

PhD DEFENCE Tuesday, March 14th, 2017

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Title: NOVEL ANTICOAGULANT NEUTRALIZING AGENTS FOR THE MANAGEMENT OF BLEEDING: STUDIES ON THE DESIGN, MECHANISM OF ACTION AND THEIR INFLUENCE ON BLOOD COAGULATION

Time and location: 9:00am PST; Room 203 of the Graduate Student Centre (6371 Crescent Road), UBC Vancouver Point Grey Campus

Supervisor: Jayachandran Kizhakkedathu

ABSTRACT

Heparins exert anticoagulation by potentiating anti-factor (F) Xa and anti-thrombin activity of antithrombin (AT), whereas oral anticoagulants (DOACs) directly target FXa or thrombin. FXa and thrombin are the key proteases required for blood clotting. Anticoagulants are therefore used for the prophylaxis and treatment of thromboembolic disorders and during surgeries. However, anticoagulation associated haemorrhage is a major concern. The only approved antidote for unfractionated heparin (UFH), protamine do have limitations including adverse cardiovascular complications. No approved antidotes are available for low molecular weight heparins, fondaparinux and direct FXa inhibitors. Therefore, there is a clinical need for antidotes that are nontoxic and efficient for the reversal of anticoagulation activity. In this thesis, we reveal the mechanism of action, hemocompatibility and efficiency of three antidote molecules that are currently under development.

UHRA: UHRA is a synthetic antidote developed by the Kizhakkedathu laboratory at the University of British Columbia. Detailed thermodynamics based on isothermal titration calorimetry (ITC) and fluorescence studies revealed the unique molecular design of UHRA, importance of steric shield offered by PEG brush, the selectivity of UHRA against heparins and its mechanism of action. The clotting studies reveal the reversal of anticoagulation activity of UHRA. Studies also show that UHRA even in the absence of heparins, do not interact with fibrinogen, alter fibrin polymerization or abrogate plasma clotting. Unlike protamine, UHRA does not incorporate into blood clots, form normal fibrin clot morphology, and the clots are stable to lysis. Studies in mice reveal that UHRA reverses UFH anticoagulant activity without the lung injury as seen with protamine. Studies confirm the superiority of UHRA compared to protamine.

Andexanet Alfa (AnXa) and PER977: AnXa is a truncated FXa recombinant protein developed by Portola Pharmaceuticals and PER977 is a small cationic molecule developed by Perosphere Pharmaceuticals. ITC confirms high affinity binding of AnXa to heparin/AT complex and to DOACs studied. PER977 shows weak binding to heparins and no binding to DOACs tested. Both antidotes do not influence fibrin polymerization, fibrin and whole blood clot architecture even in the absence of anticoagulants. Electron micrographs of blood clots containing edoxaban treated with AnXa or PER977 reveal restoration of impaired fibrin formation. However, in clotting assays PER977 failed to show antidote activity, whereas AnXa neutralized anticoagulation activity of all tested anticoagulants. IR dose of 2.3Gy are sufficient to enhance migration of both non-metastatic and metastatic breast cancer cell lines independent of EMT. By quantifying changes in the metastatic ability of tumour cells treated with a clinically relevant dose of radiation, our findings will help to determine whether there is a need for additional administration of targeted secondary therapy to minimize tumour cell dissemination.