AQX-1125, a SHIP1 Activator, Inhibits Chemotaxis *In Vitro* and Exerts Pleiotropic Anti-Inflammatory Effects in a Rodent Model of Endotoxin-Induced Pulmonary Inflammation

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Abstract

Rationale: Pharmacological modulation of the phosphoinositide 3-kinase (PI3K)/Akt pathway is an established approach to controlling inflammatory disorders. SH2-containing inositol-5'-phosphatase 1 (SHIP1) metabolizes PI(3,4,5)P3 to PI(3,4)P2. SHIP1-deficient mice exhibit pulmonary inflammation, characterized by significant granulocyte recruitment into the lung. Preclinical pharmacological activation of SHIP1 by the small molecule AQX-1125, is reported herein as an emerging innovative therapy for pulmonary inflammatory diseases.

Methods: AQX-1125 was tested in an *in vitro* enzyme assay utilizing recombinant human SHIP1 enzyme (wild-type and mutant enzyme lacking the C2 domain). As phosphoinositide signaling plays a key role in chemokinesis, the effect of AQX-1125 was tested on leukocyte chemotaxis using Boyden chambers. *In vivo*, the efficacy of AQX-1125 was tested in a model of intratracheal LPS challenge in the rat. Pharmacokinetic studies were also performed in rats.

Results: AQX-1125 induced a concentration-dependent increase in the catalytic activity of human recombinant SHIP1, an effect, that was absent after deletion of the C2 domain of the enzyme, indicating an allosteric mode of activation. AQX-1125 exerted an inhibitory effect on leukocyte chemotaxis. The greatest effect was against monocyte and B cell chemotaxis which had IC_{50} of 288 and 28 nM respectively. AQX-1125 administered to rats exhibited high oral bioavailability (85% at 30 mg/kg), a terminal half-life of approximately 5h, and high concentrations in a number of parenchymal tissues, including the lung. Consistent with its inhibitory effect on chemotaxis, the compound afforded a dose-dependent reduction of leukocyte infiltration into the bronchoalveolar lavage fluid (BALF) in a rat pulmonary inflammation model induced by LPS (43% inhibition of neutrophil influx at 30 mg/kg). This effect was associated with a reduction in the levels of multiple chemokines, cytokines and growth factors, with a characteristic signature different from that of the reference compound dexamethasone.

Conclusion: The SHIP1 activator AQX-1125 potently inhibits leukocyte chemotaxis *in vitro*, inhibits LPS-induced pulmonary inflammation and inflammatory mediator release *in vivo* and exhibits pharmacokinetics suitable for once per day oral dosing. Thus, AQX-1125 may have clinical potential for treatment of pulmonary inflammatory diseases. Proof-of-concept clinical efficacy studies are currently being initiated to assess the utility of the SHIP1 activator, AQX-1125, in human pulmonary inflammation.

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

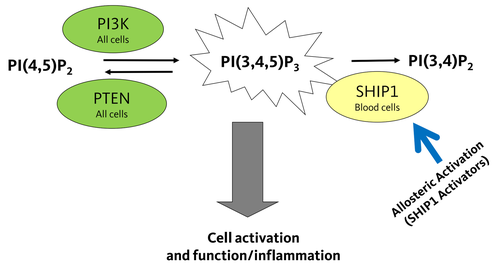

Figure 1. *SHIP1 and PI3K signalling. SHIP1 activators redirect PI3K signalling, PI3K inhibitors block PI3K signalling.*

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[1,2], 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.

Biochemical Assay: AQX-1125 Activates SHIP1

Table 1. *SHIP enzyme homology*

	hSHIP2	mSHIP1	rSHIP1
hSHIP1	51%	92%	91%
hSHIP1 C2 domain	38%	91%	90%

The low degree of homology between SHIP1 and SHIP2 confers selectivity. High homology between human and rat SHIP1 validates use of animal models for this target.

