Vs 20% when exclude and include the outliers, respectively in all patients; 11% Vs 14% when exclude and include the outliers, respectively in patients who were taking rabeprazole and the same result,

32.5%, were obtained in patients who were taking omeprazole. In the same way, comparisons of change of platelet aggregation after taking each PPI between excluded and not excluded outliers were 12.49 ± 21.43% Vs 13.52 ± 21.98%, respectively and 9.82 ± 21.43% Vs 12.04 ± 24.22%, respectively in all patients and patients who were taking rabeprazole, respectively. If outliers were excluded, the increase in MPA was not significantly different between omeprazole and rabeprazole ($p = 0.110$) while the other results of the statistical significances were similar with the data which included the outliers.

This finding is consistent with previous study which showed that omeprazole significantly decreased clopidogrel inhibitory effect on platelet $P2Y_{12}$ assessed by VASP assay compared to without omeprazole (-43.3% Vs -32.6%, $p < 0.0001$, respectively).[10] Another study confirmed this finding by reported that the antiplatelet effect when receiving clopidogrel with lansoprazole tested by ADP-induced platelet aggregation was decreased from 49% to 39% after co-treatment.[63]

The reason for this interaction involved CYP2C19, a major enzyme determinant of clopidogrel and PPIs, especially affinity of omeprazole for CYP2C19 is approximately 10 folds more than CYP3A4, therefore, omeprazole has a greater potential to interact with the metabolism of substrates for CYP2C19 than others.[9] Although rabeprazole is shown to be least affected by CYP2C19 function but might also have some potential with this interaction since rabeprazole thioether, metabolite of rabeprazole, showed lower inhibition kinetic constant (K_i) of CYP2C19, 2-8 μM, compared with K_i values of other which were 2-9, 2-9, 17-21, 69 and 1 for omeprazole, esomeprazole, rabeprazole, pantoprazole and lansoprazole, respectively.[67] Nevertheless, this information apparent lack of correlation between the *in vivo* drug-drug interaction and the predicted interaction potential from *in vitro* of rabeprazole thioether. However, some study disagreed with this finding by showing no difference in the platelet reactivity index or the ADP-induced platelet aggregation between patient taking clopidogrel with pantoprazole (n = 152; PRI = 50%; aggregation = 47 U), esomeprazole (n = 74; PRI = 54%; aggregation = 42 U), or without PPI (n = 74; PRI = 49%; aggregation = 41 U); $p = 0.382$.[62] That finding reported PPIs-clopidogrel interaction may not be a class effect.

In addition, from the previous study[64], assessed the adverse outcome of death or rehospitalization for ACS patients taking clopidogrel with PPIs found a consistent association between omeprazole (OR = 1.24, 95%CI = 1.08-1.41) and rabeprazole (OR = 2.83, 95%CI = 1.96-4.09). Moreover, Ho et al (2009)[64] found longer duration of treatment clopidogrel with PPIs was associated with increased risk of adverse outcomes. Our study showed no association between difference of time receiving PPIs and platelet aggregation (p = 0.576). The difference in the duration of intake PPIs showed trend of statistically significant (p = 0.108) in patients who were receiving PPIs for 14-30 days which might due to the difference in the percentage of achieving steady state of maximal inhibition of platelet aggregation by PPIs among patients.

5.4 Factors influencing platelet response to clopidogrel at baseline and after taking PPIs

In this study, most demographic data, co-morbidities and co-medication showed no influence on clopidogrel nonresponsiveness except for age, NSTEMI, ACEI and diuretic medication. From table 11, 12 we found that percentage of platelet aggregation was significantly higher in female and/or older patients. This higher platelet aggregation might due to the average age of female was higher than male (69.20 $\pm$ 9.29 Vs 61.10 $\pm$ 10.19, p = 0.002). In addition, patients who had a risk factor of DM and did not use any lipid lowering drug showed trend of having higher platelet aggregation but not statistically significant. Patients who have a risk factor of TVD showed trend of having lower platelet activity which might due to these patients in partially had done coronary artery bypass grafting. Moreover, using univariate analysis found that patients receiving medication of angiotensin converting enzyme inhibitors and did not receive diuretic were significantly associated with increase in MPA. However, the cytochrome P450 of furosemide or torsemide involved in metabolism of clopidogrel was not similar.

Clinical situation of widely variation in platelet aggregation of clopidogrel is caused mostly by inefficient availability of the active metabolite. This could due to patients' poor compliance associated with longer time of taking clopidogrel. In addition, maintenance dose regimen might result in lower clopidogrel blood concentration as compared with loading dose regimen and in turn resulted in lower inhibition of platelet aggregation.

Drug-Drug interaction, including lipophilic statins, may also interfere with clopidogrel-induced antiplatelet effect. However, this observation is quite controversial as larger studies have shown the lack of any interaction between lipophilic statins and clopidogrel.[53, 68] In addition, most studies did not show any negative clinical interaction with co-administration of these drugs.[54] In our study, we did not find any significant influence of either lipophilic or hydrophilic statins on platelet inhibition of clopidogrel at baseline ($p = 0.798$).

Another important factor which may have major effect on higher platelet aggregation is the genetic polymorphism of *CYP2C19*, a major determinant for clopidogrel metabolizer. Genetic polymorphism of *CYP2C19*, which is poor metabolizer phenotype, can be found in 2-5% in Caucasian, 11-23% in Asian[69] and 6.54-13.2% in Thai population.[69-71] The higher percentage of poor metabolizer could cause the active metabolite of clopidogrel to be decreased.

