Protein-S signaling- a potential target to prevent cardiac arrest induced pulmonary and atrial emboli

P. Basak, N. Kumar, S. Pilli, M. Mohammad, & R. Majumder.

Louisiana State University Health Science Center, Department of Interdisciplinary Oncology, New Orleans, Louisiana.

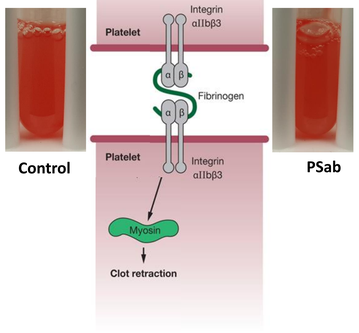
Introduction

Formation of blood clots in coronary arteries is one of the major causes of cardiac arrest. Failure of the vessel to return to patency once a thrombus has formed leads to devastating consequences. Thus, we seek to understand the process of clot retraction and the role that platelet Protein S (PS) plays in the fine balance between activation and quiescence of platelets. PS, a natural anticoagulant, is mostly secreted by the liver, but secretion directly from activated platelets has also been observed. The role of platelet PS has been unclear up until this time, and we hypothesize that it is involved in enhancement of clot retraction, in addition to its quintessential role in inhibition of the clotting cascade.

Clot retraction is facilitated by integrin αIIbβIII, and apoptotic-like pathways involving p53 and Heat Shock Protein-27 (HSP-27) have been implicated in this process. PS has a similar structure to one protein called Gas-6, which is involved is apoptosis through TAM receptor binding and has been shown to influence platelet activity. Thus, it is reasonable to infer that PS may bind this same receptor. Because PS is secreted by platelets directly into the clot, it is at the ideal location to influence platelet activation. If this is the case, we should further study this phenomenon and use our new knowledge to prevent the unnecessary outcomes of patients who have endured coronary thrombosis.

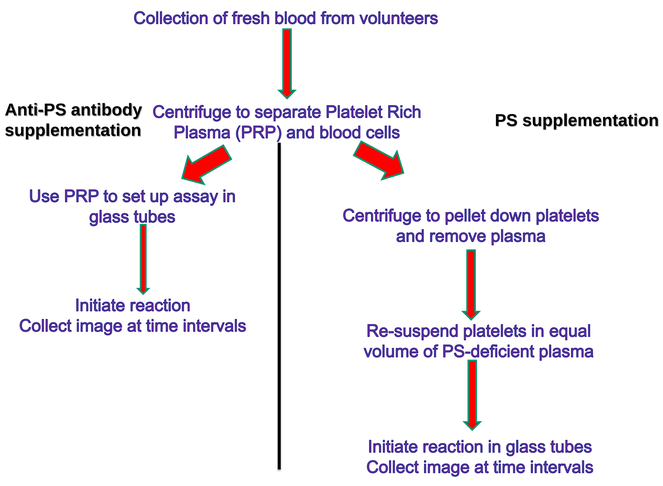
Aim Protein S signaling: a potential target to prevent cardiac arrest induced pulmonary and atrial emboli

Protein S binds and activates TAM (receptor) and initiate inside out signaling and interact with αIIbβ3 signaling which further interact with myosin a potential mechanism for its role in clot retraction.



Methods

Blood from healthy volunteers was collected in citrated tubes and platelet-rich plasma (PRP) was prepared. Clotting was initiated by adding 50 µl thrombin (20 unit/ml) to a tube with 745 µl Tyrode-HEPES Buffer, 200 µl PRP, and 5 µl red blood cells. Anti-Protein S antibodies (300 nM) were added to 6 tubes to deplete PS. To achieve a known concentration, PS was added to 300 nM to 6 tubes of platelets re-suspended in PS deficient plasma. Images and weights of the clots were taken as a function of time to measure the retraction rate. Apoptotic-like pathway induction was analyzed by immunoblots probed for phosphorylated p53, and HSP27.



Results

Figure 1a

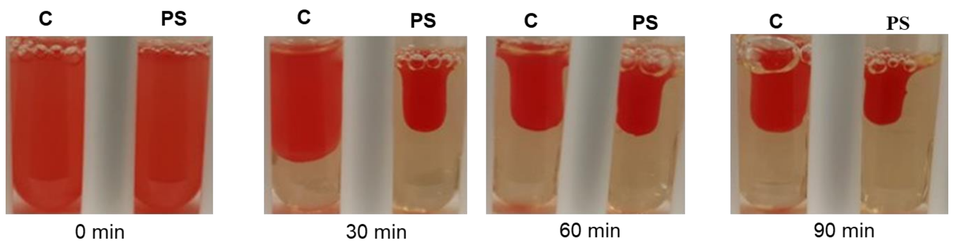


Figure 1c

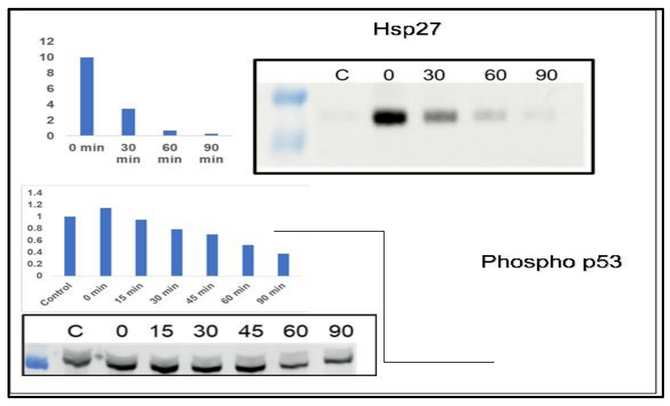


Figure 1a: Over time, the clot retracted in the presence of PS becomes dramatically smaller compared to the control. Figure 1c: The Western Blot and bar graph shows a decrease in expression of Hsp27 in the presence of PS in the given clot over time. Meanwhile, the expression of Phospho p53 increases.

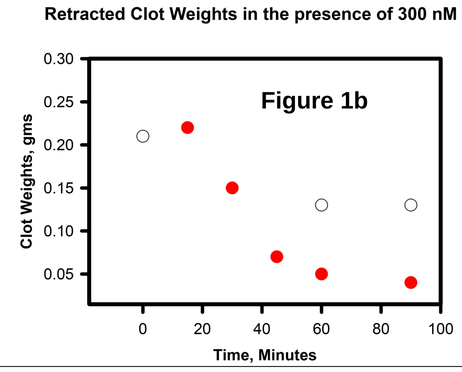
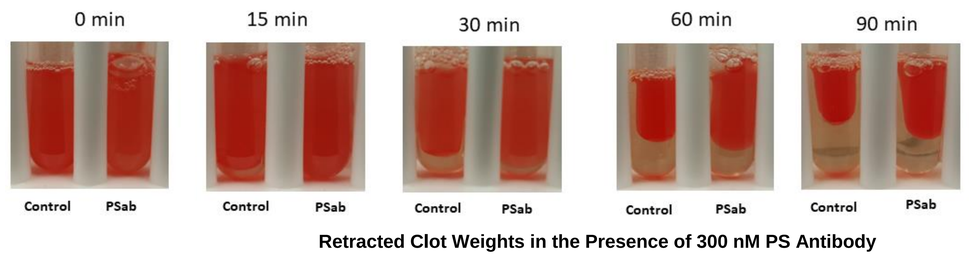


Figure 1b: The weight of the clot retracted in the presence of PS (red circles) decreases more dramatically over time than that of the control clot (open circles).



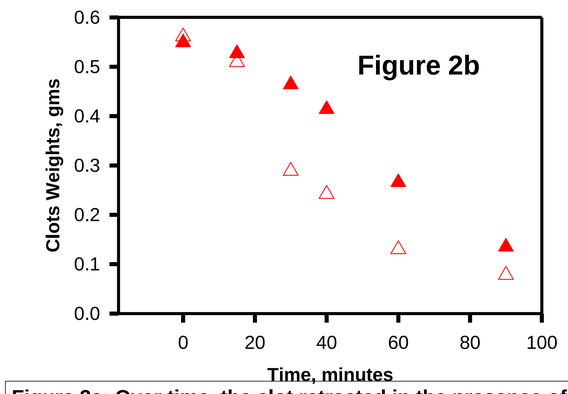


Figure 2a: Over time, the clot retracted in the presence of PS Antibody retracts much less than the control clot.
Figure 2b: The weight of the clot retracted in the presence of PS Antibody (red triangles) decreases less over time than that of the control clot (open triangles).

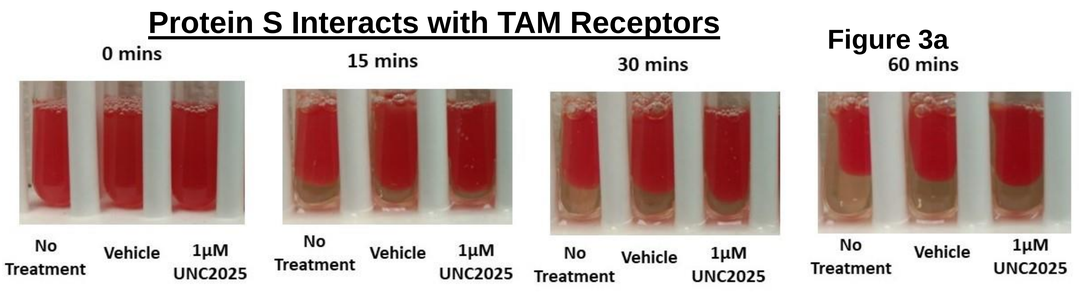
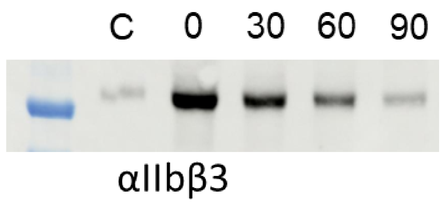


Figure 3a

Figure 3b



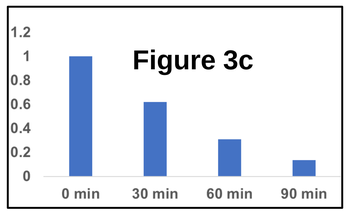


Figure 3a: Clots treated with UNC2025 are slightly bigger, showing that UNC2025 delays clot retraction. Figure 3b: Immunoblot to detect αIIbβIII integrin in the presence of PS
Figure 3c: The bar graph shows a decrease in expression of αIIbβIII in the presence of PS Antibody in the given clot over time.

Confocal Microscopy Imaging of Alexa 647 labeled Fibrin Clots

Fig. 4

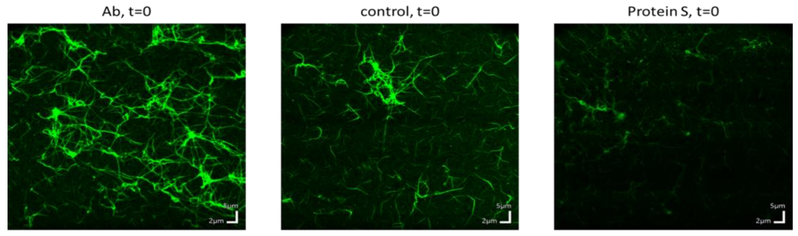


Figure 4: Fibrin fibers (green) can be clearly seen in Ab sample, fibers appear thicker and longer than in control or in sample with added PS.

Conclusion

1) Protein S supplementation accelerates clot retraction, while anti-PS antibody addition defers retraction.
2) We observed that apoptotic-like pathway proteins phospho-p53 is increased and the cell survival protein HSP-27 is decreased when clots are retracted in the presence 300 nM PS.
3) The clots retracted in the presence of UNC 2025, a TAM receptor inhibitor, show deferred retraction which indicates TAM receptor binding with PS.
4) TAM receptor binding to PS further results in the binding of PS to α IIb β3 integrin as evidenced by the decrease in expression of α IIb β3 integrin in immunoblot of the clots retracted in the presence of 300 nM PS.
5) Direct visualization of the clot shows a dramatic increase in the compaction of fibrin with PS supplementation. Thus, we conclude that PS enhances clot retraction through apoptotic-like signaling pathways, TAM receptor binding, and induction of αIIbβIII integrin expression.
6) Identifying the role of PS role in clot retraction will help us in preventing diseases like stroke, heart attack and even cardiac arrest.

This research project was supported through the LSU Health Sciences Center, School of Medicine.