Prophylactic or Therapeutic AQX-1125, a Small Molecule SHIP1 Activator, Inhibits Bleomycin-Induced Pulmonary Fibrosis in Mice

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Abstract

Rationale: SH2-containing inositol-5'-phosphatase 1 (SHIP1) dephosphorylates PI(3,4,5)P3 to yield PI(3,4)P2. SHIP1-deficient mice exhibit progressive pulmonary inflammation and develop evidence of pulmonary fibrosis. Pharmacological activation of SHIP1 has emerged as a potential novel approach to regulate pulmonary inflammation, with pre-clinical and clinical studies showing an anti-inflammatory effect of AQX-1125, a small molecule SHIP1 activator. Here we tested the effect of prophylactic or therapeutic AQX-1125 administration in a murine model of bleomycin-induced pulmonary inflammation and fibrosis.

Methods: The efficacy of AQX-1125, administered by oral gavage (3, 10 or 30 mg/kg), was assessed in bleomycin (BLM)-induced lung fibrosis in male CD-1 mice. In the prophylactic investigation, bleomycin (0.1 IU/mouse) was administered two h after the third dose of AQX-1125. For therapeutic investigation, AQX-1125 was administered starting on Day 13 after bleomycin administration (0.05 IU/mouse). AQX-1125 administration continued once per day throughout the remainder of the studies. Twenty-one-days (prophylactic investigation), or 28-days (therapeutic investigation) after bleomycin administration, mice were sacrificed and bronchoalveloar lavage (BAL) cellular content, lung edema, myeloperoxidase, TGF-β, histopathology, collagen deposition and mortality determinations were made.

Results: In the 21-day prophylactic model, AQX-1125 significantly ($p<0.05$) suppressed bleomycin-induced collagen deposition, inflammation and mortality. Moreover, therapeutically administered AQX-1125 also dose-dependently reduced the mortality to bleomycin over the duration of the study. In addition, therapeutic AQX-1125 (10 or 30 mg/kg) significantly ($p<0.05$) attenuated the bleomycin-induced collagen deposition in the airways by 43% and 82% respectively; which correlated with significantly suppressed leukocyte infiltration of the airways, lung tissue edema, myeloperoxidase activity, TGF-β concentration and the histopathology score ($p<0.05$).

Conclusion: Therapeutic or prophylactic SHIP1 activation with AQX-1125 inhibits mortality, leukocyte accumulation, edema, inflammatory mediator and collagen content of the airways in a murine model of bleomycin-induced fibrosis. Thus, AQX-1125 has the potential to be developed as a treatment for fibrotic disease.

Introduction

Targeting SHIP1

- PI3K pathway is an established target for drug development
- PI3K/SHIP1 pathway plays a key role in regulating cell migration and activation
- Targeting SHIP1 is an alternate way of modulating the PI3K pathway
- SHIP1 expression restricted to hematopoietic derived cells - limits off-target toxicity
- SHIP1 activation redirects cellular PI3K signalling, rather than preventing it

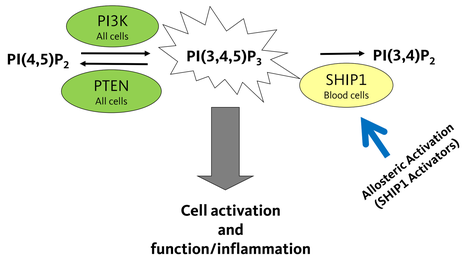


Figure 1. *SHIP1 and PI3K signalling. SHIP1 activators redirect PI3K signalling, PI3K inhibitors block PI3K signalling.*

Background

SHIP1 Activation

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 an improved drug scaffold and superior drug-like 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.

Prophylactic 21-day Bleomycin Model

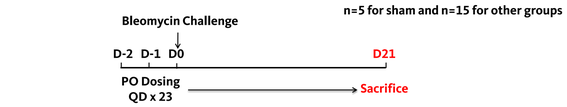


Figure 2. *Study design for 21-day BLM-induced airway inflammation challenge in mice.*

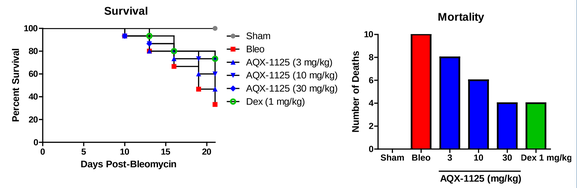
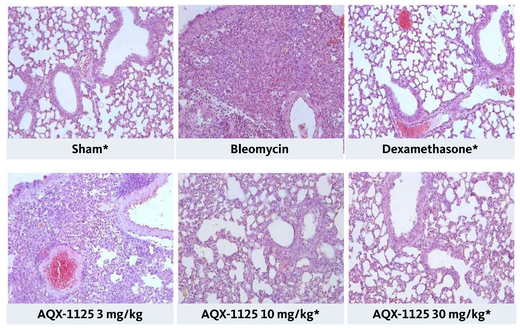


