Post-P2Y$_{12}$-receptor signalling mechanisms and platelet responses to clopidogrel

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Table of Contents  iii
List of Figures  vii
List of Tables  xii
Abstract  xiv
Declaration  xvii
Scholarships related to this thesis  xviii
Publications/presentations/abstracts related to this thesis  xix
List of Abbreviations  xxi
Acknowledgements  xxv
Table of Contents

CHAPTER 1: INTRODUCTION ................................................................. 1

1.1 Role of platelets in cardiovascular homeostasis ............................. 1

1.2 Platelet structure and function .................................................. 2

1.3 Physiological modulators of platelet function .................................. 6
  1.3.1 Anti-aggregatory modulators .................................................. 6
  1.3.2 Endothelium ........................................................................... 6
    1.3.2.1 Endothelial regulation of platelet activity ......................... 7
  1.3.3 Prostacyclin ............................................................................ 10
  1.3.4 Nitric Oxide ............................................................................ 13
  1.3.5 Adenosine ................................................................................. 13

1.4 Pro-aggregatory modulators .......................................................... 16
  1.4.1 Physical basis for platelet aggregation – focus on P2Y<sub>1</sub> and P2Y<sub>12</sub> receptor based, ADP mediated aggregation .................. 17
    1.4.1.1 Role of ADP in platelet aggregation .................................... 20

1.5 Vasomotor Stimulated Phosphoprotein (VASP) ............................... 22
  1.5.1 VASP phosphorylation ............................................................ 23
  1.5.2 Use of the 16C2 monoclonal antibody to detect Serine 239 VASP phosphorylation .......................................................... 26

1.6 Disease states with abnormal PG<sub>12</sub>/cAMP platelet responses ............ 27

1.7 Disease states associated with abnormal NO/cGMP platelet responses .... 28

1.8 Mechanism of action of P2Y<sub>12</sub> receptor antagonist drugs: thienopyridines and directly acting inhibitors ................................................................. 31

1.9. How is the efficacy of P2Y<sub>12</sub> receptor antagonists assessed? ............... 38
  1.9.1 Platelet aggregometry .............................................................. 39
  1.9.2 Flow Cytometry based techniques .......................................... 40
    1.9.2.1 The Platelet VASP Assay ................................................. 41
  1.9.2.2 VASP Fix ............................................................................ 42
  1.9.3 VerifyNow P2Y<sub>12</sub> Test ......................................................... 42
  1.9.4 Multiple Electrode Aggregometry ........................................... 43
  1.9.5 The PFA-100 system ............................................................... 43

1.10 P2Y<sub>12</sub> antagonists: clinical utility studies: impact on stent thrombosis ...... 44
  1.10.1 Basis for dual anti-aggregatory therapy .................................... 44
  1.10.2 Stent thrombosis ................................................................. 44

1.11 Clinical evidence for the use of P2Y<sub>12</sub> antagonists in cardiovascular disease .... 45

1.12 What is the evidence that efficacy of anti-aggregatory drugs (e.g. clopidogrel) is sometimes reduced? ......................................................... 48

1.13 High on treatment Platelet Reactivity ............................................. 49
1.14 What are the postulated mechanisms of high on treatment platelet reactivity?
Genomic vs non-genomic mechanisms

1.14.1 Genomic mechanisms
1.14.1.1 Pharmacogenomics
1.14.1.1.1 Role of CYP2C19 genotype
1.14.1.1.2 Role of ABCB1
1.14.1.3 Drug Interactions involving bioactivation
1.14.1.3.1 Drug interactions involving CYP2C19
1.14.1.3.1.1 Proton Pump Inhibitors (PPIs)
1.14.1.3.2 Drug interactions involving CYP3A4
1.14.1.3.2.1 Calcium Channel Blockers (CCBs)
1.14.1.3.2.2 Statins
1.14.1.4 Mutations of the P2Y12 receptor
1.14.1.5 Platelet Hyperreactivity

