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Testing the effects of Hsp90 inhibitor and mTOR inhibitor on HPIV3 and RSV replication in lung epithelial cells

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Abstract

Human parainfluenza virus 3 (HPIV3) and Respiratory syncytial virus (RSV) are common viruses that can lead to serious respiratory infections in young children and infants. As of yet, there are no vaccines or treatments for these viruses. We have shown that the natural compound, curcumin, reduces the replication of HPIV3, but RSV replication is less affected. Here, we test whether inhibitors of two cellular molecules known to be affected by curcumin, Hsp90 and mTOR1, reduce virus replication. We first assessed the toxicity of the drugs, 17AAG and Rapamycin, for uninfected lung epithelial cells lines, Beas2b and A549, using a cell viability assay. We discovered that both cell lines are sensitive to increasing concentrations of 17AAG but remain unaffected with increasing levels of Rapamycin in A549 cells. To investigate antiviral activity against RSV or HPIV3, the cells were inoculated with virus in the presence of varying concentrations of each drug, and plaque assays were performed. The results showed that overall 17AAG inhibited HPIV3 replication in both cell lines whereas rapamycin was only effective at reducing RSV replication in A549 cells. Together, these data suggests that Hsp90 inhibitors and Rapamycin represent potential therapeutic agents against RSV and HPIV3 replication.

Introduction

RSV and HPIV3 are two viruses that belong to the paramyxoviridae family. These viruses contain a negative sense, single stranded ribonucleic acid (RNA) genome and initiate infection within lung epithelial cells. The replication cycle, and protein synthesis will occur within the cytoplasm and mature virions will exit host cells by budding from the plasma membrane.

Both viruses are responsible for annual respiratory infections, especially in young children and infants. Infections with HPIV3 are second only to RSV for cause of pneumonia and bronchiolitis in children, under two years of age. Currently, there are no vaccines or effective antivirals that are available to treat these infections.

In recent years, however, curcumin, a natural product of the plant *Curcuma longa* and component of the spice, turmeric has peaked interest as a potential therapeutic agent. Studies on the compound have shown curcumin exhibits anti-inflammatory, anti-microbial, and anti-oxidant properties. In the lab, it had been demonstrated that HPIV3 replication was sensitive to curcumin exposure using THP-1 cells, a human monocyte cell line. Furthermore, in Beas2b lung epithelial cells, curcumin reduced HPIV3 replication but was ineffective at reducing virus growth in A549 cell line. RSV replication, on the other hand, was not affected by curcumin in either cell lines.

Curcumin has been shown that to act on multiple cellular signalling pathways. Two cell molecules known to be inhibited by curcumin are the protein kinase, mammalian target of Rapamycin (mTOR1) and heat shock protein 90 (hsp90). Activated signalling of mTOR 1 leads to activation of many downstream targets which promote translation, growth, DNA replication, and processes that can stimulate viral growth in host cells. Hsp90, on the other hand, is a chaperone protein that regulates the folding, assembly and maturation of many host proteins but similarly, is critical for processing of viral proteins. Therefore, both Hsp90 and mTOR1 could play significant roles in promoting RSV and HPIV3 replication and growth.

Thus the goal of these experiments was to :

- 1) to determine toxicity of the two inhibitors, Rapamycin and 17AAG, on lung epithelial cell lines - Beas2b and A549
- 2) determine the effects of these inhibitors on RSV and HPIV3 replication in A549 and BEas2b cells.

Results

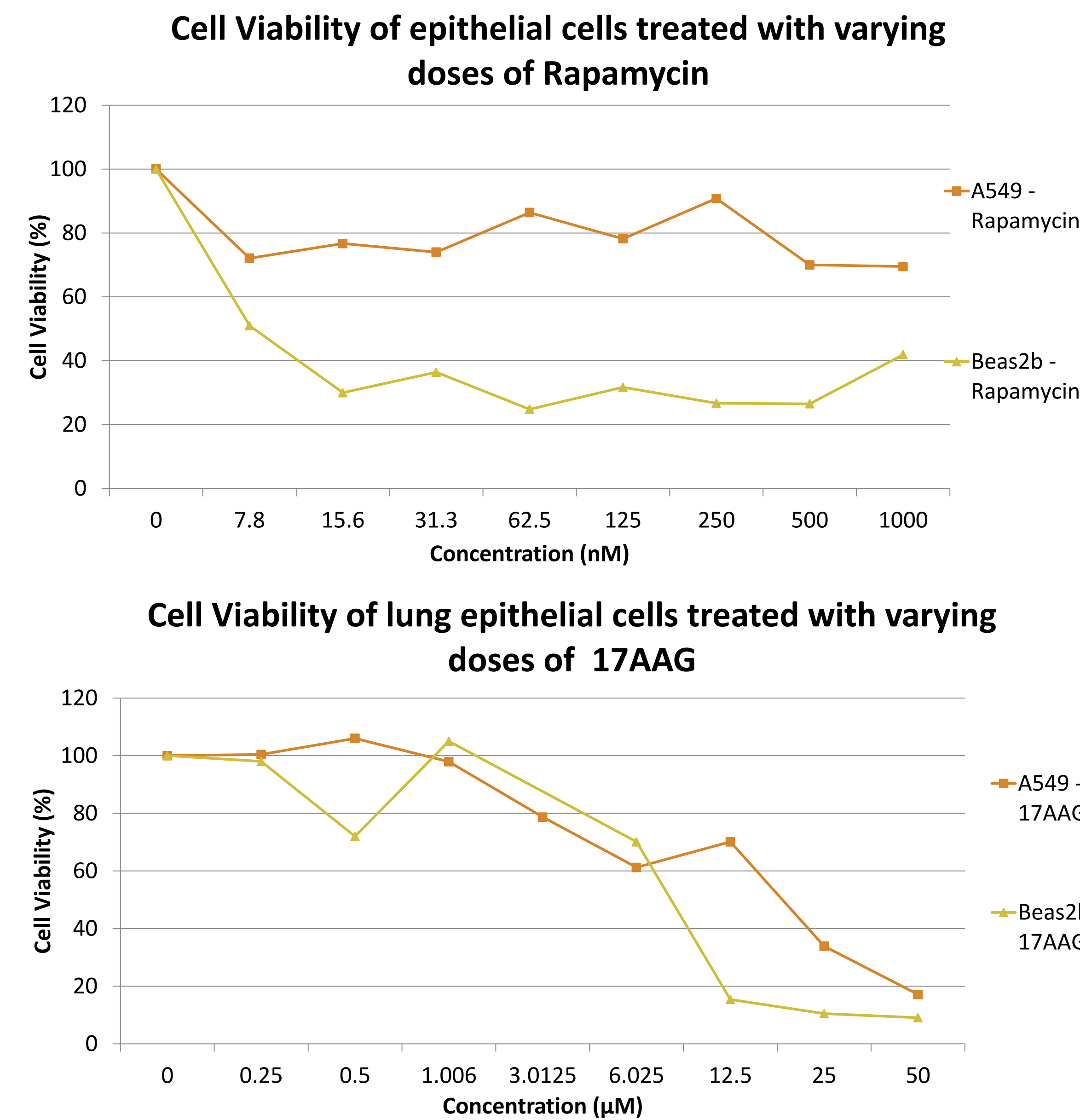


Figure 3. Cell viability of A549 and Beas2b cells exposed to varying concentrations of mTOR1 inhibitor (Rapamycin) or Hsp90 inhibitor (17AAG). **A)** A549 viability remain unaffected with increasing concentration of rapamycin but Beas2b cells exhibit reduced viability, even at lowest dose of 7.8 nM **B)** A549 and Beas2b cells exhibit significantly decreased cell viability with increasing levels of hsp90 inhibitor. Overall Beas2b cells appear to exhibit lower cell viability and higher sensitivity to inhibitors. Graph shows average viable cells relative to appropriate untreated control (p>0.05)

