The Effects Of Protease-Activated Receptors 1 and 4 In Human Platelet Activation and Inflammation

Jianying Zhang, PhD; Jorge L. Rocha, BA; Justin Hicks, BS; Melissa McLane, DO; James H-C. Wang, PhD; MaCalus V. Hogan, MD

MechanoBiology Laboratory
Foot and Ankle Injury Research Laboratory [F.A.I.R]
Department of Orthopaedic Surgery
University of Pittsburgh School of Medicine
Tendon Injuries Are Common and Costly

- Tendinopathies are the main reason for consultation for a musculoskeletal complaint
  - Approximately 30% for a general practitioner
- Commonly injured tendons in the foot and ankle
  - Achilles Tendon
  - Posterior Tibial Tendon
  - Peroneal Tendon
  - Flexor Hallucis Longus Tendon

Controversial PRP Treatment

- The efficacy of PRP treatment for tendon injury is hotly debated in orthopaedic surgery and sports medicine
- “One-size-fits-all” approach
  - Platelets used in PRP treatment are completely activated and all factors are released at once
Hypothesis:

Platelets in PRP contain pro-angiogenic and anti-angiogenic factors, these different factors can be released when the platelets are selectively activated through protease-activated receptors 1 & 4 (PAR1, PAR4)

Methods:

• Human blood obtained from 12 healthy donors
• 9 ml of blood was mixed with 1 ml of 3.8% sodium citrate and centrifuged at 500g for 10 min
• The supernatant (PRP) without the buffy coat was centrifuged at 1000g for 10 min and the resulting pellet was washed in Tyrodes-HEPES buffer and centrifuged for 10 min at 1000g
• Finally, platelets in the pellet was suspended in Tyrodes-HEPES buffer and used in experiments.
Platelet Preparation

Blood : sodium citrate

9:1

500g 10 min

Plasma

PRP

Buffy Coat

Tyrodes-HEPES buffer

Red blood cells

1000g 10 min

Platelets
PAR1 and PAR 4 Receptors on Platelets

SFLLRN – An agonist, Ligand to PAR1
GYPGQV – An agonist, Ligand to PAR4
VEGF and Endostatin in Human Platelets Induced by PAR1 or PAR4 Activation

Growth Factor (%)

Control PAR1 PAR4

VEGF Endostatin

PAR1

PAR4

VEGF Endostatin
VEGF and Endostatin Release from Human Platelets Due to Selective Activation of PAR1 or PAR4

**Graph A:**
- X-axis: Control, PAR1, PAR4
- Y-axis: VEGF (ng/ml)
- RESTING: Control
- ACTIVATED: PAR1 (by PAR1 agonist), PAR4 (by PAR4 agonist)

**Graph B:**
- X-axis: Control, PAR1, PAR4
- Y-axis: Endostatin (ng/ml)
- RESTING: Control
- ACTIVATED: PAR1 (by PAR1 agonist), PAR4 (by PAR4 agonist)
IL-1RA and HMGB1 Release from Human Platelets Due to Selective Activation of PAR1 or PAR4
Effect Of Human Platelets On Human Patellar Tendon Stem Cells Activated By PAR1 And PAR4

Control

PAR1

PAR4

Tendon-like tissue

Blood vessel
Differential Effects of Selective Activation of PAR1 or PAR4

- Platelet (resting)
- Platelet (activated)
- Angiogenic Factors (VEGF)
- Anti-angiogenic factors (Endostatin)

PAR1

PAR4

Endostatin

VEGF
PAR1 selectively regulated the release of VEGF and IL-1RA from human platelets, whereas PAR4 selectively regulated the release of endostatin and HMGB-1 from human platelets.

For acute tendon injury in young adults, one may use PAR4 activated PRP treatment to reduce vessel formation and fibrosis.

For chronic tendinopathy, there are many blood vessels formed in scar tissue, one may remove the scar tissue and then use PAR1 activated PRP to stimulate angiogenesis so that tendon healing can be enhanced.