1.14.2 Non-genomic mechanisms
1.14.2.1 Platelet Hyperreactivity
1.14.2.2 Insulin resistance
1.14.2.3 Diabetes
1.14.2.4 Cigarette Smoking
1.14.2.5 Increased BMI/body weight

1.15 Scope of the present study

CHAPTER 2: EXPERIMENTAL METHODOLOGIES

2.1 Platelet aggregometry
2.1.1 Specimen Collection
2.1.2 Platelet aggregometry methods
2.1.3 Addition of reagents and potential modulators of platelet aggregation

2.2 VASP-Phosphorylation (VASP-P) evaluation to determine VASP-PRI
2.2.1 Establishing flow cytometric analysis of VASP phosphorylation
2.2.2 Platelet VASP kit reagents
2.2.3 Method -VASP-P Protocol
2.2.4 Instrument set-up and data acquisition

2.3 Genotyping for CYP 2C19

2.4 Additional Baseline testing: Complete Blood Examination, fasting glucose and fasting insulin levels

2.5 Evaluation of platelet responsiveness to inhibitors of aggregation

CHAPTER 3: EVALUATION OF PATHWAYS INVOLVED IN PLATELET CAMP AND PI3-KINASE SIGNALLING

3.1 Introduction and Objectives

3.2 Prostaglandin E1
3.2.1 Methodological Experiments
3.2.2 Results

3.3 PI3-kinase signalling in platelets
3.3.1 Introduction ......................................................................................................................... 91
3.3.2 Wortmannin as a platelet kinase inhibitor ............................................................................. 91
3.3.3 Vehicle issues with wortmannin ............................................................................................ 94

3.4 Experiments to evaluate the contribution of PI3-kinase signalling on ADP induced platelet aggregation using the pan-PI3-kinase inhibitor wortmannin ........................................ 96
3.4.1 Methodological experiments in healthy subjects not taking clopidogrel using wortmannin ...................................................................................................................................................... 96

3.5 Results ....................................................................................................................................... 97

3.6 Discussion .................................................................................................................................. 100

CHAPTER 4: POST-P2Y₁₂ RECEPTOR DETERMINANTS OF CLOPIDOGREL EFFECT .................. 102

4.1 Introduction ................................................................................................................................ 102

4.2 Objectives of the study .............................................................................................................. 105

4.3 Study design ............................................................................................................................. 106
4.3.1 Participant selection and recruitment .................................................................................... 106

4.4 Study Protocol ........................................................................................................................... 108
4.4.1 Overview .................................................................................................................................. 108
4.4.2 Components ............................................................................................................................. 109

4.4 STATISTICAL METHODS .......................................................................................................... 112

4.5 RESULTS .................................................................................................................................... 116
4.5.1 Impact of dose-adjustment strategy .................................................................................... 118
4.5.2 Heterogeneity of platelet responsiveness to PGE₁ .............................................................. 120
4.5.3 Effect of clopidogrel on ADP-induced aggregation and VASP-PRI in subjects with and without loss of function CYP 2C19 genotypes .............................................................................................. 121
4.5.4 Primary hypotheses: Impact of platelet responses to PGE₁ and SNP and clopidogrel effect ............................................................................................................................................ 124
4.5.6 BMI/body weight and clopidogrel effect .............................................................................. 127
4.5.7. Symptomatic ischaemic heart disease and clopidogrel effect ........................................... 132
4.5.8 Investigation of ADP stimulated platelet aggregation using the pan-kinase inhibitor wortmannin ............................................................................................................................................ 134
4.5.9 Impact of Insulin Resistance ............................................................................................... 138
4.5.10 Effect of obesity on response to clopidogrel ......................................................................... 141

4.6 DISCUSSION ............................................................................................................................... 145

