BCX4161, a small molecule orally bioavailable plasma kallikrein inhibitor for the treatment of hereditary angioedema

Shanta Bantia, Jianwen Zhang, Ramanda Wilson, Cynthia Parker, Debra Kellogg, Pravin Kotian, Y.S. Babu*  
BioCryst Pharmaceuticals, Inc., 2190 Parkway Lake Drive, Birmingham, AL 35244

Background

Hereditary angioedema (HAE) is an autosomal dominant disorder characterized by episodic swelling of the skin, pharynx, larynx, GI tract, genitals and extremities. Plasma kallikrein is a proven target in the treatment of hereditary angioedema (HAE). No oral treatments for HAE are currently available.

Objective

To evaluate the preclinical characteristics of BCX4161, a novel potent small molecule inhibitor of plasma kallikrein.

Methods

A focused medicinal chemistry and structural biology approach led to the discovery of BCX4161.

The selectivity and specificity of BCX4161 was assessed in purified enzyme assays against plasma kallikrein (PK) and other serine proteases.

The ability of BCX4161 to suppress S. aureus-stimulated bradykinin (BK) production in human plasma was tested as described previously.

Kallikrein activity in ex vivo human plasma spiked with concentrations of BCX4161 was measured in a novel fluorogenic assay: briefly, prekallikrein was activated in plasma with ellagic acid activator. Active kallikrein hydrolyzed the amide bond of Z-FR-AMC, a specific, sensitive substrate for plasma kallikrein and the resulting amidolytic activity was measured in a fluorescence microplate reader.

Kallikrein inhibition was measured using this assay in plasma from rats following oral doses of BCX4161.

aPTT and PT were measured using standard assays.

Bleeding times were measured in transected rodent tail studies: BCX4161, heparin or saline control were administered via the intraperitoneal (IP) route to anesthetized rats or mice. Tails, maintained horizontally at constant temperature, were clipped 1-3 mm from the tip and bleeding times measured by blotting.

Results

BCX4161 is a potent inhibitor of human plasma kallikrein with a Ki of 0.26nM.

On-site dissociation and Ki determination studies revealed that BCX4161 is a slow-tight-binding inhibitor that forms stable enzyme/inhibitor complexes with plasma kallikrein in 2 steps.

BCX4161 has no effect on hemostasis in bleeding time studies at concentrations required for kallikrein inhibition.

Table 1: BCX4161 is a highly selective inhibitor of human plasma kallikrein with equipotent activity against 17/18proteases

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Mean IC50 (nM)</th>
<th>Selectivity index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XIIa</td>
<td>0.15</td>
<td>0.6</td>
</tr>
<tr>
<td>TF/FVIIa-dependent FX activity</td>
<td>3.28</td>
<td>13</td>
</tr>
<tr>
<td>Factor XIa</td>
<td>72.8</td>
<td>280</td>
</tr>
<tr>
<td>Tissue kallikrein</td>
<td>153</td>
<td>587</td>
</tr>
<tr>
<td>Thrombin</td>
<td>176</td>
<td>677</td>
</tr>
<tr>
<td>Plasmin</td>
<td>205</td>
<td>788</td>
</tr>
<tr>
<td>Activated protein C</td>
<td>404</td>
<td>1,554</td>
</tr>
<tr>
<td>Complement C1s</td>
<td>744</td>
<td>2,862</td>
</tr>
<tr>
<td>Factor XII</td>
<td>868</td>
<td>3,231</td>
</tr>
<tr>
<td>Tissue plasminogen activator</td>
<td>24,500</td>
<td>94,231</td>
</tr>
</tbody>
</table>

* The selectivity of BCX4161 against other serine proteases was assessed by comparing the IC50 for the assayed enzyme to the IC50 for plasma kallikrein.

BCX4161 doubles aPTT via prevention of FXII activation at 1.35 μM.

BCX4161 inhibits kallikrein activity in activated human plasma in a fluorometric assay with an IC50 = 0.006 μM.

BCX4161 inhibits FX8 production in plasma activated with S. aureus with an IC50 = 0.03 μM.

BCX4161 inhibits PK activity in activated human plasma in an ex vivo fluorogenic assay with an IC50 = 0.006 μM.

BCX4161 doubles PT via off-target inhibition of 17/18Pvita at 5.1 μM.

BCX4161 inhibits PK activity in activated human plasma in a fluorometric assay with an IC50 = 0.006 μM.

BCX4161 is a potent and specific inhibitor of plasma kallikrein with potential to be the first targeted oral prophylaxis treatment for HAE.

Further development of BCX4161 is warranted and Phase I studies are planned.

Conclusions

Acknowledgments:

The authors would like to express their sincere appreciation to Drs William Sheridan, Phil Collis and Dena Minning for many helpful discussions and suggestions.

References:

3. P-756

Presented at the 2013 AAAAI Annual Meeting Feb 22-26, 2013, San Antonio, TX

Disclosures:

All authors are current or former employees of BioCryst Pharmaceuticals, Inc.

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