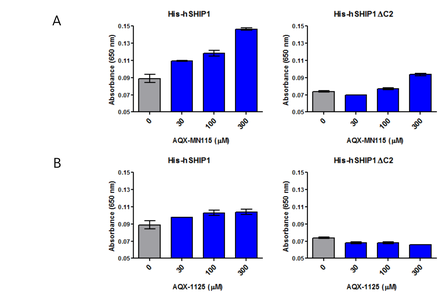

Figure 2. *AQX-MN115 and AQX-1125 activates hSHIP1 in a Malachite Green biochemical assay.*

AQX-MN115 and AQX-1125 activate human recombinant SHIP1, but not when the C2 domain is deleted, indicating allosteric activation

In Vitro: AQX-1125 Inhibits Akt Phosphorylation

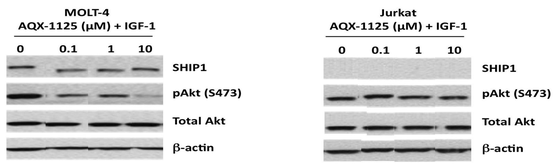

Figure 3. *MOLT-4 and Jurkat cells were treated with AQX-1125 for 30 min, followed by stimulation with IGF-1 for 30 min.*

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

In Vitro: AQX-1125 Inhibits Leukocyte Chemotaxis

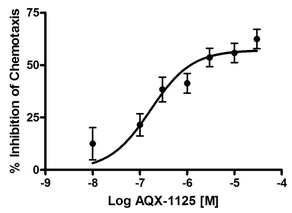

Figure 4. *AQX-1125 inhibits chemotaxis of human blood monocytes (n=4)*

Table 2. *AQX-1125 inhibits chemotaxis of various human leukocyte populations as shown below*

Cell Type	Chemokine	Chemokine Receptor	Potency of AQX-1125 (IC_{50})
Monocytes	MCP-1	CCR2	288 nM
B Cells	BCA-1	CXCR5	28 nM
Activated T Cells	IP-10 / I-TAC	CXCR3	70 nM /229 nM
Non-activated T Cells	MIP-1α	CCR1	33 nM
Neutrophils	GRO-α / IL-8	CXCR1/2	30 nM /73 nM

AQX-1125 inhibits leukocyte chemotaxis in vitro

In Vivo: Diverse Utility of AQX-1125

Table 3. *AQX-1125 has diverse in vivo utility*

In Vivo Model	Challenge	Disease	Result
Cystitis, Bladder (Rat)	Cyclophosphamide	Interstitial Cystitis	✓
Fibrosis (Mouse)	Bleomycin	Pulmonary Fibrosis	✓
Airway Inflammation (Rat)	LPS Ovalbumin	Infect./COPD Asthma	✓ ✓
Allergic Rhinitis (Mouse)	Ovalbumin	AR	✓
Airway Inflammation (Mouse)	Smoke	COPD	✓
IBD (Rat)	TNBS	IBD	✓
Psoriasis (Mouse)	Imiquimod	Psoriasis	✓
Ear Edema (Mouse)	PCA, PMA	Allergic Derm.	✓
Paw Edema (Mouse)	Carrageenan	Inflam. Pain	✓

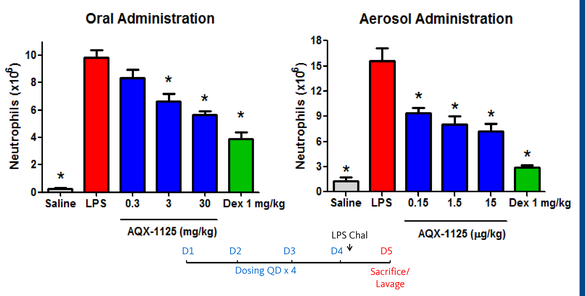

Figure 5. *AQX-1125 reduces neutrophil infiltration in a rat model of lung inflammation induced by intratracheal LPS challenge. Data are expressed as mean±SEM of n=10 determinations.*

Oral and topical AQX-1125 inhibits inflammatory cell accumulation in the BAL of rats in a model of LPS-induced pulmonary inflammation