CHAPTER 5: MODULATION OF CAMP PATHWAY: TSP-1 AND SQ 22536 .................................. 155

5.1 Thrombospondin-1 (TSP-1) ..................................................................................................... 155
5.5.1 Introduction ............................................................................................................................ 155

5.2 Signalling pathways downstream of the CD36 receptor ........................................................... 156
5.2.1 Syk dependent CD36 signalling

5.2.2 TSP-1 modulation of cAMP/PKA is CD36-dependent

5.3 TSP-1 modulates ADP-stimulated PGE_1 responses

5.3.1 Methods

5.3.2 Results

5.3.3 Discussion

5.4 Modulation of cAMP pathway using the adenylate cyclase inhibitor SQ22536

5.4.1 Introduction

5.4.2 Methods

5.4.3 Results

5.5 Discussion

CHAPTER 6: SUMMARY AND FUTURE PERSPECTIVES

6.1 Key Investigation Findings

6.2 Future perspectives

REFERENCES

APPENDIX

Published work in whole contained within this thesis (PhD hard copy only)
List of Figures

Figure 1.1  Electron Microscopy cross section of a resting human platelet…4.
Figure 1.2  Metabolism of prostacyclin from membrane phospholipids……11.
Figure 1.3  Adenosine formation after ATP release and uptake and breakdown by erythrocytes…………………………………………………………15.
Figure 1.4  ADP stimulated platelet aggregation…………………………..19.
Figure 1.5  VASP domains, interacting proteins, and PKA and PKG mediated phosphorylation sites………………………………………………….25.
Figure 1.6  Metabolic pathways of ticlopidine…………………………….32.
Figure 1.7  Metabolic pathways for clopidogrel biotransformation, including formation of active metabolite……………………………………….34.
Figure 1.8  Metabolic pathways for prasugrel biotransformation, including formation of active metabolite……………………………………….36.
Figure 1.9  Potential bases for high on treatment platelet reactivity (HTPR).50
Figure 2.1  Method for determination of inhibition of platelet aggregation using PGE₁………………………………………………………….71.
Figure 2.2  Examples of scatter plots obtained using FACS Canto II flow cytometer……………………………………………………………79.
Figure 3.1  Known effects of PGE₁ and wortmannin in human platelets……87.
Figure 3.2  Whole blood aggregometry PGE₁ concentration response curve in response to 5µM ADP………………………………………………….90.
Figure 3.3  Light Transmission Aggregometry PGE₁ dose concentration response curve in response to 5µM ADP…………………..90.
Figure 3.4 Effect of DMSO on 5µM ADP induced whole blood platelet aggregation at final concentrations of 1% and 0.1%................95.

Figure 3.5 Heterogeneity of inhibition of ADP-induced aggregation by wortmannin. Quartiles of response in the presence of 5µM ADP in 20 healthy subjects are shown.................................99.

Figure 4.1 Subject clopidogrel dose per unit weight, according to body weight in kilograms.................................................118.

Figure 4.2 Impact of clopidogrel dose per unit weight on measures of clopidogrel effect.......................................................119.

Figure 4.3 Heterogeneity of platelet responsiveness to PGE1. Basis for key evaluations.......................................................120.

Figure 4.4 Changes in ADP-induced aggregation before and after 7 days of clopidogrel therapy (A) ADP induced aggregation (B) VASP PRI following 7 days of clopidogrel therapy...............122 & 123.

Figure 4.5 Relationship between pre-clopidogrel platelet response to PGE1 and response to clopidogrel, measured via (A) ΔADP and (B) ΔVASP-P..............................................................125.

Figure 4.6 Relationship between pre-clopidogrel platelet response to SNP and response to clopidogrel, measured as (A) ΔADP and (B) ΔVASP-P..............................................................125.

Figure 4.7 Impact of variable BMI on clopidogrel response measured via (A) ΔADP and (B) ΔVASP-P......................................................128.
Figure 4.7  Impact of BMI and body weight on clopidogrel response as measured by ΔADP in (C) non-obese vs obese subjects and (D) subjects ≤80kg and >80kg. .................................128.

