I. Introduction

Factor X is a clotting protein that is made in the liver and found in the blood. Pharmaceutical companies have recently developed new oral anticoagulants (NOACs) that inhibit Factor X, such as apixaban (Eliquis), to treat and prevent life-threatening blood clots. An older anticoagulant, warfarin, is extremely difficult to dose, requires constant blood level monitoring, and has many drug and food interactions. Apixaban has none of these drawbacks, but it does lack an antidote to stop bleeding in emergencies. Our mentors are using zebrafish as animal models to search for antidotes to the NOAC, apixaban.

II. Factor X in the Coagulation Cascade

Factor X is a serine protease that acts at the end of both the intrinsic and extrinsic clotting cascades. Factor X cleaves prothrombin to form thrombin. Thrombin then cleaves fibrinogen to form the strands of fibrin that stabilize and complete the blood clot.

Coagulation Cascade

The intrinsic series of reactions is triggered by surface damage to a blood vessel, and the extrinsic cascade of reactions is triggered by trauma to both the blood vessel and surrounding tissue.

Prothrombinase Complex

Factor Xa (activated Factor X) and Factor Va (activated Factor V) combine to form the prothrombinase complex on the platelet phospholipid membrane. Prothrombin then binds to the complex, and Factor Xa cleaves prothrombin to produce thrombin. The prothrombinase complex cleaves prothrombin at 3,000 times the rate that Factor Xa does alone.

Stabilized Blood Clot

Thrombin cleaves fibrinogen to form fibrin strands, which stabilize the clot by trapping platelets and blood cells in a fibrin net.

III. Medical Conditions Treated with Anticoagulants

Deep Vein Thrombosis

Pulmonary Embolism

Atrial Fibrillation and Stroke

Deep vein thrombosis (DVT) is the formation of a blood clot in the deep veins of the leg or pelvis. Symptoms can include pain and swelling; however, there may be no symptoms. DVT requires immediate medical attention as clots can travel to the lungs and cause a life-threatening pulmonary embolism. The NOAC apixaban (Eliquis) is FDA approved to treat and prevent deep vein thrombosis, which it does by inhibiting Factor X.

Pulmonary embolism (PE) is caused by a blood clot that travels through the heart to the lungs from the leg or, in rare cases, other parts of the body. The clot damages the lung by blocking blood flow. The dark area in the diagram above indicates tissue damage. Pulmonary embolism can result in death. Anticoagulants are used to prevent and treat pulmonary embolisms. Apixaban is FDA approved to treat PE.

Atrial fibrillation (AFib) is a condition in which the heart’s natural pacemaker is out of rhythm. A stroke will occur if a blood clot leaves the heart (atrial fibrillation). AFib is associated with an increased risk of stroke and other serious health problems. Apixaban is FDA approved to prevent stroke in AFib patients.

IV. Catalytic Domain of Activated Factor X Bound to Apixaban

The blood-clotting protein Factor X is composed of a heavy protein chain and a light protein chain. The heavy chain contains the active site where prothrombin is cleaved during the last steps of the coagulation cascade. The light chain (not shown) anchors Factor X to the phospholipid membrane of platelets during the last steps of the blood clot formation. The St. Dominic SMART Team modeled the catalytic domain of activated Factor X using 3D printing technology to explore how the new oral anticoagulant apixaban (Eliquis) blocks the active site. Our mentors are in the process of searching for an antidote to apixaban using zebrafish as a model organism. Anticoagulants like apixaban are used to treat and prevent deep vein thrombosis (DVT), pulmonary embolisms (PE), and stroke in atrial fibrillation patients.

V. Structure of Apixaban

The N-H of the carbamoyl group interacts with the peptide bond carbonyl of Glu146 (Magenta). Apixaban does not interact with any specific active site amino acid resides.

VI. Apixaban versus Warfarin

In the September 15, 2011 issue of The New England Journal of Medicine, Christopher B. Granger et al. reported on the ARISTOTLE Clinical Trials involving over 18,000 patients with atrial fibrillation. Apixaban was found to be superior to warfarin in preventing stroke and systemic embolism, caused less bleeding, and resulted in lower mortality. (Funded by Bristol-Myers Squibb and Pfizer; ARISTOTLE ClinicalTrials.gov Identifier: NCT00449650)

VII. Using Zebrafish to Find an Apixaban Antidote

Lack of an antidote for apixaban can cause serious problems if a patient needs to undergo immediate surgery or has been overdosed. Our mentors are using zebrafish to screen 10,000 compounds for possible antidotes to apixaban. Zebrafish are a good model organism because they can be bred in the laboratory and can be used for testing as embryos when apixaban and antidote compounds can be added to their water. If the antidote is not effective, hemorrhaging is visible in the embryo under the microscope. In addition, zebrafish have genes for all of the coagulation proteins found in mammals (Hanumanthaiah et al. 2002). A Blast alignment comparing the amino acid sequence of Human Factor X to zebrafish Factor X showed 47% identity (the same amino acid at the same position) and 63% positively (a similar amino acid at the same position).

VIII. Summary

Factor X coagulation protein is the target of new oral anticoagulants like apixaban (Eliquis). Apixaban inhibits activated Factor X in the coagulation cascade to prevent clot formation and has been approved by the FDA to treat and prevent deep vein thrombosis and pulmonary embolism as well as to prevent strokes in atrial fibrillation patients. Apixaban’s major drawback is lack of an antidote. Our mentors are using zebrafish as a model organism to test thousands of possible antidote compounds.