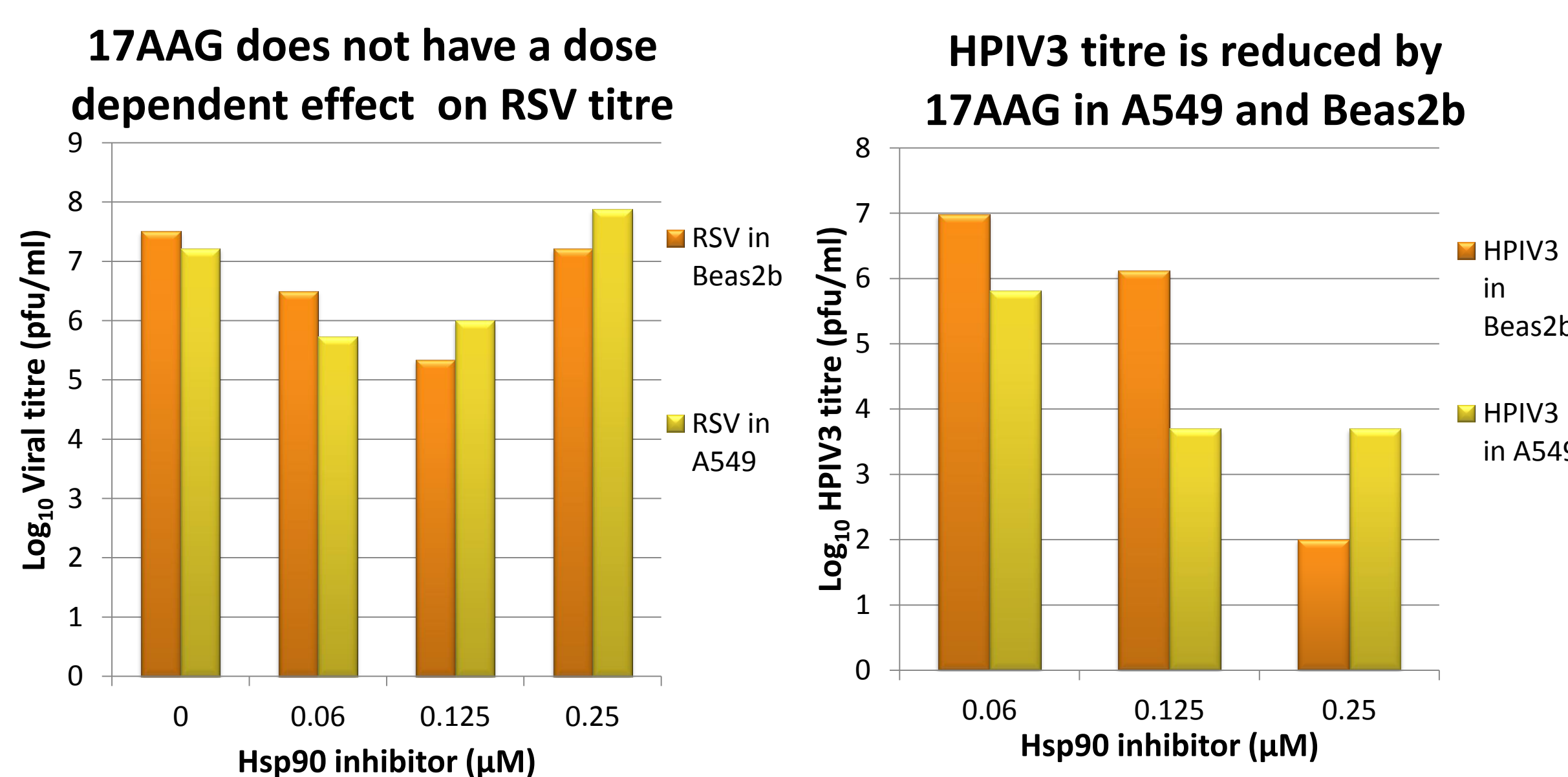


Figure 4. Hsp90 inhibitor, 17AAG effect on log viral titre in lung epithelial cells. **A)** 17AAG does not have exhibit a dose-dependent effect. RSV titre is reduced compared to untreated at concentrations of 0.06μM and 0.125μM but not 0.25μM **B)** However, HPIV3 titre is reduced with increasing levels of 17AAG in both cell lines compared to untreated

Table 1: RSV replication but not HPIV3 replication is significantly reduced by Rapamycin in A549 cells

Rapamycin (nM)	Log reduction of HPIV3 titre	Log reduction of RSV titre
10	0.16	--
25	0.21	0.39
50	0.36	3.18

Confluent wells of A549 cells were inoculated with either HPIV3 or RSV at MOI=0.1 in the absence or presence of Rapamycin, an inhibitor of mTOR1. Supernatants were harvested at day 3 p.i., and assayed for infectious virus by plaque assay. Log reduction in titre is compared to the titre of virus in untreated wells.