Figure 4.7  Impact of BMI and body weight on clopidogrel response as measured by ΔVASP-P in (E) non-obese and obese subjects and (F) subjects ≤80kg and >80kg. .................................129.

Figure 4.8  Pre clopidogrel platelet responses to (A) PGE₁ and (B) SNP vs subject BMI. .................................................................130.

Figure 4.8  Non-obese vs obese pre-clopidogrel platelet response to (C) PGE₁ and (D) SNP ...............................................................131.

Figure 4.8  Body weight ≤ 80kg and >80kg and pre-clopidogrel platelet response to (E) PGE₁ and (F) SNP .................................131.

Figure 4.9  Comparisons of healthy subjects (HS) and patients with ischaemic heart disease (IHD) measuring clopidogrel effect using (A) ΔADP and (B) ΔVASP-P .................................................................133.

Figure 4.10 Comparisons of healthy subjects (HS) and patients with ischaemic heart disease (IHD) pre-clopidogrel platelet responses to (A) PGE₁ and (B) SNP .................................................................133.

Figure 4.11 Responses of all subjects to 100nM wortmannin correlated with pre-clopidogrel platelet response to (A) PGE₁ and (B) SNP…137.

Figure 4.12 Responses of all subjects to 100nM wortmannin correlated with (A) ΔADP and (B) ΔVASP ..........................137.

Figure 4.13 HOMA-IR vs (A) ΔADP and (B) ΔVASP-P respectively.....139.
**Figure 4.14** Correlations between HOMA-IR and pre-clopidogrel platelet response to (A) PGE₁ and (B) SNP…………………………..139.

**Figure 4.15** Effect of dose per unit weight clopidogrel on (A) ΔADP and (B) ΔVASP-P, in non-obese vs obese subjects………………..143.

**Figure 4.16** Effect of obesity on pre-clopidogrel platelet response to PGE₁ vs ΔVASP-P……………………………………..…………144.

**Figure 5.1** Signalling pathway for immobilized TSP-1 and oxLDL via CD 36 …………………………………………………………………………………157.

**Figure 5.2** Modulation of PGE₁ effect on platelet cyclic AMP generation and PKA by plasma TSP-1 via the CD36 receptor……………….158.

**Figure 5.3** Platelet aggregogram. TSP-1 is able to partially reverse PGE₁ platelet response in using ADP stimulation……………….161.

**Figure 5.4** Individual subject examples of reversal of PGE₁ effect with varying concentrations of TSP-1…………………………….161.

**Figure 5.5** Whole blood aggregometry responses to ADP 2.5 µM; effects of co-incubation with (i) SQ 22536 400µM, (ii) PGE₁ to inhibit platelet aggregation by >40% (varying concentrations) and (iii) PGE₁ and SQ22536 400µM………………………166.

**Figure 5.6** Platelet rich plasma optical aggregometry responses to 5µM ADP; effects of co-incubation with (i) varying concentrations of PGE₁ (ii) PGE₁ and SQ22536 100µM and (iii) SQ22536 100µM….167.

**Figure 5.7** Theoretical effect of SQ22536 on ADP-induced platelet aggregation responses to wortmannin…………………………….169.
**Figure 5.8** Whole blood (A) and platelet rich plasma (B) aggregometry responses to 5µM ADP; effects of co-incubation with (ii) SQ22536 400 µM (iii) wortmannin 100nM (iv) wortmannin 100 nM and SQ22536 400µM…………………………………………170.
List of Tables

Table 1.1  Mechanisms of endothelial dysfunction…………………………10.

Table 1.2  Pharmacology of commonly used anti-aggregatory anti-platelet drugs………………………………………………………………………30.

