Platelet Function Beyond Hemostasis

Young-Hoon Jeong, M.D., Ph.D.

Director, Clinical Trial Center, Gyeongsang National University Hospital; Associate Professor, Department of Internal Medicine, Gyeongsang National University School of Medicine, Jinju, Korea.
## Disclosures

<table>
<thead>
<tr>
<th>Research Grants/Support</th>
<th>Honoraria/Consulting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Otsuka</td>
<td>Otsuka</td>
</tr>
<tr>
<td>Accumetrics</td>
<td>Sanofi-Aventis</td>
</tr>
<tr>
<td>Boehringer-Ingelheim</td>
<td>Daiichi Sankyo Inc</td>
</tr>
<tr>
<td>Haemonetics</td>
<td>Astrazeneca</td>
</tr>
<tr>
<td>Dong-A Pharmaceutical</td>
<td>Nanosphere</td>
</tr>
<tr>
<td>Han-Mi Pharmaceutical</td>
<td>Haemonetics</td>
</tr>
<tr>
<td></td>
<td>Han-Dok Pharmaceutical</td>
</tr>
</tbody>
</table>
Atherothrombosis: Clinical Manifestations

- Acute coronary syndromes
  - STEMI
  - NSTEMI
  - Unstable angina

- Stable CAD
- Atrial Fibrillation
- Angioplasty
- Bare metal stent
- Drug eluting stent
- CABG

- Abdominal aortic aneurysm (AAA)

- Stroke
- TIA
- Intracranial stenosis

- Carotid artery stenosis
- CEA
- Carotid stenting

- Renal artery stenosis
- Renal artery stenting

- Peripheral arterial disease
- Acute limb ischemia
- Claudication
- Amputation
- Endovascular stenting
- Peripheral bypass
- Abnormal ABI

Meadows TA, Bhatt DL. Circ Res. 2007;100:1261-1275.
# Platelet Function Beyond Hemostasis

**Diverse roles**
- Promoting inflammatory and immune response
- Maintaining vascular integrity
- Contributing wound healing

**Underlying mechanisms**
- Recruit leukocytes and progenitor cells to the sites of vascular injury or thrombosis
- Store, produce and release pro-inflammatory, anti-inflammatory and angiogenic factors and microparticles into the circulation
- Spur thrombin generation

## Disease entities related with platelets
- Endothelial dysfunction, atherosclerosis, restenosis, LV remodeling, cancer, IBD, RA, SLE, psoriasis, sepsis, acute lung injury, transplantation rejection...
# Platelet Granular and Secreted Molecules

<table>
<thead>
<tr>
<th><strong>α-Granules</strong></th>
<th><strong>Dense bodies</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Platelet-specific proteins</strong></td>
<td>ADP</td>
</tr>
<tr>
<td>Platelet factor 4</td>
<td>ATP</td>
</tr>
<tr>
<td>β-Thromboglobulin family*</td>
<td>Calcium</td>
</tr>
<tr>
<td>Multimerin</td>
<td>Serotonin</td>
</tr>
<tr>
<td><strong>Adhesive glycoproteins</strong></td>
<td>Pyrophosphate</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>GDP</td>
</tr>
<tr>
<td>von Willebrand factor</td>
<td>Magnesium</td>
</tr>
<tr>
<td>von Willebrand factor propeptide</td>
<td>Other secreted or released proteins</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Protease nexin I</td>
</tr>
<tr>
<td>Thrombospondin-1</td>
<td>Gas6</td>
</tr>
<tr>
<td>Vitronectin</td>
<td>Amyloid β-protein precursor (protease nexin II)</td>
</tr>
<tr>
<td><strong>Coagulation factors</strong></td>
<td>Tissue factor pathway inhibitor</td>
</tr>
<tr>
<td>Factor V</td>
<td>Factor XIII</td>
</tr>
<tr>
<td>Protein S</td>
<td>α1-Protease inhibitor</td>
</tr>
<tr>
<td>Factor XI</td>
<td>Complement 1 inhibitor</td>
</tr>
<tr>
<td><strong>Mitogenic factors</strong></td>
<td>High molecular weight kininogen</td>
</tr>
<tr>
<td>Platelet-derived growth factor</td>
<td>α2-Macroglobulin</td>
</tr>
<tr>
<td>Transforming growth factor-β</td>
<td>Vascular permeability factor</td>
</tr>
<tr>
<td>Endothelial cell growth factor</td>
<td>Interleukin-1β</td>
</tr>
<tr>
<td>Epidermal growth factor</td>
<td>Histidine-rich glycoprotein</td>
</tr>
<tr>
<td>Insulin-like growth factor I</td>
<td>Chemokines</td>
</tr>
<tr>
<td><strong>Angiogenic factors</strong></td>
<td>MIP-1α (CCL3)</td>
</tr>
<tr>
<td>Vascular endothelial growth factor</td>
<td>RANTES (CCL5)</td>
</tr>
<tr>
<td>Platelet factor 4 (inhibitor)</td>
<td>MCP-3 (CCL7)</td>
</tr>
<tr>
<td><strong>Fibrinolytic inhibitors</strong></td>
<td>Gro-α (CXCL1)</td>
</tr>
<tr>
<td>α2-Plasmin inhibitor</td>
<td>Platelet factor 4 (CXCL4)</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor-1</td>
<td>ENA-78 (CXCL5)</td>
</tr>
<tr>
<td><strong>Albumin</strong></td>
<td>NAP-2 (CXCL7)</td>
</tr>
<tr>
<td><strong>Immunoglobulins</strong></td>
<td>Interleukin-8 (CXCL8)</td>
</tr>
<tr>
<td><strong>Granule membrane-specific proteins</strong></td>
<td>TARC (CCL17)</td>
</tr>
<tr>
<td>P-selectin (CD62P)</td>
<td></td>
</tr>
<tr>
<td>CD63 (LAMP-3)</td>
<td></td>
</tr>
<tr>
<td>GMP 33</td>
<td></td>
</tr>
</tbody>
</table>
Endothelium-Platelet-Leukocyte Interaction