5.5 Prevalence of clopidogrel nonresponder

Our study demonstrated that the prevalence of clopidogrel nonresponder (defined as MPA > 50%) in Thai patients with CAD was 34% in patients receiving clopidogrel without PPIs and increased to 59% in patients receiving clopidogrel plus PPIs. This finding agrees with previous studies which use the same patient group and similar definition of clopidogrel nonresponder, they reported clopidogrel nonresponder in range of 22-62.5%.[45, 48, 59] However, recent study, using LD 600 mg clopidogrel with the same definition of clopidogrel nonresponsiveness found lower prevalence of clopidogrel nonresponder (14-15%).[47] The higher loading dose increased efficacy of clopidogrel in inhibiting platelet aggregation.[42, 47, 49] In addition, using clopidogrel 600 mg reloading and increased MD to 150 mg for 4 weeks in clopidogrel nonresponder group found to decreased ADP-induced platelet aggregation significantly ($83 \pm 6\%$ to $56 \pm 14\%$, $p < 0.01$) and this result was maintained throughout 4 weeks.[72] Moreover, many studies have shown an association between less platelet aggregation inhibition and more recurrent cardiovascular events, such as more stent restenosis (8.6% Vs 2.3%)[37] and more cardiac death in patients with coronary stenting (18.2% Vs 2.9%, $p = 0.006$).[43]

In our study, the prevalence of clopidogrel nonresponder among elective PCI patients and stable CAD patients who were taking clopidogrel without PPIs were 25% and 37% respectively; these prevalence of clopidogrel nonresponder were increased to 56% and 59% in patients receiving clopidogrel plus PPIs, respectively. The difference between the two groups were not statistically significant (p = 0.377, p = 0.831, respectively).

The prevalence of clopidogrel nonresponder in total patients included while receiving clopidogrel without PPIs was 34% compared with 26.7% reported by Gilard et al (2008)[10] which defined nonresponsiveness of clopidogrel as PRI > 50% as tested by VASP assay. The frequency of clopidogrel nonresponder after treated with PPIs was increased to 59%; when categorized into omeprazole group and rabeprazole group, the frequencies were 65% and 52%, respectively, (p = 0.224) compared with the result from Gilard et al (2008)[10] which found that the nonresponsiveness of clopidogrel accounted for 60.9% in undergoing PCI patients who were receiving omeprazole for 7 days.

In addition, our study confirm the result from previous study which identified DM as risk factor for nonresponsiveness of clopidogrel.[47, 59, 60] In our study, the prevalence of clopidogrel nonresponder in diabetic patients after receiving PPIs was 73% while in non-diabetic patients the prevalence was 48% (OR = 2.93, 95%CI = 1.17-7.29, p = 0.019). Before receiving PPIs, the prevalence of clopidogrel nonresponder was 41% Vs 30% in diabetic and non-diabetic patient, respectively. The prevalence of clopidogrel nonresponders found in this study are consistent with those reported by previous studies which showed the prevalence within range of 38-62.5%.[47, 48, 59] Several mechanisms account for such increased platelet aggregation in diabetic patients including increased activity of esterase, which would convert more of the clopidogrel prodrug into inactive metabolite. Another possibility is that reduced gastric motility in diabetic patients could lead to slower absorption of the prodrugs or that alterations at the megakaryocyte level changes platelet turnover and receptor expression.[47] Finally, since insulin reduces platelet aggregation by inhibition of the $P2Y_{12}$ pathway, therefore, type2 DM patients who have decreased sensitivity to insulin leading to lower cAMP levels and reduced $P2Y_{12}$ inhibition, which overall leads to increased platelet reactivity.[59]

5.6 Platelet function test for monitoring clopidogrel

Platelet function test used in our study was ADP-induced platelet aggregation assessed by light transmission aggregometry or optical (turbidimetric) platelet aggregometry which is one of the first methods developed to assess platelet function and the historical gold standard. The platelet aggregation test used to evaluate the response to clopidogrel and concomitant doses of aspirin did not interfere with the detection of prevalence of clopidogrel nonresponsiveness.[73] Moreover, in this study 20 μM ADP was used as agonist because Fitzgerald and Malee (2007)[22] found that higher concentrations of ADP induced full and irreversible platelet aggregation that was insensitive to aspirin but was inhibited by at least 90% in the presence of a $P2Y_{12}$ antagonist. Even though this method was not as specific to $P2Y_{12}$ as VASP assay[20, 21] and VerifyNow® which add prostaglandin E1 to suppress intracellular free calcium levels for reducing the platelet activation contribution from ADP to its $P2Y_1$[24], but this was a practical method in our setting.

5.7 Clinical implication

This study demonstrated that the increasing of ADP-induced platelet aggregation was not difference in patient receiving clopidogrel plus omeprazole or rabeprazole. However, since the increase in MPA was significantly when co-administered clopidogrel with either PPIs, suggested that omeprazole, a generic drug, or other PPIs should be used for patient with an indication for medication, such as a history of gastrointestinal ulcer, receiving dual antiplatelet, consistent with current guideline recommendation, rather than routine prophylactic prescription.[64, 74]

The management of clopidogrel nonresponder, ACC/AHA/SCAI 2005 guideline update for PCI recommended to increase the maintenance dose to 150 mg/day if less than 50% inhibition of platelet aggregation.[5] In addition, in patients who are receiving 75 mg MD of clopidogrel 75 mg may administer a LD of 300-600 mg clopidogrel if he/she is diagnosed as clopidogrel nonresponder and shall consider prasugrel, thienopyridine, a new agent of choice for clopidogrel nonresponder.[75] Moreover, in diabetic patients with known risk factor for nonresponsiveness may increase the MD regimen to 150 mg/day.[48] However, the risk/benefit of modifying the clopidogrel MD from 75 to 150 mg daily, or using the

alternative more potent ADP-receptor antagonist prasugrel to achieve higher platelet inhibition still needs to be further clarified.

