

## Amelioration of hemophilia by manipulating the activity of tissue factor pathway inhibitor

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4 My research will test the hypothesis that enhancing the extrinsic pathway of coagulation will ameliorate the bleeding diatheses caused by hemophilia A and B. Current treatments for these genetic disorders involve replacing the affected coagulation factor (using factor VIII or factor IX) or bypassing the tissue factor pathway (using factor VIIa). Treatment with high-dose factor VIIa has been recently introduced as an alternative approach, especially in cases where individuals have immunoreactions to exogenous factor VIII or IX. I hypothesize that manipulation of the regulation of the extrinsic pathway, specifically tissue factor pathway inhibitor (TFPI), may offer another approach to ameliorate the bleeding disorders resulting from hemophilia A and B.

TFPI is the key inhibitor in the extrinsic pathway. It inhibits the activities of both factor Xa and factor VIIa/tissue factor. Neutralizing TFPI's activity to prolong the factor VIIa/tissue factor and factor Xa activities may allow sufficient

amounts of thrombin production for clot formation in individuals with hemophilia A or B.

To test this hypothesis, I propose the following specific aims:

1. Use the "Toggle" SELEX (systematic evolution of ligands by exponential enrichment) to isolate high affinity nucleic acid ligands which will neutralize both mouse and human TFPI inhibition of factor Xa and the factor VIIa/tissue factor. I have cloned both human and mouse TFPI cDNA, and have expressed, purified and characterized the recombinant TFPI proteins. I have sufficient amounts of recombinant mouse TFPI proteins on hand for this aim.

2. Use hemophilia B mice to test the hypothesis that neutralization of TFPI with these ligands will ameliorate the bleeding disorder caused by factor VIII and/or factor IX deficiencies. The single stranded DNA aptamer will be introduced into hemophilia B mice (which are also available in our laboratory) to test this hypothesis.

The overall goal of this proposal is to improve the treatment efficacy for individuals suffering from the hemophilias. Based on the current understanding of coagulation reactions, I believe that a transient inhibitor for TFPI's activity will ameliorate the bleeding disorders involving the intrinsic pathway. A small molecule, like the aptamer described in this proposed study, which specifically inhibits TFPI's activity for a short period of time, will allow the extrinsic pathway to produce sufficient thrombin to stop bleeding. In this regard, the aptamer

will ameliorate the symptoms of not only factor VIII, but also factor IX deficiencies. Furthermore, this aptamer can be used to treat individuals who have developed antibodies against the exogenous clotting factors and cannot receive replacement therapy or gene therapy. Once the aptamer's nucleotide sequences are identified, the aptamers can be chemically synthesized, thus obviating the need for biological processing (bacterial culture, yeast culture or mammalian cell culture), making its clinical application totally free of biohazard concerns (e.g. toxins, viruses, prions). I expect that this study will initiate a new era of hope for hemophiliacs.

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*Manipulation of the regulation of the extrinsic pathway inhibitor (TFPI) may offer another approach to manage bleeding in hemophilia A and B*

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