Thrombin Generation in Human Hemophilia Plasma at Reduced Antithrombin Levels and Concomitant Factor or Bypass Agent Addition

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Background & Aims

We are currently investigating the use of RNA interference (RNAi) to target the natural anticoagulant antithrombin (AT) as a strategy to improve thrombin generation, and therefore hemostasis, in hemophilia. ALN-AT3, a subcutaneously administered investigational RNAi therapeutic targeting AT, is currently being evaluated in patients with hemophilia in a Phase 1 study (NCT02035505). Based on its mechanism of action, ALN-AT3 could potentially offer benefit to patients with hemophilia A or B, with or without inhibitors.

There were two main aims of this work. First, as factor replacement is the standard therapy in hemophilia, we investigated the dynamics of thrombin generation in hemophilia A and B plasma with varying degrees of factor replacement in the background of different levels of residual AT. Second, as bypass agents (BPAs) are used to treat bleeds in hemophilia with inhibitors, we investigated the dynamics of thrombin generation in hemophilia plasma at varying doses of BPAs in the background of different levels of residual AT.

Methods

Human severe hemophilia A and hemophilia B donor plasma was obtained and immunodepleted of AT to different concentrations and incubated for 30 min at 37 °C to deplete AT to different levels. Resultant AT levels were measured by chromogenic AT activity assay (Biophen Antithrombin 5). Thrombin generation measurements were made in the various test plasmas using the calibrated automated thrombogram (CAT) method (1 µM tissue factor, 4 mM phospholipids).

Results

Therapeutic hypothesis: rebalancing the coagulation system

Figure 1 A simplified coagulation model in hemophilia A and B the lack of FVIII and FIX, respectively, leads to a decrease in thrombin potential, resulting in a bleeding phenotype. Inhibiting antithrombin, a powerful natural anticoagulant in the pathway, has the potential to increase thrombin generation in hemophilia A or B and correct the bleeding phenotype.

Figure 2 (A) Thrombin generation in human severe HA (FVIII <1%) donor plasma depleted of AT to various levels. Control plasma was generated by adding back FVIII and AT to 100% levels. (B) Thrombin generation in human severe HB (FIX <1%) donor plasma depleted of AT to various levels. Control plasma was generated by adding back FIX and AT to 100% levels. (C) Peak thrombin values as a function of FVIII and AT level in HB donor plasma. Different amounts of FVIII were added to the plasma samples generated in (A) and thrombograms were determined. (D) Peak thrombin values as a function of FIX and AT level in HB donor plasma. Different amounts of FIX were added to the plasma samples generated in (B) and thrombograms were determined.

Figure 3 Thrombin generation in human severe HA (FVIII <1%) donor plasma at various AT activity levels and FVIIa concentrations. (A) Thrombograms at AT = 0 U/mL. (B) Thrombograms at AT = 1 U/mL. (C) Peak thrombin values determined at varying AT activity levels and concentrations of FVIIa. AT activity in donor plasma was inhibited to various levels by addition of anti-AT antibody at different concentrations, and resultant AT activity was determined by chromogenic assay.

Impact of AT and Factor V on Thrombin Generation in Hemophilia A Plasma

Figure 4 Thrombin generation in human severe HA (FVIII <1%) donor plasma at various AT activity levels and aPCC concentrations. (A) Thrombograms at aPCC = 0 U/mL. (B) Thrombograms at aPCC = 1 U/mL. (C) Peak thrombin values determined at varying AT activity levels and concentrations of aPCC. AT activity in donor plasma was inhibited to various levels by addition of anti-AT antibody at different concentrations, and resultant AT activity was determined by chromogenic assay. Anti-FVIII antibody (HTL, PAH/FVIII-S) was included in all samples at 30 BU/mL to model the inhibitor setting and to neutralize residual FVIII in the added aPCC.

Conclusions

- Both peak thrombin and endogenous thrombin potential (ETP, area under the thrombin generation curve) increased as AT decreased in hemophilia plasma, consistent with the therapeutic hypothesis that decreasing AT can increase thrombin generation in hemophilia plasma.
- Thrombin generation levels did not exceed those associated with normal conditions (100% factor, 100% AT), even when AT was fully depleted in severe hemophilia plasma.
- AT reduction led to greater thrombin generation correction than achievable with up to 1 mcg/mL rFVIIa or 1 U/mL aPCC.
- In addition, the data suggest that in the background of reduced AT a substantially lower dose of replacement factor/BPA could potentially be used to achieve a similar level of hemostatic effect.

References