**Initial capture**
- Leukocyte: PSGL-1
- Blood: RANTES/CCL5, PAF, ENA-78, Groα, IL-1β
- Platelet: P-selectin

**Released mediators**
- (Mac-1) αMβ2
- αMβ2 or αVβ3
- Fibrinogen (fibrin) thrombospondin

**Firm adhesion**
- CD36 (GP IV) αLβ2
- αMβ2
- CD40

Relationship Between Thrombosis & Inflammation
A distinct pathophysiological state of heightened platelet reactivity to ADP, platelet activation, inflammation and hypercoagulability, marks the development of symptomatic CV disease from chronic stable disease.

Anti-inflammatory & Vasoprotective Effects of P2Y$_{12}$ Receptor Inhibitors

↑ Nitric Oxide
↑ Prostaglandin I$_2$
↓ Platelet-leukocyte aggregates
↓ P-selectin
↓ RANTES
↓ CD40 ligand
↓ CRP
↓ Tissue factor expression

Platelet Function:
Platelet-Leukocyte Aggregates and Inflammation
Effect of Cilostazol on HS-CRP
Type 2 DM patients with PAOD (n = 192)

Clopidogrel on PLT Activation and Inflammation

Symptomatic CAD on Aspirin: 5-week Clopidogrel (n = 77) vs. Placebo (n = 26)

- sCD40L, ng/ml
- RANTES, pg/ml
- hsCRP, mg/L
- NOx, µM

Heitzer T et al. ATVB 2006;26:1648-52.
Prasugrel vs. Clopidogrel on Platelet Activation

Stable CAD on Aspirin: 4-week Prasugrel (n = 55) vs. Clopidogrel (n = 55)

Prasugrel vs. Clopidogrel on Coagulation Activation

Stable CAD on Aspirin: 4-week Prasugrel (n = 55) vs. Clopidogrel (n = 55)

Relationship Between Inflammation and PFT

Stable CAD on Chronic DAPT (n = 1,223)
Relationship Between Fibrinogen and PFT

Stable CAD on Chronic DAPT (n = 1,223)

Platelet Function:

Endothelial Dysfunction and Atherosclerosis
Clopidogrel on Endothelial NO Bioavailability
Symptomatic CAD on Aspirin: 5-week Clopidogrel (n = 77) vs. Placebo (n = 26)

Heitzer T et al. ATVB 2006;26:1648-52.
HD Clopidogrel on Endothelial NO Bioavailability

PCI-treated Patients: 75 mg vs. 150 mg Clopidogrel (n = 50, 30-day cross-over)

Relationship btw Endothelial Dysfunction and HPR

PCI-treated Patients on Chronic DAPT (n = 103): HPR ≥ 230 PRU

RHI (Reactive Hyperemia Index): peripheral ED

Relationship Between Atheroma Burden and HPR

IVUS imaging in PCI-treated Patients (n = 335): PRU > 230 (32.5%)

* Adjustments for age, sex, DM, and CRF
Platelet Function:

Post-injury or Post-stent Neointimal Hyperplasia
CYP2C19 SNP on Intra-stent Thrombi and TLR

Follow-up OCT imaging in DES-treated Patients on DAPT (n = 125)

Thrombelastography (TEG®) Hemostasis System

- Whole blood test
- Measures global hemostasis
  - From clot initiation to clot lysis
  - Net effect of components

**Diagram:**
- Coagulation
- Fibrinolysis
- Platelets (MA)
- Platelet-Fibrin Clot strength (G)
- Enzymatic (R)
- Fibrinogen (K, α)
- Thrombolysins (Ly30, EPL)
- Clotting time
- Clot kinetics
- Clot stability
- Clot breakdown
Influence of HPR (LTA) and MA$_{KH}$ (TEG) on MACE

PCI-treated Patients on DAPT (n = 197): 2-year F/U (MACE, 12.7%)