Table 4. *AQX-1125 reduces LPS-induced inflammatory mediator content in rat BAL*

Analyte	Saline	LPS	AQX-1125 (10 mg/kg)
Myeloperoxidase (ng/mL)	2±0*	26±2	17±3*
Leukemia Inhibitory Factor (pg/mL)	71±16*	1447±204	648±25*
Stem Cell Factor (pg/mL)	68±5*	175±15	111±12*
Endothelin-1 (pg/mL)	5±0*	10±1	8±1*
Monocyte Chemotactic Protein-1 (pg/mL)	15±7*	382±77	190±51*
Monocyte Chemotactic Protein-3 (pg/mL)	18±8*	314±64	149±46*
Macrophage Inflammatory Protein-1β (pg/mL)	534±170*	48360±3044	39380±2794*
Macrophage Derived Chemokine (pg/mL)	12±2*	165±56	35±14*
Fibroblast Growth Factor-Basic (ng/mL)	6±1*	7±1	1±1*
Lymphotactin (pg/mL)	10±1*	33±3	22±3*

In Vivo: Pharmacokinetics of AQX-1125

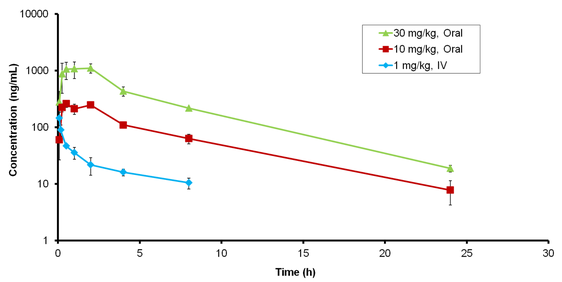

Figure 6. *Pharmacokinetics of AQX-1125 in male Sprague Dawley rats. Data are expressed as mean±SD.*

AQX-1125 has a dose proportional pharmacokinetic profile

In Vivo: Pharmacokinetics of AQX-1125

Table 5. *Pharmacokinetics of AQX-1125 in male Sprague Dawley rats*

	IV 1 mg/kg	PO 10 mg/kg	PO 30 mg/kg
T_{max} (hr)	0.083	1.00	1.17
C_{max} (ng/mL)	144	267	1230
AUC_{inf}/D	268	176	227
$t_{½}$ (hr)	5.6	5.2	4.5
F (%)	N/A	66	85

Clinical Development of AQX-1125

Phase I: Pharmacokinetics of AQX-1125 in healthy human volunteers

- AQX-1125 is well tolerated in HHV's (SAD/MAD)
- PK is dose-proportional
- Terminal half-life is ~22hr
- No food effects detected on AUC
- Oral bioavailability is high
- PK supports once-a-day dosing

Phase II: Allergen and LPS Challenge

- Allergen Challenge (Asthma POC)
  - Respiratory Clinical Trials Group, London, UK - PIs: Drs. Leaker & O'Connor
  - Cross-over study (1 active dose + placebo); 22 mild asthmatics, 7 days dosing
  - Lung function, sputum leukocytes and analyte endpoints
- LPS Challenge (COPD POC)
  - Celerion, Belfast, UK - PI: Drs. Smith & Elborn
  - Two parallel groups, cross-over study (2 active doses + placebo); 40 NH volunteers, 7 days dosing
  - Sputum leukocytes and analyte endpoints

Phase 1 Clinical Data are presented: AQX-1125, A SHIP1 Activator In Clinical Development For Pulmonary Inflammation: Pharmacokinetics, Metabolism And Tolerability In Healthy Human 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 per day dosing, inhibits the PI3K pathway resulting in an inhibition of Akt phosphorylation and reduced chemotaxis. As shown here, orally or topically administered AQX-1125 inhibits LPS-mediated airway inflammation and inflammatory mediator production *in vivo*. In addition to the effects on LPS-mediated airway inflammation, AQX-1125 has significant and diverse *in vivo* utility in allergen and smoke-induced airway inflammation, pulmonary fibrosis, as well as inflammatory disorders of the skin, bladder and gut. These data indicate that AQX-1125 has clinical potential in multiple inflammatory disorders such as asthma, COPD, pulmonary fibrosis, cystitis, etc. Phase IIa proof-of-concept studies are underway and will be instrumental in determining the potential human clinical therapeutic utility of the compound.

References

1. Helgason *et al*. Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span. *Genes & Dev.* 1998;12:1610-1620.
2. Ong *et al*. Small molecule agonists of SHIP1 inhibit the phosphoinositide 3-kinase pathway in hematopoietic cells. B*lood.* 2007;110:1942-1949.

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