Inhibitors, Agonists, Screening Libraries

BIOLOGICAL ACTIVITY:

Alvelestat (AZD9668) is a novel, oral inhibitor of neutrophil elastase (NE) with the pIC50 of 7.9 for Human NE.

IC50 Value: 7.9 ± 0.12 (pIC50, Human NE); 4.9 nM (Ki value, Human NE) [1]

Target: Neutrophil elastase

in vitro: AZD9668 had a high binding affinity for human NE (KD = 9.5 nM) and potently inhibited NE activity. The calculated pIC50 (IC50) and Ki values for AZD9668 for human NE were 7.9 (12 nM) and 4.9 nM, respectively. In contrast to earlier NE inhibitors, the interaction between AZD9668 and NE was rapidly reversible. AZD9668 was also highly selective for NE over other neutrophil-derived serine proteases. In cell-based assays, AZD9668 inhibited plasma NE activity in zymosan-stimulated whole blood. In isolated human polymorphonuclear cells, AZD9668 inhibited NE activity on the surface of stimulated cells and in the supernatant of primed, stimulated cells. AZD9668 showed good crossover potency to NE from other species [1].

in vivo: Six hundred and fifteen patients were randomised: placebo (302), AZD9668 60 mg bid (313). AZD9668 showed no effect on lung function: change in mean pre-bronchodilator FEV1 versus placebo was 0.01L (95% confidence interval: -0.03, 0.05; p=0.533). AZD9668 did not significantly improve respiratory signs and symptoms, SGRQ-C score or time to first exacerbation. Adverse events were similar for AZD9668 and placebo [2]. AZD9668 was well tolerated at single doses up to 150 mg and multiple doses up to 70 mg twice daily. PK were dose linear; median time to peak plasma concentration was reached at 0.5 - 1.5 hours and the short elimination half-life was consistent with twice daily dosing. Steady state was reached by Day 2 of twice daily dosing with negligible accumulation. Approximately 40% of AZD9668 was eliminated renally as unchanged compound. Ex vivo zymosan-stimulated inhibition of NE activity was dose-dependent, with maximal inhibition achieved at 60 mg [4].

Toxicity: A total of 838 patients were randomised to AZD9668 5 mg bid (212 patients), 20 mg bid (206 patients), 60 mg bid (202 patients) or placebo (218 patients). AZD9668 showed no effect on lung function, respiratory signs and symptoms, QoL or biomarkers [3].

Clinical trial: Phase II study of a neutrophil elastase inhibitor (AZD9668) in patients with bronchiectasis.

PROTOCOL (Extracted from published papers and Only for reference)

Kinase assay [1] The binding kinetics between AZD9668 and human NE are analyzed using a BIAcore T100 instrument and a direct binding assay. NE (100 μg/ml in 10 mM acetate buffer, pH 4.5), preincubated for 10 min with AZD9668 (1 μM) to maintain active site availability, is immobilized to a CMS sensor chip surface by amine coupling. An activated and deactivated CMS chip surface using amine coupling is used as a control surface. After equilibration with running buffer (0.1 M Tris-HCl, pH 7.4, containing 0.5 M NaCl buffer, pH 7.4, with 1% DMSO; AZD9668 is injected over the immobilized enzyme at a flow rate of 50 μl/min, and the association rate is determined. After 1 min, running buffer is applied to the surface, and the dissociation rate is determined over 5 min. The rate of complex (AB) formation between AZD9668 (A) and the immobilized NE (B) during the sample injection is given by: \( \frac{d[AB]}{dt} = kon[A][B] - koff[AB] \). Cell Assay [1] In the whole-blood assay, citrate anti-coagulated human whole blood is incubated with AZD9668...
for 15 min before the addition of opsonized zymosan (final concentration 0.75 mg/ml). NE activity is measured in cell-free plasma after the addition of NE substrate (71 μM final concentration in 0.1 M Tris-HCl, pH 7.4 containing 0.5 M NaCl) and incubation for 60 min at room temperature. Animal administration [1] Human NE (250 U/ml and 1 ml/kg body weight), dissolved in 9 mg/ml NaCl, is given to female C57BL/6J BomTac mice intratracheally 1 h after oral administration of AZD9668 in drug vehicle (0.5% hydroxypropyl methylcellulose in citrate buffer). Nondrug-treated control and NE-treated animals are administered either 9 mg/ml NaCl or human NE as appropriate 1 h after administration of drug vehicle. Four hours later, the mice are sacrificed by an overdose of pentobarbital and subjected to BAL. The BAL fluid is then centrifuged and the cell pellet is resuspended in 1 ml of deionized water to lyse the red blood cells. Hemorrhage is defined as the concentration of hemoglobin in BAL cell lysate and calculated by determining the absorbance at 412 nm and extrapolating the values from a hemoglobin reference curve.

References: