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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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, suggesting 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 approximately 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.

Conclusions: 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.

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