# Exploring the Impact of Plasmin Inhibitors on Clotting Characteristics as a Novel Therapeutic for Thromboembolic Events

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**Background:**
Thromboembolisms are common, life-threatening emergencies. Systemic hemorrhaging post-thrombolytic treatment is an adverse reaction, highlighting the need for safer clot-digesting therapeutics. Our research focuses on an endogenous fibrinolytic enzyme, plasmin. We investigated how co-delivery of plasmin and pentamidine, a reversible bivalent plasmin inhibitor, impacts clotting and thrombolysis. Plasmin and pentamidine delivery were tested across ex-vivo stagnant viscoelastic assays (thromboelastography, TEG) and shear-based clot formation and digestion assays (Chandler Loop). This research aims to optimize safer clot-digesting therapeutics that minimize adverse bleeding.

**Methods:**
Consenting healthy volunteers (n=13) donated whole blood into citrated tubes using an approved IRB protocol. Whole blood was analyzed via Chandler Loop (20 RPM at 37C for 60min) and TEG (37C for 90min). Pentamidine (0-800µM) and plasmin (0-0.1mg/mL) were tested independently and mixed measuring: clot mass, clot strength (MA), R-time, angle, and K.

**Results:**
At increasing pentamidine concentrations, R-time and K increased while MA, angle, clot weight, and clot length decreased. At increasing plasmin concentrations, R-time, K, and angle remained unchanged while MA, clot weight, and clot length decreased. Concentrations >700µM pentamidine and >0.1mg/mL plasmin inhibited clotting. In the presence of fixed pentamidine (50µM in TEG, 200µM in Chandler), the initial impact to clot formation resembled 50µM of pentamidine alone followed by limited impact at increasing plasmin concentrations with a flat decrease in clot MA (18.3%), clot length (19.5%), and clot weight (18.9%) and increase in R-time (43.4%) and K (50.6%).

**Conclusion/Impact:**
Increasing plasmin concentrations results in increased clot digestion while increasing pentamidine concentrations functions more akin to an anticoagulant preventing clot formation. Co-administered plasmin with pentamidine demonstrates how an inhibitor can be used to deliver an active clot digesting enzyme. Leveraging the results presented herein, and the principles of multivalency, plasmin inhibitory molecules can be developed to create safer and more effective direct fibrinolytics for clinical use.

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