ATTENUATING THE COAGULATION CASCADE WITH ANNEXIN A5

Abstract—Membrane displayed phosphatidylserine (PS) on platelets, microparticles, endothelial cells and erythrocytes is the platform for the coagulation cascade and annexin A5's great affinity for PS allows it to bind to it and form a shield that can block the cascade. In spite of this significant anticoagulant ability annexin A5 has no therapeutic anticoagulant application. Why?

When thrombin is present clotting begins and when there is trauma or inflammation the tissue factor pathway is activated and thrombin is produced. In addition to clot formation this thrombin activates the blood coagulation cascade components that can exponentially increase thrombin production, a feedback cycle that is unsustainable and dangerous and requires control. This control is manifested in the prothrombinase and tenase complexes where two of the thrombin activated cascade components, factors V (Thiagarajan P[1], Tait JF)and VIII (Ahmad SS), are required to adhere to PS on the membranes of activated platelets in order for the cascade to begin. These complexes are checkpoints where protein C and the annexin A5 molecule modulate the cascade, protein C by inactivating factors V and VIII and annexin A5 by competing with them for PS access. Protein C is activated by thrombin but annexin A5 doesn't require activation and the mechanism for its increased levels during clotting is not known.

The annexin A5 molecule is known for its anticoagulant ability and for its great affinity for PS that is so pronounced that its labeled molecules are used in both the lab and clinically to identify it. Considerable amounts of annexin A5 are present in cell cytoplasm and only small amounts are normally present in plasma (Tzima E) where its half-life is a brief +/- 4 minutes (Rand ML). How annexin A5 gets from cell cytoplasm to plasma is not known but what is known in that exposure to both thrombin and collagen cause cytoplasmic annexin A5 to rapidly migrate to the platelet's membrane (Tzima E) while these same stimuli cause PS display on its surface (Heemskerk JW). When clotting occurs in conditions such as trauma or a myocardial infarct the annexin A5 levels rise acutely, but transiently (Matsuda R). Both clotting and inflammation appear to be the driving forces for elevation of annexin A5 molecules but the mechanism for this has not been determined. Annexin A5's levels are chronically elevated in the procoagulant and inflammatory sickle cell disease and they increase further during a sickle cell crisis (van Tits LJ). In this disease these elevated levels may be the result of its cytoplasmic release due to the sickle erythrocyte's rapid hemolytic breakdown. The procoagulant state in sickle cell disease appears to be the result of the premature senescence of 30-40% of sickle erythrocytes that causes them to display PS on their surface (Galili U). Much remains to be discovered about annexin A5 but its documented ability to form a shield over membrane displayed PS that prevents binding of factors V and VIII appears to explain its anticoagulant ability.

Two in vivo rabbit studies comparing heparin and annexin A5 best demonstrate annexin A5's anticoagulant effectiveness and safety. In the first study a carotid artery was traumatized until the blood flow in it was reduced by 50% at which time the trauma was stopped and a "sufficient amount" of both agents were given to maintain blood flow for 180 minutes (Thiagarajan P[1], Benedict CR). At that time the annexin A5 clot that formed weighed 1/3 less and contained approximately 40% less fibrin and platelets. This study also showed that blocking annexin A5's access to endothelial displayed PS prevented its

anticoagulant effect. Of special importance was the fact that 3 times the amount of annexin A5 that was required to maintain blood flow didn't cause bleeding while the amount of heparin required for arterial patency caused significant bleeding

In the other rabbit study (Van Ryn-McKenna J) that compared heparin and annexin A5, a single injection of each was given after cessation of trauma to a jugular vein and it was found that the annexin A5 clot that formed contained 60% less fibrin while the heparin had no effect. Both of these agents have short half-lives and this surprising finding suggests that heparin was removed before it had any significant effect while annexin A5 rapidly adhered to membrane displayed PS and persisted.

In conclusion it appears that annexin A5 can form a shield over membrane displayed PS that blocks the coagulation cascade by preventing factors V and VIII's access to PS. Theoretically a sufficient amount of it could rapidly and completely cloak all membrane displayed PS and halt the coagulation cascade without involving the tissue factor pathway to thrombin while any unbound annexin A5 would be rapidly eliminated. The local arterial damage present in the first study (Thiagarajan P[1], Benedict CR), where a rapidly growing thrombus threatened to completely occlude an artery, is similar to conditions found early in a myocardial infarct and annexin A5's success in that study suggests that it may be able to do the same, including reduced likelihood of bleeding, when given early in the treatment of an infarct . Annexin A5's rapid elimination in its unbound state, while a safety factor, would limit its use to initial short term thrombus management. Whether it has advantages over current infarct therapy could only be answered by clinical trials.

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