Table 2.1  Intraassay coefficients of variability (CV) for VASP-PRI at baseline and after 7 days of clopidogrel (1) within 4 hours and (2) at 24 hours post-sampling…………………………………………………………74.

Table 2.2  Interassay coefficients of variability (CV) for VASP-PRI at baseline and after 7 days of clopidogrel (1) within 4 hours and (2) at 24 hours and (3) 48 hours post-sampling………………………………74.

Table 2.3  CYP2C19 gene single nucleotide polymorphisms (SNPs) detected and their reference sample numbers……………………………………80.

Table 3.1  Known kinases and pro-aggregant signalling blocked by differing concentrations of wortmannin in human platelets……………….93.

Table 3.2  Participant demographics: Healthy subjects participating in the wortmannin pilot study………………………………………………………97.

Table 4.1  Participant demographics – The clinical study…………………..118.

Table 4.2  Multivariate Analysis – Primary hypothesis: independent determinants of the two measures of clopidogrel response tested, \( \Delta \text{ADP} \) and \( \Delta \text{VASP-P} \)…………………………………………………………126.

Table 4.3  Participant demographics – wortmannin substudy: investigation of ADP stimulated platelet aggregation using the pan-kinase inhibitor wortmannin……………………………………………………………………135.
Table 4.4  Multivariate analysis: correlates of $\Delta$VASP-P. Only PGE$_1$ response was found to be an independent correlate of $\Delta$VASP-P.

Table 4.5  Participant demographics of subjects undergoing analysis for (1) HOMA-IR vs (i) pre-clopidogrel response to PGE$_1$ and SNP and (ii) clopidogrel effect (Chapter 4.5.9) and (2) effect of obesity in response to clopidogrel (Chapter 4.5.10).
Abstract

Anti-aggregatory agents such as clopidogrel limit platelet responses to adenosine diphosphate (ADP) by blocking the P2Y\textsubscript{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 variability 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\textsubscript{12} receptor activation by ADP is linked via Gi\alpha 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\textsubscript{1} function as physiological activators of adenylate cyclase and have been shown to potentiate the effects of P2Y\textsubscript{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\textsubscript{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\textsubscript{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

September 2014
Scholarships and Awards related to this thesis

Australian Postgraduate Award, awarded for 2009, interrupted for maternity leave, received until March 2013

TQEH Research Foundation Scholarship 2010
Publications/presentations/abstracts related to this thesis


Nooney VB, Hurst NL, Chirkov YY, Horowitz JD, Acute effects of clopidogrel are predicted by integrity of prostacyclin signalling, European Society of Cardiology Meeting, Amsterdam, The Netherlands, 2013. Poster Presentation