Figure 3. *Efficacy of AQX-1125 on survival in a 21-day BLM-induced lung fibrosis model.*

AQX-1125 suppresses bleomycin-induced mortality



*p<0.05 vs Bleo Challenge

Figure 4. *Histopathologic representation of AQX-1125 dose response in BLM-induced lung model*

AQX-1125 inhibits bleomycin-induced lung histopathological changes

Table 1. *Efficacy of AQX-1125 on additional endpoints in the therapeutic 21-day BLM-induced lung fibrosis model.*

Endpoint	Sham	Bleo	AQX-1125 (30 mg/kg, Oral)	Dex (1 mg/kg, IP)
MPO (U/g tissue)	66±3*	542±15	193±15*	99±5*
Edema (Wet:Dry Weight)	2.04±0.19*	5.30±0.18	2.70±0.09*	2.33±0.13*
BAL Leukocytes (x10^3 Cells)	207±5*	1015±69	504±31*	356±11*
Macrophages	207±5*	663±52	352±.18*	285±11*
Lymphocytes	0*	225±22	99±15*	43±6*
Neutrophils	0*	121±19	52±6*	30±4*
Eosinophils	0*	6.1±2.6	1.5±0.8	0.6±0.4*
Collagen (µg/mg)	3.4±0.2*	17.8±0.4	9.2±0.6*	5.4±0.2*

AQX-1125 inhibits bleomycin-induced lung histopathological changes

AQX-1125 (10 or 30 mg/kg) significantly inhibited the BLM-induced total leukocyte infiltration of the airways by 59% and 63%, respectively. AQX-1125 dose-dependently (3, 10 or 30 mg/kg) reduced the mortality to BLM over the duration of the study and significantly inhibited the BLM-mediated increase in edema, myeloperoxidase activity, histopathology scores, collagen accumulation and inflammatory marker accumulation in the lung.

Therapeutic 28-day Bleomycin Model

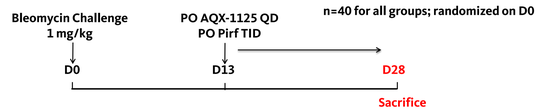
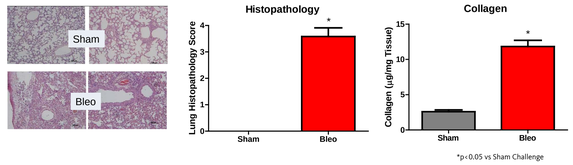


Figure 5. *Study design for 28-day BLM-induced airway inflammation challenge in mice.*



*p<0.05 vs Sham Challenge

Figure 6. *BLM-induced therapeutic lung fibrosis model at day 13 prior to AQX-1125 administration.*

Disease burden on Day 13 with expected 30% mortality

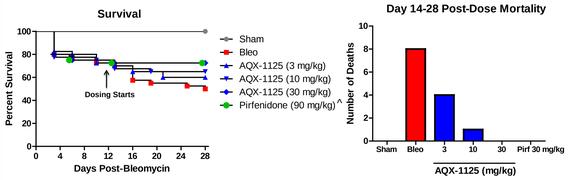
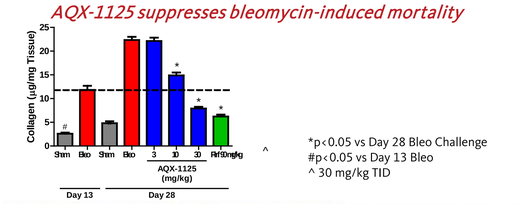


Figure 7. *Efficacy of AQX-1125 on survival in a 28-day BLM-induced therapeutic lung fibrosis model.*

AQX-1125 suppresses bleomycin-induced mortality



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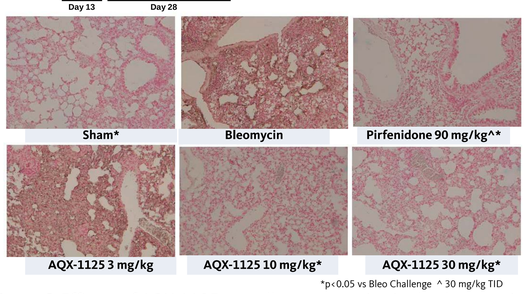
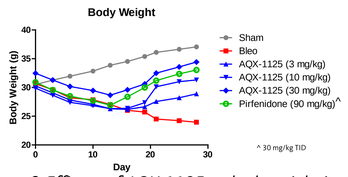
*p<0.05 vs Day 28 Bleo Challenge
#p<0.05 vs Day 13 Bleo
^ 30 mg/kg TID