5.8 Limitation

Limitations of this study included small sample size and one method of determining clopidogrel nonresponsiveness. Future studies are recommended. First, to examine and compare different concentrations of ADP and different methods for determining clopidogrel nonresponsiveness. Second, to find out the association between platelet reactivity and genetic polymorphism of *CYP2C19* in Thai population. Third, to extend the study to other groups of population to identify the prevalence of clopidogrel nonresponder in different groups of patients

CHAPTER VI

CONCLUSION

Patients with acute coronary syndrome consisted of patients with UA, NSTEMI and STEMI and after coronary stenting patients who are receiving clopidogrel and aspirin dual therapy are commonly concomitantly treated with PPIs to prevent the gastrointestinal side effect. Clopidogrel is a thienopyridine prodrug requiring several biotransformation steps, mediated mainly by cytochrome P-450; especially CYP3A4/5, CYP2C9, CYP2C19, CYP2B6 and CYP1A2 to active metabolites. The principle isoenzymes involved in the metabolism of PPIs including the most commonly used PPI, i.e., omeprazole are CYP2C19 and CYP3A4. Therefore, interaction may occur and this may reduce the effect of clopidogrel.

The purposes of this study were to determine the effect of rabeprazole and omeprazole on the antiplatelet action of clopidogrel plus aspirin, to investigate the prevalence of clopidogrel nonresponder in patients with CAD and compare the rate of clopidogrel nonresponsiveness before and after receiving each PPI.

All consecutive patients with angiographic diagnosis of CAD were recruited. The study consisted of patients who had taken clopidogrel 75 mg/day at least 5 days and patients who received loading dose of clopidogrel 300 mg and aspirin 300-325 mg before underwent PCI. All patients were treated with aspirin 81-325 mg/day at least 7 days prior to the study. The patients were randomized into two treatment groups: 20 mg/day omeprazole or 20 mg/day rabeprazole for at least 2 weeks. Effect of clopidogrel on platelet aggregation was measured by light transmission aggregometry using ADP 20 μM as agonist. Clopidogrel nonresponder was defined as ADP 20 μM-induced maximal platelet aggregation > 50%. Platelet aggregation test was assessed before and after receiving PPIs for at least 2 weeks.

Of the 87 patients enrolled during August 2008 to March 2009, there were 43 patients taking omeprazole and 44 patients taking rabeprazole. Overall, 18% were scheduled for elective PCI patients and 82% were stable CAD. This study found that concomitant use of combination of aspirin and clopidogrel plus omeprazole or rabeprazole were significantly associated with higher platelet aggregation (32.5% Vs 14%, respectively) but did not

significantly different between the two drugs (p = 0.519). Average value of MPA after ADP 20 μM stimuli compared between before and after receiving PPIs was 38.53 $\pm$ 20.16% and 52.05 $\pm$ 19.46%, respectively. Using pair-t test to compare platelet aggregation before and after taking PPIs when categorized into quartiles revealed that only quartile 1 and quartile 2 showed significantly increased; $p < 0.0001$, $p < 0.0001$, respectively. In the 3rd quartile, the MPA of patients receiving omeprazole only was significantly increased but not in patients receiving rabeprazole. There were no significant difference including demographic, clinical characteristic and co-medication in both groups except for BMI of which the patients in the rabeprazole group was higher than that of the omeprazole group.

This study showed the influence of several factors on platelet response to clopidogrel**,** there was a significantly higher percentage of platelet aggregation induced by ADP 20 μM in female at baseline. However, change in platelet aggregation and percent increased of MPA after receiving PPIs were not significantly different between genders. Age, namely, older patient associated with higher platelet aggregation; and platelet count, namely, patients with lower platelet count correlate with increase in platelet aggregation after receiving PPIs. Using univariate analysis found that patients receiving medication of angiotensin converting enzyme inhibitors and did not receive diuretic were significant associated with increase of MPA and if use Scheffe post hoc comparison found that loop diuretic was significantly associated with decreasing of platelet aggregation compared with patients who did not receive any diuretic medication (p = 0.028).

This study demonstrated high prevalence of clopidogrel nonresponder (defined as MPA > 50%), 34%, prior to taking PPIs and increased to 59% after receiving PPIs as tested by ADP 20 μM-induced maximal platelet aggregation in CAD patients. In addition, the results showed higher frequency of clopidogrel nonresponder in patients who were taking omeprazole than patients who were taking rabeprazole, however, this difference was not statistically significant (65.1% Vs 52.3%, p = 0.224). From univariate analysis, this study found diabetes mellitus to be a risk factor for clopidogrel nonresponsiveness (OR = 2.93, 95%CI = 1.17-7.29, p = 0.019).

In future, larger prospective study is needed to determine how these platelet responses in functional tests associate with cardiovascular outcomes. Moreover, platelet function test might prove beneficial in tailoring individual antiplatelet medication especially in patients with known risk factor for nonresponsiveness.

REFERENCES

[1] Neal, B.; Chapman, N. and Patel, A. Managing the global burden of cardiovascular disease. Eur Heart J 4 (2002): F2-F6.

[2] กลุ่มภารกิจด้านข้อมูลข่าวสารและสารสนทศสุขภาพ สำนักนโยบายและยุทธศาสตร์ สำนักงานปลัดกระทรวงสาธารณสุข. ตาราง 2.3.4 จำนวนและอัตราตายต่อประชากร 100,000 คน จำแนกตามสาเหตุที่สำคัญ พ.ศ. 2545-2549 [online]. Available from: http://203.157.10.15/Moph/LinkClick.aspx?fileticket=8K8LkNWFKv4%3d&tabid=57&mid=431. [2008, June 12].

[3] Srimahachota, S.; Kanjanavanit, R. and Boonyaratavej, S. Demographic, management practice and in-hospital outcomes of Thai Acute Coronary Syndrome Registry (TACSR): The difference from the western world. TACSR's abstracts : The Heart Association of Thailand under the Royal Patronage of HM the King (2006): 10-14.

