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. BCX4161 is an orally bioavailable potent inhibitor of plasma kallikrein.
- Ellagic acid activator (EAA) activates prekallikrein (PKK) in plasma to kallikrein.
- Identification of specific substrates to measure kallikrein activity would permit use of a pharmacodynamic assay to measure the activity of BCX4161 in human plasma.

Objective

- To develop a sensitive and selective assay to determine kallikrein activity in activated plasma from normal and HAE subjects.

Methods

- Ellagic acid activator (EAA) activates prekallikrein (PKK) in plasma to kallikrein.
- Kallikrein hydrolyzes the R-AMC amide bond (“amidolytic activity”) in a peptide-based fluorogenic substrate, Z-FR-AMC, releasing the highly fluorescent ("amidolytic activity") in a peptide-based fluorogenic substrate, Z-FR-AMC, releasing the highly fluorescent 7-amino-4-methylcoumarin (AMC) group.
- The kinetics of amidolytic activity were measured in a fluorescence microplate reader in plasma from normal donors and HAE patients; the inhibitory activity of BCX4161 and C1INH was assessed.

Results

- Amidolytic activity was stimulated by EAA in a dose-dependent manner in normal human plasma; however, in PKK-deficient plasma, EAA-stimulated amidolytic activity was completely abolished (Figure 1), indicating that Z-FR-AMC is a kallikrein selective substrate. Amidolytic activity could be restored in a dose-dependent manner by addition of purified human PKK (Figure 2).
- Figure 1: Z-FR-AMC is a selective substrate for plasma kallikrein.
- Figure 2: Amidolytic activity is restored in PKK-depleted plasma after reconstitution with purified human PKK.

Conclusions

- A sensitive and selective fluorogenic assay was developed to monitor ex-vivo kallikrein activity in human plasma.
- This assay demonstrated that BCX4161 was ~15 times more potent than C1INH as an inhibitor of plasma kallikrein.
- This assay was used as a pharmacodynamic biomarker of drug effect in a Phase 1 study of BCX4161 to aid in dose selection for further trials (see Poster P-138).

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

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