THE EFFECT OF RV1162 (PUR1800), A NOVEL NARROW SPECTRUM KINASE INHIBITOR, ON VIRAL REPLICATION AND VIRAL-INDUCED INFLAMMATION IN PRIMARY HUMAN AIRWAY CELLS

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Introduction

RV1162 is a novel inhaled narrow-spectrum kinase inhibitor (NSKI) targeting three families of 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, underlying the need for alternative therapies. In addition to anti-inflammatory effects, preliminary 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.

This poster summarize results of two studies, the first assessing the anti-viral activity of RV1162 in respiratory syncytial virus (RSV)-infected primary human bronchial epithelial cells (HBEC) from healthy volunteers and the second assessing the anti-inflammatory effects of RV1162 in Poly (I:C)-stimulated primary HBECs from healthy volunteers and COPD patients. Poly (I:C) is a TLR3 agonist that mimics the effects of a viral infection, resulting in a cellular inflammatory response.

Study #1

Primary Human Bronchial Epithelial Cells (HBEC) were harvested from healthy volunteers and grown in 96 well plates for at least 48 hours prior to infection. Cells were infected with 100µL of a solution containing Respiratory Syncytial Virus (RSV) strain A2 and maintained for 1 hour at 37^{0}C for adsorption. Cells were then washed with PBS and fresh media and incubated for 4 days. Cells were treated for 2 hours prior to infection and 1 hour post viral washout with a control solution (DMSO), RV1162 in solution at up to 5 dose levels, or Ribavarin at 10µg/mL as a positive control. Cell viability was assessed for each dose group and compared to vehicle control cells *via* crystal violet staining as demonstrated in Table 1 and Figure 1 below.

Table 1. Effects of RV1162 and Ribavarin on HBEC Viability

Conc. (µg/mL)	0.0016	0.008	0.04	0.2	10
RV1162	70.3 (13.3)	86.3 (14.9)	91.5 (19.1)	81.3 (20.6)	-
Ribavarin	-	-	-	-	60.8 (13.3)

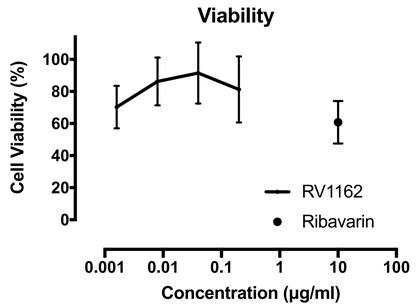

Figure 1. Graphical representation of effects on cell viability.
Cell viability, expressed as mean % cell survival ± SD, across 4 replicate tests, was reduced by the efficacious dose of Ribavarin with increased viability at all RV1162 doses.

After 4 days of incubation, viral load was assessed by measurement of RSV F-Protein expression as detected by ELISA. Harvested cells were fixed with 4% formaldehyde in PBS and exposure to anti-RSV F-fusion antibody, followed by an HRP-conjugated secondary antibody. Once the reaction as stopped, the resultant signal was determined calorimetrically in a microplate reader. These data are shown below in Table 2 and in Figure 2.

Table 2. Inhibition of RSV F-Protein Expression in HBECs

Conc. (µg/mL)	0.00032	0.0016	0.008	0.04	0.2	10
RV1162	1.2 (3.4)	4.4 (20.4)	62.0 (10.1)	89.6 (7.5)	88.7 (8.9)	-
Ribavarin	-	-	-	-	-	94.5 (5.8)

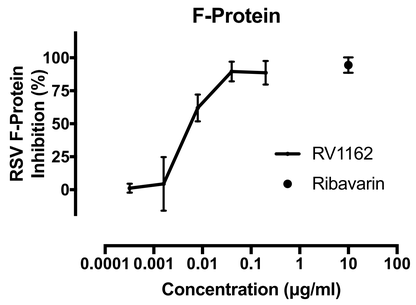

Figure 2. Graphical representation of RSV F-Protein inhibition
RV1162 RSV F-Protein expression, expressed as mean % inhibition ± SD, across 4 replicates resulted in an IC_{50} of 6.4 ng/mL. The standard dose of Ribavarin showed similar efficacy but with reduced cell viability. These data demonstrate the ability of RV1162 to inhibit the viral replication in HBECs at relatively low concentrations with minimal effects on cell viability over 4 days of incubation.

Study #2

HBECs were harvested from healthy volunteers and COPD patients and cultured in an air liquid interface (ALI) in 24-well plates. Cells were pretreated with DMSO as a negative control, 2 dose levels of fluticasone propionate or 2 dose levels of RV1162 at the basal surface for 2h. The basal and apical surfaces were stimulated with 50ng/mL Poly (I:C) in DMSO containing the appropriate doses of fluticasone or RV1162. Exposure to Poly (I:C) resulted in increased cytokine expression after incubation for 24h at 37^{0}C and 5% CO_2. The effect of Poly (I:C) on each analyte was measured and expressed as percent reduction versus control (DMSO) treated cells. Both HBECs from healthy volunteers and COPD patients responded in a similar manner to the stimulus. The inflammatory cytokine release was assessed *via* a Luminex kit for IL-6, TNFα, CXCL8, eotaxin, MIP1-β, G-CSF and GM-CSF in basal supernatant as well as apical TNFα. These data are shown in Figure 3. RV1162 ■ ; Fluticasone propionate ■

