Nanomedicine Targeting to Activated Neutrophil-Platelet Complexes as a Novel Treatment for DVT

Anirban Sen Gupta[1], Michelle Cruz[1], Jurgis Alvikas[2], Nicole Masters[3], Shannon Halderman[2], Kara L Bane[1], Maria de la Fuente[1], Marvin Nieman[1], Keith Neeves[3], Matthew D Neal[2], Evi Stavrou[1]

[1] Case Western Reserve University, [2] University of Pittsburgh, [3] University of Colorado Denver

Statement of Purpose: Deep vein thrombosis (DVT) and pulmonary embolism (PE) collectively referred to as venous thromboembolism (VTE), constitute a leading cause of cardiovascular death. The standard of care for DVT is anticoagulation therapies that inhibit different components of the coagulation cascade towards a common downstream goal of attenuating fibrin generation and hence limit thrombus growth. However, due to their mechanism of systemic action, anticoagulant therapies are associated with increased risks of off-target bleeding. Therefore, there is an urgent need for the development of strategies to treat DVT more safely without compromising efficacy. In this framework, we rationalize that a safer and highly efficacious treatment for DVT can be achieved by downregulating the '*upstream mechanistic trigger'* (initiation phase) of coagulation processes in a targeted manner rather than by systemically inhibiting downstream components of the coagulation process. Several recent studies have established that aberrant neutrophil activation and neutrophil extracellular trap formation (NETosis) constitute a primary driving mechanism of DVT, that then leads to platelet recruitment, establishment of neutrophil-platelet complexes (NPCs) as well as NET-induced activation of coagulation factors. Therefore we hypothesize that a nanoparticle technology that can be targeted site-specifically to developing NPC site to deliver drugs that can modulate neutrophil hyperactivity and downregulate NETosis, will be highly effective in attenuating neutrophil-driven coagulation initiation mechanisms in DVT, thereby minimizing the need for downstream anticoagulation. To this end, we developed a unique nanoparticle system that specifically binds to activated NPC via combination targeting of neutrophil elastase (for binding active neutrophils) and P-selectin (for binding active platelets), and delivers a NETosis downregulating drug hydroxychloroquine (HCQ). This nanomedicine system was evaluated in vitro and in a mouse DVT model in vivo for targeted therapeutic efficacy.

Methods: Cholesterol, Distearoyl phosphatidylcholine (DSPC), Polyethylene glycol-modified distearoyl phosphatidylethanolamine (DSPE-PEG) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). An alpha-antitrypsin derived neutrophil elastase binding peptide (NEBP) and a P-selectin binding peptide (PBP) were custom synthesized from Genscript (Piscataway, NJ, USA). Lipid-tethered Rhodamine B fluorescent probe was obtained from Setareh Biotech (Eugene, OR, USA). HCQ was obtained from Sigma-Aldrich. DSPC, cholesterol, DSPE-PEG-NEBP and DSPE-PEG-PBP were self-assembled into hybrid liposomal vesicles, ~ 200 nm in diameter, using thin film rehydration and extrusion technique. For in vitro studies human neutrophils and platelets were isolated from blood drawn using IRB-approved protocol from healthy donors. For in vivo studies a mouse inferior vena cava (IVC) stasis model of DVT was used, using IACUC-approved protocol. NPC-targeting was evaluated using microfluidics with immunofluorescence-based analysis. In vitro targeted therapeutic effect was evaluated by NETosis extent evaluation using citrullinated histone as a marker. In vivo therapeutic efficacy was evaluated by excised clot weight at 24 hrs post-treatment.

Results: In vitro microfluidic studies established high capability of the combination peptide-modified nanoparticles to bind to DVT-relevant activated NPC site. In vitro delivery of HCQ using the nanoparticles significantly reduced neutrophil activation and NETosis. In vivo, delivery of HCQ-loaded targeted nanoparticles to mouse IVC ligation (stenosis) site significantly reduced thrombus weight. The nanoparticles had an in vivo circulation half-life of approximately 12 hrs and did not cause any systemic distress. Overall, these results demonstrate the promise of this novel strategy to attenuate neutrophil-driven mechanisms in DVT.

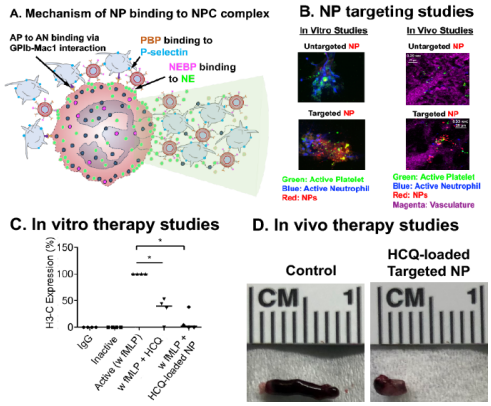

Fig 1. Schematic and representative targeting and therapy results for NPC-targeted HCQ-loaded NPs.

Conclusions: Activated neutrophils and their interaction with activated platelets, leading to NETosis and coagulation initiation, have been implicated as driving mechanisms of DVT pathophysiology. However, till date no therapeutic strategy has been explored in downregulating NETosis as a potential treatment for DVT. This is because systemically neutrophil downregulation is not clinically feasible or translatable due to potential risk of systemic immunocompromise. We developed a unique nanomedicine strategy to target disease-associated activated neutrophils and platelets, and thereby deliver a NETosis attenuating drug to evaluate the potential of this strategy as a treatment for reducing coagulation and thrombus growth in DVT. Our studies demonstrate the success of this strategy and warrant further studies of this approach for treatment of neutrophil-driven pathologies.

Acknowledgement: The research was supported by funding from NIH R01 513988.