A Novel Polymeric Antidote for Clinically used Parenteral Anticoagulants

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Introduction:

Parenteral anticoagulants such as unfractionated heparin (UFH) and low molecular weight heparins (LMWHs) play a vital role in treatment and prophylaxis of thromboembolic complications. Life threatening bleeding associated with UFH therapy in patients, undergoing major invasive surgeries makes heparin reversal a mandatory postoperative procedure. Shortcomings of traditional heparin anticoagulants led to the development of fondaparinux with superior pharmacokinetic and safety profile. Nonetheless, haemorrhage is the major adverse effect associated with all available anticoagulants and could be life threatening in geriatric, pediatric populations and patients with coagulation disorders and renal insufficiency. The only clinically approved reversal agent available is protamine sulphate which neutralizes UFH. However, life threatening cardiovascular adverse reactions of protamine and ineffectiveness in completely neutralizing novel anticoagulants restricts its use as an antidote. Hence, there is an imperative need to develop biocompatible and efficient universal antidote which could neutralize all available anticoagulants. Here, we report development of hyperbranched polyglycerol (HPG) based biocompatible and multivalent cationic macromolecular heparin reversal agent capable of reversing *in vitro* anticoagulation effects of all clinically available parenteral anticoagulants.

Methods:

Macromolecular heparin antidotes (MHA) with HPG core modified with binding groups capable of eliciting positive charges in physiological pH and polyethylene glycol chains (PEG) which prevents non-specific interaction with blood components were synthesized. (Figure-1)

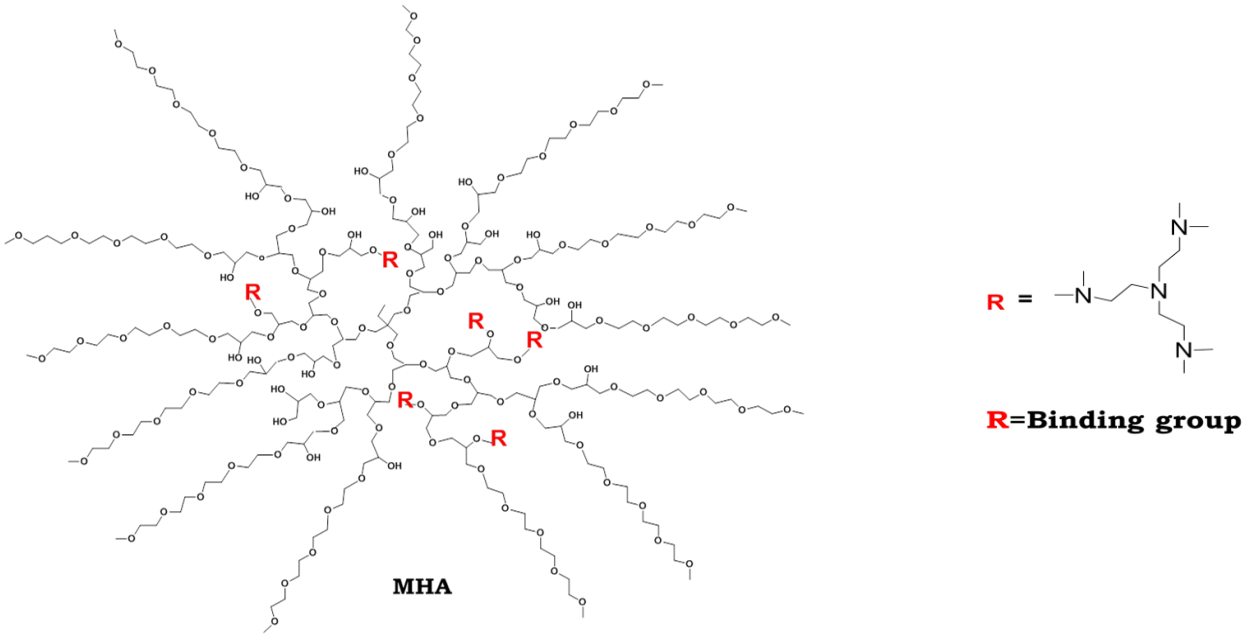

Figure: 1 Design of macromolecular heparin antidote (MHA)

Synthesized polymeric antidotes were fully characterized by gel permeation chromatography (GPC), nuclear magnetic resonance (NMR) and conductometric titrations. UFH and LMWHs neutralization by polymeric antidotes and biocompatibility was assessed by performing activated partial thromboplastin time (aPTT) assay in anticoagulated and normal human platelet poor plasma respectively. Biocompatibility was also assessed by performing erythrocyte aggregation, hemolysis and complement activation assays. Thromboelastography (TEG) and chromogenic factor Xa assay were performed to investigate the fondaparinux neutralization capability of polymeric antidotes in human whole blood and platelet poor plasma respectively. The thermodynamics of binding interactions between cationic polymeric antidotes and negatively charged groups on fondaparinux was studied by Isothermal titration Calorimetry (ITC).

