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

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Category: Basic Sciences/Biologics

Keywords: Tendon Injury, Platelet-Rich Plasma (PRP),Protease-Activated Receptors (PAR), PAR 1, PAR 4, HMGB-1, IL-1RA, VEGF, Endostatin

Introduction/Purpose: Tendon injuries occur frequently and cost billions of health care dollars annually. Recently, there has been an increase in the use of platelet-rich plasma (PRP) to treat tendon injuries. However, the efficacy of PRP treatment is controversial due to inconsistent results from human clinical trials. It is thought that variations in PRP preparation contribute to these inconsistencies. Specifically, platelets in PRP contain pro-angiogenic (e.g. VEGF) or anti-angiogenic (e.g. endostatin) factors, which may differentially affect the healing of tendon injuries. It is known that these factors are selectively released after platelet activation by specific receptors. Therefore, in this study we investigated the effect of protease-activated receptors 1 and 4 (PAR1 and PAR4) in platelet activation and inflammation.

Methods: Platelet preparation – Human blood was obtained from 12 healthy donors and 9 ml of blood was mixed with 1 ml of 3.8% sodium citrate and centrifuged at 500g for 10 min. Then, 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 activation – About 100 µl of platelet from above was activated with 5 µl 1 mM PAR1-activating peptide (PAR1-AP) or PAR4-activating peptide (PAR4-AP) at 25°C for 10 min. Then, the mixture was centrifuged at 1000g for 10 min, and the levels of VEGF, endostatin, IL-1RA and HMGB-1in the supernatant was determined by ELISA. Platelets without activators were used as controls.

Results: PAR1 induced angiogenic effects in human platelets. PAR1 activated platelets released 3 times more VEGF than when activated with PAR4 (Fig. 1A). However, PAR4 activated platelets released 7 times more endostatin than the PAR1 activated platelets (Fig. 1B). Further, PAR1 induced anti-inflammatory effects in human platelets; it did not change IL-1R-A (Fig. 2A) but decreased HMGB-1 levels (Fig. 2B). In contrast, PAR4 stimulated inflammatory effects in human platelets by lowering IL-1-RA and increasing HMGB-1 levels.

Conclusion: Our findings indicate that PAR1 induces angiogenetic and anti-inflammatory effects in human platelets, while PAR4 has anti-angiogenetic and inflammatory effects. Of significance is HMGB-1, which is constitutively expressed in the nuclei of most mammalian cells. Under cellular stress, HMGB1 is released into the extracellular matrix and activates the immune response thus acting as a danger-signal. Both PAR1 and PAR4 selectively regulated the release of VEGF and endostatin, and IL-1RA and HMGB-1 from human platelets. Therefore, the role of PAR1 and PAR4 on human platelet activation and inflammation should be considered prior to the use of PRP to treat tendon injuries.

Foot & Ankle Orthopaedics, 1(1)
DOI: 10.1177/ 2473011416S00297
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