Table 2. Inhibition of mTOR1 with Rapamycin has no effect on HPIV3 and RSV replication in Beas2b epithelial cells

Rapamycin (pM)	Log reduction of HPIV3 titre	Log reduction of RSV titre
10	4.97	0.15
25	0.061	0.34
50	0.30	0.08

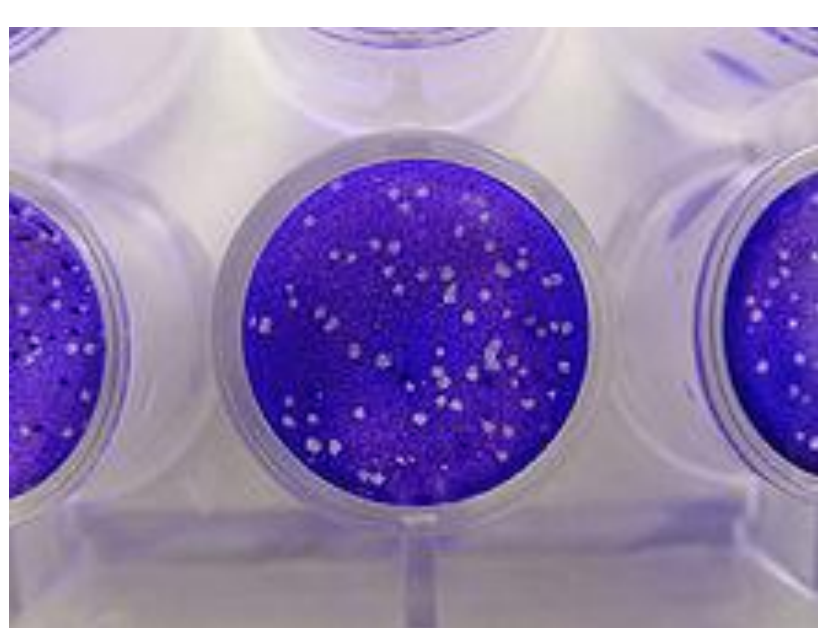
Confluent wells of Beas2b cells were inoculated with either HPIV3 or RSV at MOI=0.1 in the absence or presence of Rapamycin, an inhibitor of mTOR1. Supernatants were harvested at day 3 p.i., and assayed for infectious virus by plaque assay. Log reduction in titre is compared to the titre of virus in untreated wells.

Conclusions

- ✓ Lung epithelial cells become less viable with increasing levels of 17AAG.
- ✓ A549 cells are unaffected at the concentrations of Rapamycin tested, but Beas2b cells are quite sensitive and show reduced viability at even the lowest dose.
- ✓ HPIV3 replication and growth is negatively affected by Hsp90 inhibitor in a dose dependent manner in both types of lung epithelial cells.
- ✓ RSV replication appears to be inhibited at some doses of the Hsp90 inhibitor (17AAG), but not at the highest dose used.
- ✓ Rapamycin inhibits RSV replication at concentration above 50nM in A549 cells but has no effect on HPIV3 or RSV replication in Beas2b cells.
- ✓ No conclusions can be made from the results without repeating the experiments.

Experimental design

Figure 1. Plaque Assay



Confluent monolayers of A549 or Beas2b cells in 24 well plates were inoculated with either RSV or HPIV3 at MOI=0.1 in the presence of varying concentrations of each inhibitor. After incubation for 3 days, supernatants were collected and assayed for infectious virus using plaque assay method.

- ✓ This method involved preparing dilutions of samples and adding them onto confluent cell monolayer for replication
- ✓ The results are formation of small round plaques (clear areas) in the monolayer as the virus replicates and kills off surrounding area.
- ✓ The monolayer are stained to visualize and quantify the plaques

Figure 2. MTT Assay



To assess cell viability and cytotoxicity of lung epithelial cells to drug inhibitors

- ✓ A549 and Beas2b cells were set up on 96 well plate.
- ✓ Serial dilutions of appropriate inhibitor and media added to plates and incubated for 3 days.
- ✓ MTT is added to wells and is reduced by metabolic active cells → forms purple color
- ✓ Absorbance is read with spectrophotometer at 540nm
- ✓ Therefore, the less intense color = less viable or active cells.

Future directions

- ✓ Repeat cell viability experiment with broader concentrations of Rapamycin in Beas2b cells in order to identify a dose suitable for further experiments
- ✓ Confirm findings by repeating all experiments
- ✓ Study the time dependent inhibition of RSV and HPIV3 by Hsp90 inhibitor
- ✓ Explore antiviral replication further using higher concentrations of Rapamycin

Acknowledgements

Dr. Kathryn Wright, BMI, University of Ottawa
Undergraduate Research Opportunity Program (UROP)

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