Results:

Developed antidotes completely neutralized the *in vitro* anticoagulation effects of UFH and LMWHs over a broad concentration ranging from 0.025mg/mL to 1mg/mL (Figure- 2). On the other hand protamine failed to completely reverse anticoagulation effects of UFH and also showed anticoagulation effect above 0.1mg/mL. Antidote at 0.1 mg/mL was able to completely normalize TEG parameters of 1.2 IU fondaparinized blood and diminished 85% of anti FXa activity of fondaparinux in chromogenic FXa assay (Figure-3). Antidotes also exhibited excellent hemocompatibility compared to protamine as they did not induce hemolysis, erythrocyte aggregation or activate the complement system. Thermodynamic parameters obtained from ITC experiments provided evidence for multivalent interactions between fondaparinux and antidotes.

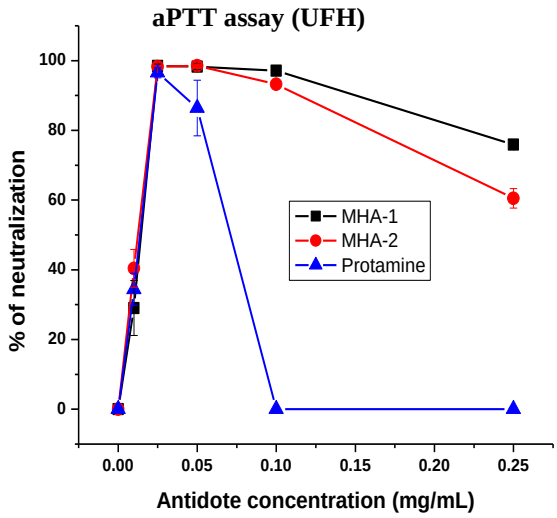

Figure- 2: aPTT assay in human platelet poor plasma demonstrating complete neutralization of UFH by antidotes.

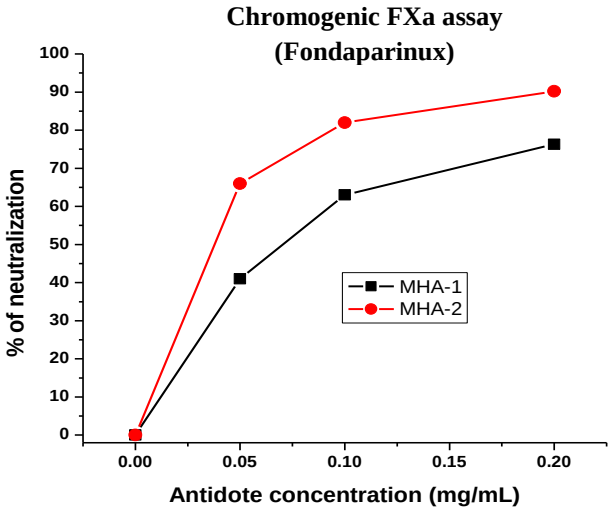

Figure- 3: Chromogenic FXa assay in fondaparinized human platelet poor plasma demonstrating 95% anti FXa diminishing activity of antidotes.

Conclusion and future directions:

We have developed a novel, biocompatible, multivalent and efficient macromolecular heparin reversal agent capable of reversing *in vitro* anticoagulation effects of all clinically available parenteral anticoagulants. Polymer molecular weight and number of cationic charges per polymer molecule are the major determinants of multivalent binding which in turn strongly affect the anticoagulant neutralization efficiency of antidotes *in vitro*. Studies using bleeding and thrombosis animal models are required to further confirm *in vitro* anticoagulant neutralization capability of developed polymeric antidotes. Development of novel binding group and modification on polymer design to improve efficacy without compromising biocompatibility is under progress.