Ischemia-driven TVR: 76% of MACE

"Platelet function or Clot strength" is associated with post-injury neointimal hyperplasia

Sustained P2Y$_{12}$ Inhibition by Ticagrelor to Prevent Subsequent Neointima

Figure 6. Representative carotid artery neointima sections in C57BL/6 mice treated with vehicle alone (A), ticagrelor before injury only (B), ticagrelor postinjury only at 4 and 24 hours (C), and ticagrelor before injury and 4 hours postinjury (D).
Platelet Function:

Post-MI Left Ventricular Remodeling
Role of Platelets in Mediating Inflammatory Responses for Post-MI LV Remodeling

Platelet-Leukocyte Accumulation in Infarced Myocardium (C57BL/6 mice)

Randomized treatment started 2 hrs after MI and lasted for 3 days

- Low-dose clopidogrel: 15/5/5 mg/kg vs.
- High-dose clopidogrel: 50/15/15 mg/kg vs.
- Prasugrel: 5/5/5 mg/kg vs.
- PD (platelet depletion) by CD41 antibody

Role of Platelets in Mediating Inflammatory Responses for Post-MI LV Remodeling
Platelet-Leukocyte Accumulation in Infarcted Myocardium (C57BL/6 mice)

Acute phase: LV rupture

Chronic phase: LV remodeling

Novel Role of Platelet Reactivity and Inflammation in LV Remodeling Following STEMI

REMODELING Study: PPCI-treated STEMI Patients on DAPT (n = 150)

LV Remodeling: a relative >20% increase in LV EDV between baseline and 1-month F/U

LVR by PRU quartile

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>10.8%</td>
</tr>
<tr>
<td>2nd</td>
<td>23.1%</td>
</tr>
<tr>
<td>3rd</td>
<td>27%</td>
</tr>
<tr>
<td>4th</td>
<td>35.1%</td>
</tr>
</tbody>
</table>

P = 0.015

LVR by hs-CPR quartile

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st</td>
<td>12.5%</td>
</tr>
<tr>
<td>2nd</td>
<td>16.7%</td>
</tr>
<tr>
<td>3rd</td>
<td>35.1%</td>
</tr>
<tr>
<td>4th</td>
<td>32.4%</td>
</tr>
</tbody>
</table>

P = 0.012

Cross-talk btw Platelet Reactivity and Inflammation in LV Remodeling Following STEMI

REMODELING Study: PPCI-treated STEMI Patients on DAPT (n = 150)

Predictors of LV Remodeling

<table>
<thead>
<tr>
<th>Predictor</th>
<th>p value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 65 year old</td>
<td>0.145</td>
<td>2.73 (0.71, 10.50)</td>
</tr>
<tr>
<td>Female.</td>
<td>0.259</td>
<td>2.08 (0.58, 7.41)</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>0.559</td>
<td>1.55 (0.36, 6.71)</td>
</tr>
<tr>
<td>Anemia</td>
<td>0.367</td>
<td>1.72 (0.53, 5.62)</td>
</tr>
<tr>
<td>PRU &lt; 248 and hs-CRP &lt; 1.4 mg/L vs.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRU ≥ 248 and hs-CRP &lt; 1.4 mg/L</td>
<td>0.010</td>
<td>5.99 (1.54, 23.26)</td>
</tr>
<tr>
<td>PRU &lt; 248 and hs-CRP ≥ 1.4 mg/L</td>
<td>0.001</td>
<td>12.50 (2.72, 58.82)</td>
</tr>
<tr>
<td>PRU ≥ 248 and hs-CRP ≥ 1.4 mg/L</td>
<td>&lt; 0.001</td>
<td>14.08 (3.61, 55.56)</td>
</tr>
<tr>
<td>LVID_{diastole} (per 1mm increment)</td>
<td>0.095</td>
<td>0.92 (0.83, 1.02)</td>
</tr>
<tr>
<td>LVEDV index (per 1mL/m² increment)</td>
<td>0.008</td>
<td>0.86 (0.77, 0.96)</td>
</tr>
<tr>
<td>LVESV index (per 1mL/m² increment)</td>
<td>0.029</td>
<td>0.84 (0.73, 0.98)</td>
</tr>
<tr>
<td>Post-PCI slow flow (TIMI flow 2)</td>
<td>0.170</td>
<td>3.06 (0.62, 15.11)</td>
</tr>
</tbody>
</table>

Platelet Function in CV Disorder

Key roles in “Atherothrombosis”

- “Platelet activation & aggregation” are related with hemostasis and thrombosis, endothelial dysfunction and atherosclerosis, inflammatory cascade, vascular, post-stent and myocardial repair…

- “Antiplatelet therapy” has potentials to prevent and control athero-thrombosis through multidisciplinary pathways.
Risk-Benefit Balance in Antiplatelet Therapy

“Classic Concept”

“New Concept”

Repair
Inflammation
Vasoprotection
Thanks for your attention