Novel Small-Molecule Inhibitors Targeting Complement Factor D for Therapy of Paroxysmal Nocturnal Hemoglobinuria

The complement system is a pivotal player in multiple hematological conditions that include paroxysmal nocturnal hemoglobinuria (PNH). The current standard of care for PNH is intravenous infusion of eculizumab, a humanized monoclonal antibody that targets the terminal complement protein C5 and thereby efficiently impairs intravascular hemolysis. However, an oral therapeutic would be highly desirable for chronic/long-term therapy of PNH. Furthermore, a significant fraction of PNH patients in clinical practice respond incompletely to eculizumab due to exhaustion of extracapillary hemolysis occurring through C3 opsonization. Additionally, a non-responsive sub-population has been identified with a rare genetic polymorphism in C5 that renders the variant incapable of binding eculizumab. Therefore, an unmet medical need remains for regimens that improve efficacy and that can be administered orally. To achieve this goal, we initiated a discovery program for small molecule inhibitors of complement factor D (fD), a serine protease that is the rate limiting enzyme of the alternative complement pathway. We have discovered novel inhibitors of fD that possess high potency and specificity as well as pharmacokinetics that allow for oral dosing in non-human primates. Herein, we present a biochemical characterization of two ACH lead compounds (Compound A and D) and studies of their activities in various complement-mediated processes.

METHODS

- fD proteolytic activity was evaluated biochemically using both a nonspecific synthetic substrate and a natural substrate comprising C3b and complement factor B (fB). In the latter assay, the cleavage product fB was quantified with a commercial ELISA kit (BD).
- The alternative pathway (AP) hemolysis assay was conducted with rabbit erythrocytes and 8% normal human serum (NHS) in the presence of 10 mM MgCl2.
- AP-mediated terminal pathway activation in NHS was further assessed with the Wieslab AP ELISA assay that measures terminal complement complex (TCC) deposition after AP activation.
- fC3 fragment deposition on the surface of rabbit erythrocytes was assayed by flow cytometry with a FITC-conjugated anti-fC3 antibody after incubation with 20% C5-depleted NHS.
- Human PNH-like erythrocytes were prepared with anti-CD55 and CD59 monoclonal antibodies. AP-mediated hemolysis was assessed with normal or acidified (pH 6.4) AB donor serum in the presence of 2% NHS and 2.5 mM MgCl2. Inhibition by compound was evaluated using 62.5% acidified serum (the final concentration after adding an equal volume of erythrocytes in buffer to 1:8 diluted serum).
- PK and PD studies were conducted in cynomolgus monkeys with Compound A. Compound plasma concentrations were measured by LC-MS/MS. Complement AP activity in serum was measured ex vivo with Wieslab AP ELISA assay.

RESULTS

- ACH compounds bind to fD with pM affinity.
- ACH fD inhibitors demonstrate potent inhibition of fB cleavage with IC50 values of 35 and 8.5 nM for Compounds A and D, respectively.
- ACH fD inhibitors show potent inhibition of AP-mediated terminal pathway activation as assessed by multiple endpoints.
- ACH fD inhibitors block C3 fragment deposition effectively (<5% of cells stained positive at 0.3 µM) (Fig. 4A and 4B).
- Compounds (CP-40) achieved the same level of effectiveness at 30 µM (Fig. 4C).

CONCLUSIONS

- ACH compounds bind to fD with pM affinity.
- ACH fD inhibitors demonstrate potent inhibition of AP-mediated terminal pathway activation and are derived from data fitting of time-course of SPR signals for both Compound A and D.
- ACH fD inhibitors are highly specific. Unlike the broad spectrum serine protease inhibitor FUT-175, ACH fD inhibitors showed no effect in AP hemolysis assay with rat plasma.
- The steeper dose-response of ACH fD inhibitors results in a 7- to 27-fold greater potency than anti-C5 mAb as measured by IC90 (Table 2 & Fig. 3B).
- ACH fD inhibitors blocked C3 fragment deposition effectively (<5% of cells stained positive at 0.3 µM) (Fig. 4A and 4B).
- Compartment (CP-40) achieved the same level of effectiveness at 30 µM (Fig. 4C).

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Disclosures

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