[4] Anderson, J.L.; Adams, C.D.; Antman, E.M.; Bridges, C.R.; Califf, R.M.; Casey, D.E., et al. ACC/AHA 2007 guidelines for the management of patients with unstable angina/Non-ST-Elevation myocardial infarction: A report of the American college of cardiology/American heart association task force on practice guidelines (writing committee to revise the 2002 guidelines for the management of patients with unstable angina/Non-ST-Elevation myocardial infarction. Circulation 116 (2007): e148-e304.

[5] Smith, S.C.; Feldman, T.E.; Hirshfeld, J.W.; Jacobs, A.K.; Kern, M.J.; King, S.B., et al. ACC/AHA/SCAI 2005 guideline update for percutaneous coronary intervention: A report of the American college of cardiology/American heart association task force on practice guidelines (ACC/AHA/SCAI writing committee to update the 2001 guidelines for percutaneous coronary intervention). Circulation 113 (2006): e166-e286.

[6] Brandt, J.; Close, S.; Iturria, S.; Payne, C.; Farid, N.; Ernest, C., et al. Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel. J Thromb Haemost 5 (2007): 2429-2436.

[7] Hulot, J.-S.b.; Bura, A.; Villard, E.; Azizi, M.; Remones, V.r.; Goyenvalle, C., et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. BLOOD 108 (2006): 2244-2247.

[8] Savi, P.; Pereillo, J.; Uzabiaga, M.; Combalbert, J.; Picard, C.; Maffrand, J., et al. The metabolism of clopidogrel is catalyzed by human cytochrome P450 3A and is inhibited by atorvastatin. Thromb Haemost 84 (2000): 891-896.

[9] Ishizaki, T. and Horai, Y. Review article: cytochrome P450 and the metabolism of proton pump inhibitors - emphasis on rabeprazole. Aliment Pharmacol Ther 13 (1999): 27-36.

[10] Gilard, M.; Arnaud, B.; Cornily, J.-C.; Gal, G.L.; Lacut, K.; Calvez, G.L., et al. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin. J Am Coll cardiol 51 (2008): 256-260.

[11] Pace, F.; Annese, V.; Prada, A.; Zambelli, A.; Casalini, S.; Nardini, P., et al. Rabeprazole is equivalent to omeprazole in the treatment of erosive gastro-oesophageal reflux disease. Dig Liv Dis 37 (2005): 741–750.

[12] Dekkers, C.; Beker, J.; Thjodleifsson, B.; Gabryelewicz, A.; Bell, N. and Humphries, T. Comparison of rabeprazole 20 mg versus omeprazole 20 mg in the treatment of active duodenal ulcer: a European multicentre study. Aliment Pharmacol Ther 13 (1999): 179-186.

[13] WHO collaborating centre for drug statistics methodology. Drugs for peptic ulcer and gastro-oesophageal reflux disease [online]. Available from: http://www.whocc.no/atcddd/indexdatabase/index.php?query=A02BC [2008, June 26].

[14] McNicol, A. and Israels, S.J. Platelets and anti-platelet therapy. J Pharmacol Sci 93 (2003): 381 – 396.

[15] Rang, H.P.; Dale, M.M.; Ritter, J.M. and Moore, P.K. Haemostasis and thrombosis. in Pharmacology, Loanhead: Churchill livingstone, 2003. pp. 314-329.

[16] Wientong, P. The frequency of aspirin resistance and factors associated with platelet response to aspirin type2 diabetic patients. Doctor dissertation, Pharmaceutical care, Faculty of Pharmaceutical Sciences, Chulalongkorn University. 2006.

[17] Willoughbya, S.; Holmesa, A. and Loscalzob, J. Platelets and cardiovascular disease. Eur J Cardiovasc Nursing 1 (2002): 273-288.

[18] Raju, N.C.; Eikelboom, J.W. and Hirsh, J. Platelet ADP-receptor antagonists for cardiovascular disease: past, present and future. Nat Clin Pract Cardiovasc Med 5 (2008): 766-780.

[19] Kottke-Marchant, K. and Corcoran, G. The Laboratory Diagnosis of Platelet Disorders. Arch Pathol Lab Med 126 (2002): 133–146.

[20] Harrison, P.; Frelinger, A.L.; Furmanb, M.I. and Michelson, A.D. Measuring antiplatelet drug effects in the laboratory. Thromb Res 120 (2007): 323-336.

[21] Michelson, A.D. Methods for the Measurement of Platelet Function. Am J Cardiol 103 [suppl] (2009): 20A-26A.

[22] Fitzgerald, D.J. and Maree, A. Aspirin and clopidogrel resistance. Hematology (2007): 114-120.

[23] Michelson, A.D.; Frelinger, A.L. and Furman, M.I. Current options in platelet function testing. Am J Cardiol 98 [suppl] (2006): 4N–10N.

[24] Malinin, A.; Pokov, A.; Spergling, M.; Defranco, A.; Schwartz, K.; Schwartz, D., et al. Monitoring platelet inhibition after clopidogrel with the VerifyNow-P2Y12R rapid analyzer: The VERIFY Thrombosis risk ASsessment (VERITAS) study. Thromb Res 119 (2007): 277—284.

[25] Moshfegh, K.; Redondo, M.; Julmy, F.; Wuillemin, W.A.; Gebauer, M.U.; Haeberli, A., et al. Antiplatelet effects of clopidogrel compared with aspirin after myocardial infarction: Enhanced inhibitory effects of combination therapy. J Am Coll Cardiol 36 (2000): 699 –705.

[26] Farrell, T.P.; Hayes, K.B.; Sobel, B.E. and Schneider, D.J. The lack of augmentation by aspirin of inhibition of platelet reactivity by ticlopidine. Am J Cardiol 83 (1999): 770–774.

[27] Geiger, J.; Teichmann, L.; Grossmann, R.; Aktas, B.; Steigerwald, U.; Walter, U., et al. Monitoring of clopidogrel action: Comparison of methods. Clin Chem 51 (2005): 957-965.

