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Describing the molecular mechanism by which the A2 protein improves survival in mice with endotoxemia.

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Von Willebrand factor (VWF) is an essential protein that mediates the platelet adhesion to the exposed subendothelium surface and significantly contributes to arterial thrombosis and stroke. Secreted VWF from the stimulated endothelium as long strings remain anchored to the surface of the endothelial cells and interact with circulating platelets, leading to vessel occlusion. We have shown that the isolated A2 domain of VWF (A2 protein) contains bind activity for the A1 domain, vimentin, and fibrin(ogen). In fact, as a result of those interactions, the A2 protein impairs platelet adhesion to VWF and fibrin(ogen), formation of VWF strings on the endothelium, and fibrin polymerization. The A2 protein was effective in preventing fibrin-rich microvascular thrombosis and improving survival (100%) of mice with endotoxemia (LPS)-induced disseminated intravascular coagulation (DIC). Thus, a goal was to identify the binding sites of A1 domain, vimentin, and fibrin in the A2 protein and dissect the mode of action by which the A2 protein recuperates the sick animals. To determine the amino acid residues that are important in the recognition for vimentin, A1 and fibrin, I used the crystal structure of the A2 domain as a guide for point mutations. Two mutations, E1523A and E1567A were introduced in the A2 protein. The purified mutants were tested for their binding capacity for the ligands using ELISA. Moreover, the A2 mutants were tested in mice with endotoxemia (LPS). We are now demonstrating that E1523A mutation had a reduced binding affinity for Vimentin and A1 domain. Strikingly, E1567A and E1523A mutations significantly abolished the binding to fibrin(ogen). Importantly, both mutations failed to replicate the beneficial effect of the wild type A2 protein in improving survival of mice with LPS-induced DIC. Together, these findings suggest that the A2 protein impairs microvascular thrombosis in mice with endotoxemia mainly via its interaction with fibrin.

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