PUR1800 (RV1162), A NOVEL NARROW SPECTRUM KINASE INHIBITOR, BUT NOT FLUTICASONE, REDUCES TNFα-INDUCED CYTOKINE RELEASE BY PRIMARY BRONCHIAL EPITHELIAL CELLS FROM HEALTHY VOLUNTEERS AND COPD PATIENTS

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Abstract

PUR1800 is a dry powder iSPERSE formulation of RV1162, a narrow-spectrum kinase inhibitor (NSKI), targeting p38 MAPK, Src and Syk kinases. PUR1800 is being developed as an inhaled anti-inflammatory therapy for COPD. The anti-inflammatory effects of PUR1800 were tested by measuring inhibition of TNFα-induced cytokine release from primary human bronchial epithelial cells (HBEC) collected from healthy volunteers (HV) and COPD patients. Cells were incubated for 2h with RV1162, fluticasone propionate (FP) or vehicle before stimulation with 50 ng/mL TNFα for 4h. Supernatant was analyzed for CXCL8 and IL-6 using R&D Systems Human Duoset® ELISA kits. TNFα stimulated CXCL8 and IL-6 production in HV HBEC cells by 2.9- and 3.0-fold and in COPD HBEC cells by 1.9- and 1.8-fold, respectively. In both cell types, FP partially inhibited cytokine release, failing to achieve an IC₅₀ value up to a concentration of 2 nM. RV1162 inhibited CXCL8 and IL-6 release in a concentration-dependent manner in both HV and COPD HBECs. In HV HBEC cells, the IC₅₀ values for CXCL8 and IL-6 were 3.3 and 2.8 nM and in COPD HBEC cells, the same cytokine IC₅₀ values were 0.85 and 1.7 nM respectively. These data demonstrate the potential of PUR1800 as an anti-inflammatory therapy in COPD patients.

Overview

RV1162 is a novel inhaled narrow-spectrum kinase inhibitor (NSKI) targeting the inflammatory kinases, p38 MAPK, Src and Syk. PUR1800 is being developed as a dry powder iSPERSE formulation of RV1162 for the treatment of acute exacerbations of COPD (AECOPD). Exacerbations, often secondary to viral infection, can significantly worsen symptoms, leading to hospitalization with long lasting or permanent impairment of lung function. AECOPD are often poorly responsive to corticosteroids, underlining the need for alternative therapies. In addition to anti-inflammatory effects, preclinical studies with RV1162 have demonstrated potential for anti-viral activity. The ability of RV1162 to inhibit both inflammation as well as viral activity suggests a possible role in prevention and treatment of AECOPD.

Methods

Primary airway epithelial cells were obtained from 3 different healthy subjects and 3 different patients with COPD (Asterand (Royston, UK) or Lonza (Basel, Switzerland)), and maintained in BEGM media. Cells were exposed to RV1162, fluticasone propionate or vehicle (DMSO) across a range of doses 2h prior to stimulation with 50ng/mL TNFα. Cells were incubated for 4h at 37°C and 5%CO₂. Supernatants were collected and frozen at -20°C prior to analysis.

The levels of IL-8 and IL-6 were determined by ELISA using R&D Systems’ Human CXCL8 and IL-8 Duoset® Elisa Kits. Costar 96 well high binding plates were treated with capture antibody diluted in Dulbecco’s phosphate buffered solution (PBS) overnight. Plates were washed (0.05% Tween20 in PBS) and blocked for one hour with 1% Bovine serum albumin (Fisher Scientific UK Ltd, Loughborough, UK) in PBS. Supematant and standards were added to a final volume of 100L and plates were incubated at room temperature for 2 hours. ELISA plates were washed and processed following the manufacturer’s recommended protocol.

Fold increases in IL-8 and IL-6 following TNFα were calculated by comparing unstimulated cells to cells stimulated with TNFα and treated with vehicle. The percent inhibition of RV1162 and fluticasone were calculated for each concentration by comparison to the vehicle control. Results of duplicate dilutions and plates were averaged together for each subject and mean percent inhibition across the 3 subjects were determined. The 50% inhibitory concentrations (IC₅₀) were determined from the resultant concentration-response curves.

Results

TNFα-induced IL-8 and IL-6 production by epithelial cells from healthy subjects

TNFα-stimulated IL-8 and IL-6 production in normal bronchial cells by 2.9±0.8 fold and 3.0±0.9 fold, respectively (Figure 1). Fluticasone propionate only partially inhibited both IL-8 and IL-6 production, but its maximum inhibitory effects were below 35%. In contrast, RV1162 inhibited both IL-8 and IL6 release in a concentration dependent manner, and IC₅₀ values were 3.3 and 2.8 nM, respectively (Figure 2).

TNFα-induced IL-8 and IL-6 production by epithelial cells from COPD patients

TNFα-stimulated IL-8 and IL-6 production in the COPD bronchial cells by 1.9±0.2 fold and 1.8±0.2 fold respectively (Figure 1). Basal levels of IL-8 and IL6 were higher in COPD cells than in healthy cells (IL-8: 296.2±36.2 pg/mL vs 215 ±5.3 pg/mL; IL-6: 42.1±1.0 pg/mL vs 17.6±7.7 pg/mL; Figure 3). Fluticasone propionate inhibited both IL-8 and IL-6 production, but its maximum inhibitory effects were below 43%. In contrast, RV1162 inhibited both IL-8 and IL6 release in a concentration-dependent manner, and IC₅₀ values were 0.85 and 1.7 nM, respectively (Figure 4).

Conclusions

• TNFα stimulated IL-8 and IL-6 release from primary human bronchial epithelial cells derived from healthy subjects and patients with COPD
• TNFα-induced IL-8 and IL-6 release was modestly inhibited by fluticasone propionate, suggesting that these culture systems are glucocorticoid insensitive
• RV1162 inhibited the release of TNFα-induced IL-8 and IL-6 in a concentration dependent manner with IC₅₀ values ranging from 0.85 to 3.3 nM