Preclinical Characterization of BCX7353, an Oral Plasma Kallikrein Inhibitor, for the Treatment of Hereditary Angioedema

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Background

Hereditary angioedema (HAE) develops in individuals with deficiency or dysfunction of CI inhibitor (C1NH), and is characterized by episodic swelling of the skin, pharynx, larynx, GI tract, genitals and extremities

Bradykinin, generated from plasma kallikrein-mediated cleavage of high molecular weight kininogen (HK), is the major mediator of acute HAE attacks

Plasma kallikrein is a proven target in the treatment of hereditary angioedema

BCX7353, a potent small molecule inhibitor of plasma kallikrein, is being evaluated in a phase 2 clinical efficacy trial for the prophylactic treatment of HAE (APA01-1, NCT02879729)

Objective

To evaluate the effects of BCX7353 on isolated plasma kallikrein & other serine proteases, kallikrein activity in ex-vivo activated HAE plasma, cleavage of HK in ex-vivo activated normal and HAE plasma, and kallikrein activity and release of bradykinin from the surface of human umbilical endothelial cells (HUVEC)

Methods

A. Activity of isolated human plasma kallikrein was measured in a colorimetric assay using S2302 as substrate, and for other serine proteases using specific reagents (Table 1).

B. Kallikrein activity in ex vivo human plasma activated with elagic acid (EAA) was measured in a fluorogenic assay with a specific substrate, Z-FL-FMK (Table 1 & Fig. 4).

C. Cleavage of HK in normal (Fig. 2) and HAE plasma (Fig. 3) was determined by Western blot analysis using mouse monoclonal antibody against HK light chain.

D. Kallikrein activity for HUVEC coated with HK/prekallikrein (PKK) was measured in a colorimetric assay using S2302 as substrate (Fig 5 Panel A).

E. Bradykinin release from endothelial cells: Confluent HUVEC were coated with HK, and the cells were washed and incubated with prekallikrein. Prekallikrein is activated to kallikrein after binding to endothelial cells, and then cleaves HK and releases bradykinin into the supernatant. Lisinopril was added to prevent the breakdown of bradykinin. Bradykinin Concentrations were determined by Elisa (Fig 5 Panel B).

Figure 1: BCX7353 inhibition of plasma kallikrein prevents cleavage of HK and release of bradykinin 2

Figure 2: BCX7353 inhibits cleavage of HK in “neat” normal plasma upon activation of the contact pathway by 5% EAA

Figure 3: BCX7353 inhibits cleavage of HK in “neat” HAE plasma upon activation of the contact pathway by 0.15% EAA

Table 1. In vitro potency and specificity of BCX7353 for plasma kallikrein

<table>
<thead>
<tr>
<th>Assay system for on-target activity against plasma kallikrein</th>
<th>[BCX7353] (nM)</th>
<th>Mean IC50 (SEM)</th>
<th>Relative to IC50 for Plasma Kallikrein</th>
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<td>Tissue kallikrein (PKK)</td>
<td>53.6 ± 1.2</td>
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Figure 4: In HAE patient plasma BCX7353 is ~14 times more potent than C1NH in inhibiting plasma kallikrein

Figure 5: BCX7353 inhibits kallikrein activity and bradykinin release from HK-prekallikrein coated endothelial cells

Conclusions

BCX7353 potently inhibits kallikrein activity, cleavage of HK in normal and HAE plasma, and suppresses the release of bradykinin after contact system activation on endothelial cells.

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References:
