AQX-1125, a Modulator of the SHIP1/PI3K Pathway, Suppresses Chemotaxis and Inflammation

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Abstract

Rationale: AQX-1125, a novel small-molecule allosteric activator of SHIP1, was evaluated for in vitro and in vivo anti-inflammatory activity.

Methods: AQX-1125 was tested in an in vitro Boyden chamber chemotaxis assay. The efficacy of AQX-1125 on reducing ear thickness and myeloperoxidase content was evaluated in vivo in the phorbol 12-myristate 13-acetate (PMA)-induced ear edema mouse model. AQX-1125 was administered orally for 3 days at 1, 10 and 30 mg/kg prior to application of PMA. The efficacy of AQX-1125 was also tested in a rat ovalbumin-mediated airway inflammation model, administered orally for 4 days at 0.1, 1 and 10 mg/kg before airway challenge. The degree of inhibition of leukocyte infiltration and mediator release in the bronchoalveolar lavage fluid (BALF) was measured.

Results: AQX-1125 is a potent inhibitor of in vitro leukocyte chemotaxis. In vivo, AQX-1125 at 30 mg/kg significantly reduced ear edema and myeloperoxidase content in the PMA model. At 1 mg/kg, AQX-1125 significantly reduced the total number of leukocytes recovered in the BALF of animals sensitized and challenged with ovalbumin. AQX-1125 also reduced the level of the inflammatory mediators IL-1α and IL-11, in the BALF.

Conclusions: AQX-1125 potently inhibits leukocyte chemotaxis in vitro and myeloperoxidase (neutrophil) accumulation in vivo. AQX-1125 also inhibits leukocyte accumulation and inflammatory mediator release in the BALF in a model of allergic airway inflammation. These data suggest that AQX-1125 has clinical potential for treatment of allergic and inflammatory diseases such as asthma and COPD.

Introduction

Targeting SHIP1

- PI3K pathway is one of the most active areas in BioTechPharma
- SHIP1 is an ideal drug target
- PI3K/SHIP1 pathway plays a key role in regulating cell migration and activation
- SHIP1 expression restricted to hematopoietic derived cells - limits off-target toxicity
- SHIP1 is a novel target distinct from the extensive PI3K investigations
- Redirects cellular PI3K signalling, rather than prevent it

Background

SHIP enzymes

Good homology between human and rodent SHIP1

- nSHIP1 and mSHIP1 domain
- nSHIP1 and mSHIP1 domain
- nSHIP1 and mSHIP2 domain
- nSHIP1 and mSHIP2 domain

Figure 2. SHIP enzymes – 51% homology between nSHIP1 and mSHIP2, and 38% homology between the nSHIP1C2 and mSHIP2C2 domains. This reduced homology between SHIP1 and SHIP2 confers selectivity

SHIP1 Knockout

- Smallest size
- Splenomegaly and C
- Inflammatory infiltrate of the lungs

SHIP1 Activation

Pelorol was the first generation SHIP1 activator isolated. Analogues of Pelorol, AQX-016A and AQX-MN100, were synthesized with greater SHIP1-activating properties. These small molecules activate SHIP1 through an allosteric mechanism, via interaction with the C2 domain, and are anti-inflammatory in cellular and murine models.

AQX-1125 is a small molecule next generation SHIP1 activator. It has many of the biological effects of the earlier generation SHIP1 activators, but has more drug-like properties.

Cellular and In Vivo Models

- Akt phosphorylation in T cell lines – Western blotting
- Leukocyte chemotaxis in vitro
- PMA-induced ear edema in mice
- Ovalbumin-mediated allergic airway inflammation in rats

Results

AQX-1125 inhibits Akt phosphorylation in SHIP1-proficient MOLT-4, but not in SHIP1-deficient Jurkats

Figure 4. MOLT-4 and Jurkats cells were treated with AQX-1125 for 30 min, followed by stimulation with IGF-1 for 30 min

AQX-1125 inhibits OVA-induced inflammatory mediator content in Brown Norway rat BALF

Figure 6. (A) Male Brown Norway rats were sensitized to OVA, followed by oral AQX-1125 administration and OVA challenge. BAL was performed and resulting data shown expressed as means±SEM of (B) BAL leukocyte and (C) eosinophil counts, *p<0.05 vs OVA.

AQX-1125 inhibits OVA-induced allergic airway inflammation in Brown Norway rats

Figure 5. Human blood leukocytes were treated with AQX-1125 for 30 min, followed by induction of chemotaxis with chemokines listed.

Pharmacokinetics of AQX-1125 in rats

Figure 8. Female ICR dosed orally with AQX-1125 (1, 10, 30 mg/kg) were challenged by topical application of PMA to the ear. Six h post-challenge edema was assessed by measuring the change in ear thickness (A) and tissue MPO content was measured (B). *p<0.05 vs OVA. Data are expressed as mean±SEM.

Summary

SHIP1 is a novel drug target which controls PI3K-driven cellular migration and activation. SHIP1’s restriction to hematopoietic cells and poor homology with SHIP2 reduces the likelihood of off-target, off-tissue toxicity. AQX-1125, a small molecule with PK properties suited to once per day dosing, inhibits the PI3K pathway through activation of SH2-containing inositol-5'-phosphatase 1 (SHIP1), resulting in a reduction of pAkt in T and B lymphocytes. AQX-1125 has significant in vivo anti-inflammatory activity, in models of allergic inflammation. These data indicate that AQX-1125 has significant clinical potential in inflammatory disorders such as asthma.

References


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