[28] Hochholzer, W.; Trenk, D.; Frundi, D. and Neumann, F.-J. Whole blood aggregometry for evaluation of the antiplatelet effects of clopidogrel. Thromb Res 119 (2007): 285-291.

[29] Gurbel, P.A. and Tantry, U.S. Clopidogrel resistance? Thromb Res 120 (2007): 311–321.

[30] Beckerath, N.v.; Taubert, D.; Pogatsa-Murray, G.; Schömig, E.; Kastrati, A. and Schömig, A. Absorption, metabolization, and antiplatelet effects of 300-, 600-, and 900-mg loading doses of clopidogrel. Circulation 112 (2005): 2946-2950.

[31] Angiolillo, D.J.; Fernandez-Ortiz, A.; Bernardo, E.; Alfonso, F.; Carlos Macaya, M.; Bass, T.A., et al. Variability in individual responsiveness to clopidogrel: Clinical implications, management, and future perspectives. J Am Coll Cardiol 49 (2007): 1505-1516.

[32] Bhatt, D.L.; Fox, K.A.A.; Hacke, W.; Berger, P.B.; Black, H.R.; Boden, W.E., et al. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. N Engl J Med 354 (2006): 1706-1717.

[33] Mehta, S.R.; Yusuf, S.; Peters, R.J.G.; Bertrand, M.E.; Lewis, B.S.; Natarajan, M.K., et al. Effects of pretreatment with clopidogrel and aspirin followed by long-term therapy in patients undergoing percutaneous coronary intervention: the PCI-CURE study. Lancet 358 (2001): 527-533.

[34] Yusuf, S.; Zhao, F.; Mehta, S.R.; Chrolavicius, S.; Tognoni, G. and Fox, K.K. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med 345 (2001): 494-502.

[35] Oqueli, E.; Hiscock, M. and Dick, R. Clopidogrel Resistance. Heart, Lung and Circulation 16 (2007): S17–S28.

[36] Nguyen, T.A.; Diodati, J.G. and Pharand, C. Resistance to clopidogrel: A review of the evidence. J Am Coll Cardiol 45 (2005): 1157– 1164.

[37] Buonamici, P.; Marcucci, R.; Migliorini, A.; Gensini, G.F.; Santini, A.; Paniccia, R., et al. Impact of platelet reactivity after clopidogrel administration on drug-eluting stent thrombosis. J Am Coll cardiol 49 (2007): 2312-2317.

[38] Müller, I.; Besta, F.; Schulz, C.; Massberg, S.; Schönig, A. and Gawaz, M. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. Thromb Haemost 89 (2003): 783–787.

[39] Gurbel, P.A.; Bliden, K.P.; Hiatt, B.L. and O'Connor, C.M. Clopidogrel for coronary stenting: response variability, drug resistance, and the effect of pretreatment platelet reactivity. Circulation 107 (2003): 2908-2913.

[40] Mobley, J.E.; Bresee, S.J.; Wortham, D.C.; Craft, R.M.; Snider, C.C. and Carroll, R.C. Frequency of nonresponse antiplatelet activity of clopidogrel during pretreatment for cardiac catheterization. Am J Cardiol 93 (2004): 456–458.

[41] Matetzky, S.; Shenkman, B.; Guetta, V.; Shechter, M.; Bienart, R.; Goldenberg, I., et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. Circulation 109 (2004): 3171-3175.

[42] Gurbel, P.A.; Bliden, K.P.; Hayes, K.M.; Yoho, J.A.; Herzog, W.R. and Tantry, U.S. The relation of dosing to clopidogrel responsiveness and the Incidence of high post-treatment platelet aggregation in patients undergoing coronary stenting. J Am Coll Cardiol 45 (2005): 1392-1396.

[43] Geisler, T.; Langer, H.; Wydymus, M.; Gohring, K.; Zurn, C.; Bigalke, B., et al. Low response to clopidogrel is associated with cardiovascular outcome after coronary stent implantation. Eur Heart J 27 (2006): 2420-2425.

[44] Cuisset, T.; Frere, C.; Quilici, J.; Morange, P.-E.; Nait-Saidi, L.; Mielot, C., et al. High post-treatment platelet reactivity is associated with a high incidence of myonecrosis after stenting for non-ST elevation acute coronary syndromes. Thromb Haemost 97 (2007): 282-287.

[45] Bliden, K.P.; DiChiara, J.; Tantry, U.S.; Bassi, A.K.; Chaganti, S.K. and Gurbel, P.A. Increased risk in patients with high platelet aggregation receiving chronic clopidogrel therapy undergoing percutaneous coronary intervention: Is the current antiplatelet therapy adequate? J Am Coll Cardiol 49 (2007): 657–666.

[46] Angiolillo, D.J.; Fernandez-Ortiz, A.; Bernardo, E.; Ramırez, C.; Sabate, M.; Banuelos, C., et al. High clopidogrel loading dose during coronary stenting: effects on drug response and interindividual variability. Eur Heart J 25 (2004): 1903–1910.

[47] Erlinge, D.; Varenhorst, C.; Braun, O.Ö.; James, S.; Winters, K.J.; Jakubowski, J.A., et al. Patients with poor responsiveness to thienopyridine treatment or with diabetes have lower levels of circulating active metabolite, but their platelets respond normally to active metabolite added Ex Vivo. J Am Coll Cardiol 52 (2008): 1968–1977.

[48] Angiolillo, D.J.; Shoemaker, S.B.; Desai, B.; Yuan, H.; Charlton, R.K.; Bernardo, E., et al. Randomized comparison of a high clopidogrel maintenance dose in patients with diabetes mellitus and coronary artery disease. Results of the Optimizing Antiplatelet Therapy in Diabetes Mellitus (OPTIMUS) Study. Circulation 115 (2007): 1-8.

[49] Cuisset, T.; Frere, C.; Quilici, J.; Morange, P.-E.; Nait-Saidi, L.; Carvajal, J., et al. Benefit of a 600-mg loading dose of clopidogrel on platelet reactivity and clinical outcomes in patients with non–ST-segment elevation acute coronary syndrome undergoing coronary stenting. J Am Coll Cardiol 48 (2006): 1339–1345.

[50] Price, M.J.; Endemann, S.; Gollapudi, R.R.; Valencia, R.; Stinis, C.T.; Levisay, J.P., et al. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. Eur Heart J 29 (2008): 992-1000.

[51] Angiolillo, D.J. Variability in responsiveness to oral antiplatelet Therapy. Am J Cardiol 103[suppl] (2009): 27A-34A

[52] Müller, I.; Besta, F.; Schulz, C.; Li, Z.; Massberg, S. and Gawaz, M. Effects of statins on platelet inhibition by a high loading dose of clopidogrel. Circulation 108 (2003): 2195-2197.

[53] Serebruany, V.L.; Midei, M.G.; Malinin, A.I.; Oshrine, B.R.; Lowry, D.R.; Sane, D.C., et al. Absence of Interaction Between Atorvastatin or Other Statins and Clopidogrel. Arch Intern Med 164 (2004): 2051-2057.

[54] Angiolillo, D.J. and Alfonso, F. Clopidogrel–Statin interaction. J Am Coll Cardiol 50 (2007): 296-298.

[55] Nguyen, T.; Frishman, W.H.; Nawarskas, J. and Lerner, R.G. Variability of response to clopidogrel possible mechanisms and clinical implications. Cardiology in Review 14 (2006): 136-142.

[56] Bliden, K.P.; DiChiara, J.; Lawal, L.; Singla, A.; Antonino, M.J.; Baker, B.A., et al. The association of cigarette smoking with enhanced platelet inhibition by clopidogrel. J Am Coll Cardiol 52 (2008): 531-533.

[57] Giustia, B.; Goria, A.M.; Marcuccia, R.; Saracinia, C.; Sestinia, I.; Panicciaa, R., et al. Cytochrome P450 2C19 loss-of-function polymorphism, but not CYP3A4 IVS10 + 12G/A and P2Y12 T744C polymorphisms, is associated

with response variability to dual antiplatelet treatment in high-risk vascular patients. Pharmacogenetics Genom 17 (2007): 1057–1064.

[58] Lev, E.I.; Patel, R.T.; Guthikonda, S.; Lopez, D.; Bray, P.F. and Kleiman, N.S. Genetic polymorphisms of the platelet receptors P2Y12, P2Y1 and GP IIIa and response to aspirin an clopidogrel. Thromb Res 119 (2007): 355—360.

[59] Angiolillo, D.J.; Fernandez-Ortiz, A.; Bernardo, E.; Ramırez, C.; Sabate, M.; Jimenez-Quevedo, P., et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. Diabetes 54 (2005): 2430–2435.

[60] Hochholzer, W.; Trenk, D.; Bestehorn, H.-P.; Fischer, B.; Valina, C.M.; Ferenc, M., et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. J Am Coll Cardiol 48 (2006): 1742–1750.

[61] McCall, K.E.L. and Kennerleyl, P. Proton pump inhibitors - differences emerge in hepatic metabolism. Digest Liver Dis 34 (2002): 461-467.

[62] Siller-Matula, J.M.; Spiel, A.O.; Lang, I.M.; Kreiner, G.; Christ, G. and Jilma, B. Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel. Am Heart J 157 (2009): 148.e141-148.e145.

[63] Small, D.S.; Farid, N.A.; Payne, C.D.; Weerakkody, G.J.; Li, Y.G.; Brandt, J.T., et al. Effects of the proton pump inhibitor lansoprazole on the pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel. J Clin Pharmacol 48 (2008): 475-484.

[64] Ho, P.M.; Maddox, T.M.; Wang, L.; Fihn, S.D.; Jesse, R.L.; Peterson, E.D., et al. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. JAMA 301 (2009): 937-944.

[65] Forgacs, I. and Loganayagam, A. Overprescribing proton pump inhibitors. BMJ 336 (2008): 2-3.

[66] Canadian Agency for Drugs and Technologies in Health. Proton pump inhibitor [online] Available from: http://www.cadth.ca/index.php/en/compus/current-topics/ppis [2009, 22 March].

[67] Li, X.-Q.; Andersson, T.B.; Ahlstrom, M. and Weidolf, L. Comparison of inhibitory effects of the proton pump-inhibiting drugs omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole on human cytochrome P450 activities. Drug Metab Dispos 32 (2004): 821-827.

[68] Müller, I.; Besta, F.; Schulz, C.; Li, Z.; Massberg, S. and Gawaz, M. Effects of statins on platelet inhibition by a high loading dose of clopidogrel. Circulation 108 (2003): 2195-2197.

[69] Tassaneeyakul, W.; Mahatthanatrakul, W.; Niwatananun, K.; Na-Bangchang, K.; Tawalee, A.; Krikreangsak, N., et al. *CYP2C19* genetic polymorphism in Thai, Burmese and Karen populations. Drug Metab Pharmacokinet 21 (2006): 286-290.

[70] Tassaneeyakul, W.; Tawalee, A.; Tassaneeyakul, W.; Kukongviriyapan, V.; Blaisdell, J.; Goldstein, J.A., et al. Analysis of the CYP2C19 polymorphism in a North-eastern Thai population. Pharmacogenetics 12 (2002): 221-225.

[71] Yamada, S.; Onda, M.; Kato, S.; Matsuda, N.; Matsuhisa, T.; Yamada, N., et al. Genetic differences in CYP2C19 single nucleotide polymorphisms among four Asian populations. J Gastroenterol 36 (2001): 669–672.

[72] Matetzky, S.; Fefer, P.; Shenkman, B.; Varon, D.; Savion, N. and Hod, H. Effectiveness of reloading to overcome clopidogrel nonresponsiveness in patients with acute myocardial infarction. Am J Cardiol doi:10.1016/j.amjcard.2008.04.028 (2008).