Vivek Nooney, Nicola Hurst, John Horowitz, Jeffrey Isenberg, Yuliy Chirkov, Thrombospondin-1 exerts bidirectional effects on prostacyclin signalling in human platelets: implications regarding clopidogrel resistance, World Congress of Cardiology Meeting, Melbourne Australia, 2014
List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
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<tbody>
<tr>
<td>ABCB1</td>
<td>ATP Binding Casette B1</td>
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<tr>
<td>ACS</td>
<td>Acute coronary syndrome</td>
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<tr>
<td>ADP</td>
<td>Adenosine Diphosphate</td>
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<td>ADMA</td>
<td>Asymmetric dimethylarginine</td>
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<tr>
<td>Akt</td>
<td>Previously Protein kinase B (PKB)</td>
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<td>ANCOVA</td>
<td>Analysis of covariance</td>
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<td>ATP</td>
<td>Adenosine Triphosphate</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>BSA</td>
<td>Body surface area</td>
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<tr>
<td>cAMP</td>
<td>Cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CAD</td>
<td>Coronary Artery Disease</td>
</tr>
<tr>
<td>CBE</td>
<td>Complete Blood Examination</td>
</tr>
<tr>
<td>CCB</td>
<td>Calcium channel blocker</td>
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<tr>
<td>CCF</td>
<td>Congestive cardiac failure</td>
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<tr>
<td>cGMP</td>
<td>Cyclic guanosine monophosphate</td>
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<td>CHD</td>
<td>Coronary Heart Disease</td>
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<td>CV</td>
<td>Coefficient of Variance</td>
</tr>
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<td>CYPs</td>
<td>Cytochrome P450s</td>
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<tr>
<td>DAG</td>
<td>Diacylglycerol</td>
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<tr>
<td>DAPT</td>
<td>Dual anti-platelet therapy</td>
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<tr>
<td>DMSO</td>
<td>Dimethyl Sulfoxide</td>
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<td>GPCR</td>
<td>G protein coupled receptor</td>
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<tr>
<td>HOMA-IR</td>
<td>Homeostasis Model Assessment of Insulin Resistance</td>
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<tr>
<td>HTPR</td>
<td>High on treatment platelet reactivity</td>
</tr>
<tr>
<td>IHD</td>
<td>Ischaemic heart disease</td>
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<tr>
<td>IMS</td>
<td>Invaginated membrane system</td>
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<tr>
<td>IP</td>
<td>Prostacyclin receptor</td>
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<tr>
<td>IP₃</td>
<td>Inositol (1,4,5) trisphosphate</td>
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<tr>
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<td>Insulin Resistance</td>
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<tr>
<td>NO</td>
<td>Nitric Oxide</td>
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<tr>
<td>P-AM</td>
<td>Prasugrel active metabolite</td>
</tr>
<tr>
<td>PARs</td>
<td>Protease-activated Receptors.</td>
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<tr>
<td>PCI</td>
<td>Percutaneous coronary investigation</td>
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<td>PCOS</td>
<td>Polycystic Ovarian Syndrome</td>
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<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>PDK-1</td>
<td>Phosphatidylinositol-dependent kinase 1</td>
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<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PGE₁</td>
<td>Prostaglandin E₁</td>
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<tr>
<td>PGI₂</td>
<td>Prostaglandin I₂ or prostacyclin</td>
</tr>
<tr>
<td>PI3-kinase</td>
<td>Phosphoinositide 3-kinase</td>
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<td>PIP₂</td>
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<td>Plk</td>
<td>Polo like kinase</td>
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<tr>
<td>PPIs</td>
<td>Proton pump inhibitors</td>
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<tr>
<td>PRI</td>
<td>Platelet reactivity index</td>
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<tr>
<td>PRP</td>
<td>Platelet Rich Plasma</td>
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<tr>
<td>PVD</td>
<td>Peripheral vascular disease</td>
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<tr>
<td>ROC</td>
<td>Receiver Operating Curve</td>
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<td>SAP</td>
<td>Stable Angina Pectoris</td>
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<td>SD</td>
<td>Standard Deviation</td>
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<tr>
<td>sGC</td>
<td>Soluble guanylate cyclase</td>
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<td>SNP</td>
<td>Sodium nitroprusside</td>
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<td>SQ22536</td>
<td>9-(Tetrahydro-2-furanyl)9H-purin-6-amine</td>
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<td>SPA</td>
<td>Spontaneous Platelet Aggregation</td>
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<td>ST elevation myocardial infarction</td>
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<td>TF</td>
<td>Tissue Factor</td>
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<td>Type 2 diabetes mellitus</td>
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<td>Acronym</td>
<td>Full Name</td>
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</tr>
<tr>
<td>TSP-1</td>
<td>Thrombospondin-1</td>
</tr>
<tr>
<td>VASP</td>
<td>Vasomotor-stimulated phosphoprotein</td>
</tr>
<tr>
<td>vWF</td>
<td>Von Willebrand Factor</td>
</tr>
<tr>
<td>WBA</td>
<td>Whole Blood Aggregometry</td>
</tr>
</tbody>
</table>
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