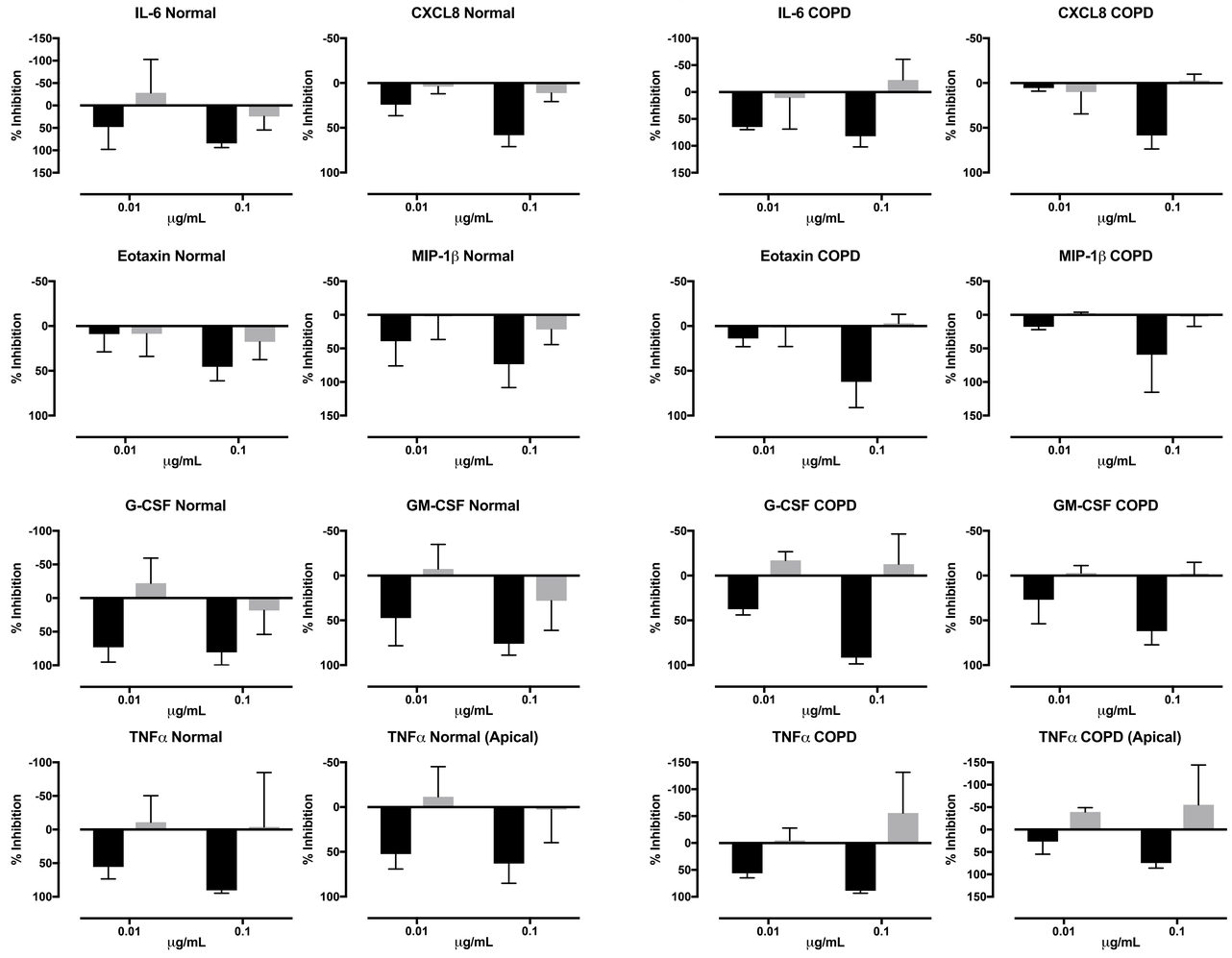

Figure 3. Reductions in cytokine response with RV1162 or Fluticasone treatment after Poly (I:C) stimulation in HBECs.
Data represent the mean % reduction (across 4 normal or 3 COPD replicates ±SD) in each of the cytokines at 0.01 or 0.1µg/mL of each compound 24 hours after exposure to 50ng/mL Poly (I:C) relative to DMSO controls. The majority of the cytokines were measured in the basal supernatant with the exception of TNFα, which was also measured in the apical supernatant. Data demonstrate a dose dependent reduction in the maximum cytokine levels relative to those from cells stimulated with Poly (I:C) and treated with DMSO as a negative control.

Conclusions

- These data indicate that RV1162 shows activity against both viral replication, as well as, against the inflammatory response of human bronchial epithelial cells to viral infection.
- The anti-inflammatory effects were equally effective in cells from healthy volunteers and COPD patients
- Combined, these data suggest that RV1162 may be an effective treatment for viral-induced exacerbation in COPD.