[73] Snoep, J.D.; Hovens, M.M.C.; Eikenboom, J.C.J.; Bom, J.G.v.d.; Jukema, J.W. and Huisman, M.V. Clopidogrel nonresponsiveness in patients undergoing percutaneous coronary intervention with stenting: a systematic review and meta-analysis. Am Heart J 154 (2007): 221-231.

[74] Bhatt, D.L.; Scheiman, J.; Abraham, N.S.; Antman, E.M.; Chan, F.K.L.; Furberg, C.D., et al. ACCF/ACG/AHA 2008 expert consensus document on reducing the gastrointestinal risks of antiplatelet therapy and NSAID Use: A report of the American college of cardiology foundation task force on clinical expert consensus documents. Circulation 118 (2008): 1894-1909.

[75] Ang, L. and Mahmud, E. Monitoring oral antiplatelet therapy: is it justified? Ther Adv Cardiovasc Dis 2 (2008): 485-496.

APPENDIX

Table 20: Data of individual patients

No.	Sex	Age (years)	BMI (Kg/m^2)	Patient's group	PPIs	Cl_{Cr} (ml/min)	Platelet count (x $10^3/mm^3$)	% MPA at Baseline	% MPA after treatment	Duration of taking PPIs (days)
1	Male	64	25.78	PCI	Omeprazole	49.76	197	54.0	48.2	18
2	Male	65	32.27	CAD	Rabeprazole	62.50	279	28.9	61.2	39
3	Female	70	28.44	PCI	Rabeprazole	97.79	207	63.6	76.1	32
4	Male	64	17.65	PCI	Omeprazole	38.45	298	17.9	78.5	30
5	Male	74	27.55	PCI	Rabeprazole	45.83	229	33.0	23.4	22
6	Male	72	19.38	PCI	Rabeprazole	38.56	160	23.0	68.7	23
7	Female	61	25.72	PCI	Omeprazole	62.95	169	18.5	68.8	26
8	Female	61	25.00	PCI	Omeprazole	74.61	217	35.2	102.1	26
9	Male	57	26.37	PCI	Omeprazole	74.93	190	6.6	33.4	39
10	Male	61	24.38	PCI	Rabeprazole	49.74	224	57.5	59.0	20
11	Male	63	24.57	CAD	Omeprazole	58.41	279	53.6	94.5	27
12	Male	54	23.59	PCI	Omeprazole	70.58	236	47.5	54.0	43
13	Female	56	24.80	PCI	Rabeprazole	61.98	199	40.1	36.6	35
14	Male	64	28.52	PCI	Rabeprazole	64.21	204	37.1	34.9	31
15	Male	49	23.53	CAD	Omeprazole	85.94	206	40.8	34.9	26
16	Male	57	22.49	PCI	Rabeprazole	62.44	285	49.3	31.0	18

Table 20: Data of individual patients (continue)

No.	Sex	Age (years)	BMI (Kg/m^2)	Patient's group	PPIs	Cl_{Cr} (ml/min)	Platelet count (x $10^3/mm^3$)	% MPA at Baseline	% MPA after treatment	Duration of taking PPIs (days)
17	Male	69	27.97	PCI	Rabeprazole	96.15	240	41.4	62.0	18
18	Male	65	23.24	PCI	Omeprazole	83.33	324	31.8	46.6	14
19	Male	61	29.67	PCI	Omeprazole	73.67	132	55.4	56.9	25
20	Male	57	26.18	CAD	Rabeprazole	105.19	249	16.8	63.7	24
21	Male	41	22.23	CAD	Omeprazole	85.25	391	59.9	60.5	35
22	Male	75	26.13	CAD	Rabeprazole	43.33	182	23.1	71.0	26
23	Male	50	26.12	CAD	Omeprazole	125.00	203	31.5	63.8	33
24	Male	61	27.17	CAD	Rabeprazole	99.85	215	9.1	37.8	38
25	Female	72	21.60	CAD	Omeprazole	29.97	336	33.3	52.7	49
26*	Male	54	31.96	CAD	Omeprazole	103.92	378	52.5	-	-
27	Male	51	29.38	CAD	Rabeprazole	76.07	198	35.8	36.4	42
28	Male	43	20.76	CAD	Omeprazole	73.48	320	33.3	71.1	14
29	Male	65	30.11	CAD	Rabeprazole	75.76	230	28.3	59.5	49
30	Female	77	26.22	CAD	Omeprazole	62.69	313	69.9	74.1	46
31	Female	73	22.51	CAD	Rabeprazole	37.39	214	36.9	77.8	15
32	Male	51	27.55	CAD	Rabeprazole	92.71	456	4.1	4.6	27

* Excluded because lack of blood sample

Table 20: Data of individual patients (continue)

No.	Sex	Age (years)	BMI (Kg/m^2)	Patient's group	PPIs	Cl_{Cr} (ml/min)	Platelet count (x $10^3/mm^3$)	% MPA at Baseline	% MPA after treatment	Duration of taking PPIs (days)
33	Male	87	22.23	CAD	Omeprazole	35.11	217	55.2	55.4	27
34[f]	Male	64	25.00	CAD	Rabeprazole	67.56	205	1.9	67.7	40
35[*]	Male	59	22.23	CAD	Omeprazole	73.13	285	44.0	-	-
36	Male	71	24.24	CAD	Rabeprazole	57.50	189	61.7	73.2	26
37	Female	75	22.77	CAD	Omeprazole	29.60	215	57.5	51.7	30
38[*]	Male	66	25.91	CAD	Rabeprazole	76.06	200	21.7	-	-
39	Female	53	30.54	CAD	Omeprazole	96.84	265	58.0	75.1	31
40	Male	52	27.61	CAD	Rabeprazole	85.56	325	68.4	51.5	90
41	Female	76	18.67	CAD	Omeprazole	26.44	313	52.4	37.4	56
42	Female	48	25.00	CAD	Rabeprazole	139.02	320	13.3	71.0	87
43	Female	80	24.97	CAD	Rabeprazole	28.33	264	62.5	25.5	15
44	Male	47	29.41	CAD	Omeprazole	92.57	296	5.0	6.0	25
45	Male	64	30.11	CAD	Rabeprazole	70.37	286	21.3	27.0	55
46	Male	58	27.36	CAD	Omeprazole	60.74	168	24.5	37.1	32
47	Male	58	26.03	CAD	Rabeprazole	79.72	267	64.3	60.4	39
48	Male	53	23.57	CAD	Omeprazole	110.26	224	35.9	39.2	70

