Post-$P2Y_{12}$-receptor signalling mechanisms and platelet responses to clopidogrel

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Abstract

Anti-aggregatory agents such as clopidogrel limit platelet responses to adenosine diphosphate (ADP) by blocking the $P2Y_{12}$ receptor and have pivotal roles in the prevention of coronary artery stent thrombosis. However anti-aggregatory responses to such agents vary between individuals and this may predispose to stent thrombosis: the bases for this variablility are incompletely understood. Loss of function CYP2C19 genotypes, impeding clopidogrel bioactivation, have received considerable attention in the literature. However, this alone does not account for the increased rates of lack of response to clopidogrel commonly seen in diseases such as diabetes, obesity and acute coronary syndromes. Furthermore, assessment of aggregation on treatment, while clinically useful, does not precisely measure response to anti-aggregatory therapy, and standardisation of drug dosing may lead to relative underdosing in obese individuals.

$P2Y_{12}$ receptor activation by ADP is linked via Giα protein, to inhibition of adenylate cyclase and thus suppression of cyclic AMP formation. This results in nett pro-aggregatory effects. Hence clopidogrel acts in part by reversing this cascade.

A number of prostanoids including prostacyclin and prostaglandin E_1 function as physiological activators of adenylate cyclase and have been shown to potentiate the effects of $P2Y_{12}$ inhibitors. Furthermore the integrity of the prostanoid/adenylate cyclase signalling pathway is impaired in both cardiovascular disease states and diseases predisposing to cardiovascular disease such as obesity and diabetes. Cyclic AMP formation contributes, together with

that of cyclic GMP, to phosphorylation of vasomotor stimulated phosphoprotein (VASP) an effector pathway for ADP effects and their inhibition.

The principal hypothesis tested in this thesis was that adenylate cyclase signalling integrity predicted 7 day response to clopidogrel.

This was tested by measuring response to clopidogrel (both regarding changes in aggregation and in VASP phosphorylation) in both normal subjects and patients with known ischaemic heart disease. Integrity of prostanoid/adenylate cyclase signalling was measured via pre-clopidogrel platelet response to PGE_1. Putative determinants of clopidogrel response, including genotype and cGMP formation, were evaluated via univariate followed by multivariate analyses, and it was found that PGE_1 response (but not genotype) strongly predicted clopidogrel response.

In related analyses, it was also shown that

(i) weight adjusted clopidogrel dosing could be utilised to show that variable BMI does not markedly influence response to clopidogrel and that

(ii) presence of symptomatic cardiac disease was not a significant cause of variability in response.

Finally, exploratory analyses were undertaken to evaluate:-

(i) the role of insulin resistance

(ii) the putative involvement of the phosphoinositide 3-kinase (PI3-kinase) pathway and

(iii) the matricellular protein thrombospondin-1 (TSP-1) as further modulators of clopidogrel effect.

Overall the data demonstrate marked inter-individual heterogeneity of responsiveness to clopidogrel, which is engendered at least in part, by variable post-receptor signal transduction.

Declaration

I, Nicola Leigh Hurst, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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Nicola Hurst

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List of abbreviations

ABCB1	ATP Binding Casette B1
ACS	Acute coronary syndrome
ADP	Adenosine Diphosphate
ADMA	Asymmetric dimethylargine
Akt	Previously Protein kinase B (PKB)
ANCOVA	Analysis of covariance
ATP	Adenosine Triphosphate
BMI	Body Mass Index
BSA	Body surface area
cAMP	Cyclic adenosine monophosphate
CAD	Coronary Artery Disease
CBE	Complete Blood Examination
CCB	Calcium channel blocker
CCF	Congestive cardiac failure
cGMP	Cyclic guanosine monophosphate
CHD	Coronary Heart Disease
CV	Coefficient of Variance
CYPs	Cytochrome P450s
DAG	Diacylglycerol
DAPT	Dual anti-platelet therapy
DMSO	Dimethyl Sulfoxide

DNA	Deoxyribosenucleic acid
ENT 1	Equilibrative nucleoside transporter 1
E-NTPDases	Ectonucleoside triphosphate dephosphorylases
GP	Glycoprotein
GPCR	G protein coupled receptor
HOMA-IR	Homeostasis Model Assessment of Insulin Resistance
HTPR	High on treatment platelet reactivity
IHD	Ischaemic heart disease
IMS	Invaginated membrane system
IP	Prostacyclin receptor
IP_3	Inositol (1,4,5) trisphosphate
IR	Insulin Resistance
MACE	Major Adverse Coronary Events
MFI	Median Fluorescence Intensity
MI	Myocardial Infarction
MLCk	Myosin light chain kinase
NO	Nitric Oxide
P-AM	Prasugrel active metabolite
PARs	Protease-activated Receptors.
PCI	Percutaneous coronary investigation
PCOS	Polycystic Ovarian Syndrome
PCR	Polymerase chain reaction
PDK-1	Phosphadylinositol-dependent kinase 1

PGE_1	Prostaglandin E_1
PGI_2	Prostaglandin I_2 or prostacyclin
PI3-kinase	Phosphoinositide 3-kinase
PIP_2	Phosphatidylinositol-4,5-bisphosphate
PKA	Protein kinase A
PKC	Protein kinase C
PKG	Protein Kinase G
PLC	Phospholipase C
Plk	Polo like kinase
PPIs	Proton pump inhibitors
PRI	Platelet reactivity index
PRP	Platelet Rich Plasma
PVD	Peripheral vascular disease
ROC	Receiver Operating Curve
SAP	Stable Angina Pectoris
SD	Standard Deviation
sGC	Soluble guanylate cyclase
SNP	Sodium nitroprusside
SQ22536	9-(Tetrahydro-2-furanyl)9*H*-purin-6-amine
SPA	Spontaneous Platelet Aggregation
STEMI	ST elevation myocardial infarction
TF	Tissue Factor
T2DM	Type 2 diabetes mellitus

TSP-1	Thrombospondin-1
VASP	Vasomotor-stimulated phosphoprotein
vWF	Von Willebrand Factor
WBA	Whole Blood Aggregometry

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