*p<0.05 vs Bleo Challenge ^ 30 mg/kg TID

Figure 8. *Efficacy of AQX-1125 on collagen expression in the therapeutic 28-day BLM-induced lung fibrosis model.*

AQX-1125 reduces bleomycin-induced lung collagen content



^ 30 mg/kg TID

Figure 9. *Efficacy of AQX-1125 on body weight in the therapeutic 28-day BLM-induced lung fibrosis model.*

AQX-1125 suppresses bleomycin-mediated decrease in body weight

Therapeutic 28-day Bleomycin Model

Table 2. *Efficacy of AQX-1125 on additional endpoints in the therapeutic 28-day BLM-induced lung fibrosis model.*

Endpoint	Sham	Bleo	AQX-1125 (30 mg/kg, QD)	Pirfenidone (30 mg/kg, TID)
MPO (U/g tissue)	72±1*	908±34	302±5*	298±5*
Edema (Wet: Dry Weight)	1.92±0.04*	8.22±0.18	3.26±0.09*	3.31±0.11*
BAL Leukocytes (x10^6 Cells)	0.20±0.003*	1.00±0.03	0.34±0.01*	0.34±0.01*
TGF-β (histo score)	0±0*	7.14±0.18	0.23±0.01*	0.11±0.01*
HSP 47 (histo score)	0±0*	7.12±0.15	0.10±0.01*	0.07±0.01*
Collagen (µg/mg)	4.8±0.4*	22.4±0.7	7.9±0.4*	6.2±0.4*
Hydroxyproline (µg/mg)	3.8±0.1*	11.1±0.3	5.4±0.1*	5.0±0.1*

AQX-1125 suppresses bleomycin-induced changes in additional endpoints

Therapeutically administered AQX-1125 (3,10 or 30 mg/kg) significantly inhibited the BLM-induced total leukocyte infiltration by 11%, 54% and 83%, respectively, and dose-dependently reduced the mortality to BLM over the duration of the study. In addition, therapeutic AQX-1125 (10 or 30 mg/kg) significantly attenuated the BLM-induced collaged deposition, lung tissue edema, myeloperoxidase activity, histopathology score and inflammatory marker expression in the lung.

Clinical Development of AQX-1125

Phase II: Studies

- Interstitial Cystitis/Bladder Pain Syndrome "LEADERSHIP" Study
  - Parallel group, randomized, double-blind, placebo-controlled, multicenter study
  - 6 weeks dosing
  - Bladder pain and IC/BPS symptom endpoints
- COPD Exacerbation "FLAGSHIP" Study
  - Parallel group, randomized, double-blind, placebo-controlled, multicenter study
  - 12 weeks dosing
  - Exacerbation rates and COPD symptom endpoints

Phase IIa clinical data are presented:
"The Effects of The Novel SHIP1 Activator AQX-1125 on Allergen-Induced Responses in Mild to Moderate Asthma"

And

"The Effects of AQX-1125, a Selective Oral SHIP1 Activator on Lipopolysaccharide-Induced Cellular and Biochemical Changes in Sputum From Healthy Volunteers"

Summary

SHIP1 is a novel drug target which controls PI3K-driven cellular migration and activation. SHIP1's preferential expression in hematopoietic cells and low degree of homology with SHIP2 reduces the likelihood of off-target, off-tissue toxicities. AQX-1125, a small molecule allosteric SHIP1 activator, with PK properties suited to once daily dosing, inhibits the PI3K pathway resulting in an inhibition of Akt phosphorylation and reduced chemotaxis. AQX-1125 has high water-solubility, good oral bioavailability, a terminal half-life suited to once per day dosing, minimal metabolism, and distributes to the lung at high concentrations[1]. As shown here, orally administered AQX-1125 inhibits bleomycin-mediated airway inflammation and fibrosis. In addition to the current anti-fibrotic data, AQX-1125 has demonstrated efficacy in several rodent models of pulmonary inflammation[2], supporting the view that SHIP1 activators, such as AQX-1125, may have significant therapeutic potential in fibrotic disease. Phase II studies are underway and will be instrumental in determining the potential human clinical therapeutic utility of the compound.

References

1. Stenton et al. (2012a). Characterization of AQX-1125, a small molecule SHIP1 activator. Part 1. Effects on inflammatory cell activation and chemotaxis *in vitro* and pharmacokinetic characterization *in vivo*. *Br J Pharmacol* in press
2. Stenton et al. (2012b). Characterization of AQX-1125, a small molecule SHIP1 activator. Part 2. Efficacy studies in pulmonary inflammation models *in vivo*. *Br J Pharmacol* in press