[*]Excluded because lack of blood sample, [f] Outlier

Table 20: Data of individual patients (continue)

No.	Sex	Age (years)	BMI (Kg/m^2)	Patient's group	PPIs	Cl_{Cr} (ml/min)	Platelet count (x $10^3/mm^3$)	% MPA at Baseline	% MPA after treatment	Duration of taking PPIs (days)
49	Male	62	27.68	CAD	Omeprazole	66.67	244	38.8	59.7	67
50	Female	80	21.00	CAD	Rabeprazole	21.72	332	35.0	35.0	48
51	Male	74	24.38	CAD	Rabeprazole	62.33	282	41.2	45.8	61
52	Male	60	16.85	CAD	Omeprazole	87.04	352	26.2	47.1	29
53	Male	56	22.79	CAD	Omeprazole	67.08	210	5.6	22.4	15
54	Male	76	20.20	CAD	Rabeprazole	32.59	211	31.4	63.9	16
55	Male	56	24.91	CAD	Omeprazole	78.17	201	64.0	60.0	14
56	Male	75	24.61	CAD	Rabeprazole	51.70	210	57.0	50.4	14
57	Male	73	19.53	CAD	Omeprazole	42.30	305	14.3	48.7	77
58	Male	49	22.72	CAD	Rabeprazole	78.13	285	37.9	23.7	73
59	Male	71	27.34	CAD	Omeprazole	55.90	230	41.3	59.8	56
60	Female	68	31.59	CAD	Omeprazole	121.83	235	33.9	14.9	14
61	Male	58	30.12	CAD	Rabeprazole	121.01	228	13.1	8.2	14
62	Male	63	25.10	CAD	Rabeprazole	53.47	177	15.4	44.5	19
63[f]	Male	63	26.99	CAD	Rabeprazole	83.42	199	4.6	38.2	32
64	Male	42	24.22	CAD	Omeprazole	86.62	211	24.2	49.0	33

[f] Outlier

Table 20: Data of individual patients (continue)

No.	Sex	Age (years)	BMI (Kg/m^2)	Patient's group	PPIs	Cl_{Cr} (ml/min)	Platelet count (x $10^3/mm^3$)	% MPA at Baseline	% MPA after treatment	Duration of taking PPIs (days)
65	Male	51	22.92	CAD	Omeprazole	79.79	198	19.2	59.2	56
66	Male	70	24.22	CAD	Omeprazole	48.61	187	19.3	50.3	71
67	Female	68	29.14	CAD	Rabeprazole	60.00	337	39.1	45.3	33
68	Male	55	21.97	CAD	Rabeprazole	81.33	227	33.5	59.9	62
69	Male	59	23.83	CAD	Omeprazole	65.25	561	76.5	62.4	14
70	Male	67	23.44	CAD	Rabeprazole	67.59	284	62.5	57.6	70
71	Male	57	22.15	CAD	Omeprazole	61.48	238	51.8	52.4	32
72	Male	63	24.98	CAD	Rabeprazole	60.60	358	75.8	73.0	70
73	Female	72	22.22	CAD	Omeprazole	44.60	294	52.2	62.7	35
74	Male	70	25.25	CAD	Omeprazole	45.37	250	31.6	43.7	36
75	Female	82	20.00	CAD	Omeprazole	38.52	225	39.2	70.2	31
76	Male	33	31.64	CAD	Rabeprazole	144.90	234	56.2	50.6	21
77	Male	71	27.25	CAD	Rabeprazole	56.03	223	17.2	69.9	33
78	Female	68	25.39	CAD	Rabeprazole	92.08	194	71.0	53.9	14
79	Male	52	25.61	CAD	Rabeprazole	129.21	303	8.0	34.7	49
80	Male	71	26.95	CAD	Omeprazole	82.66	245	72.2	95.7	26

Table 20: Data of individual patients (continue)

No.	Sex	Age (years)	BMI (Kg/m^2)	Patient's group	PPIs	Cl_{Cr} (ml/min)	Platelet count (x $10^3/mm^3$)	% MPA at Baseline	% MPA after treatment	Duration of taking PPIs (days)
81	Male	71	25.00	CAD	Omeprazole	55.76	287	34.6	35.3	28
82	Female	76	21.88	CAD	Rabeprazole	52.89	227	64.5	62.2	19
83	Male	76	25.71	CAD	Rabeprazole	41.48	352	63.9	41.0	14
84	Male	67	21.91	CAD	Omeprazole	45.29	272	71.7	82.5	56
85[f]	Male	41	26.93	CAD	Rabeprazole	95.00	219	1.1	29.0	14
86	Male	70	25.78	CAD	Omeprazole	49.36	159	61.1	58.5	30
87	Male	58	25.00	CAD	Rabeprazole	56.07	185	31.5	19.4	34
88	Male	57	33.75	CAD	Omeprazole	97.46	209	41.7	65.3	35
89	Female	68	23.44	CAD	Omeprazole	51.00	326	52.3	54.4	28
90	Male	70	25.78	CAD	Rabeprazole	53.47	300	21.0	45.9	26

[f] Outlier

VITAE

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