Epithelix

in vitro Respiratory Solutions

Efficacy of antiviral drugs in a human *in vitro* nasal epithelium model (MucilAir™)

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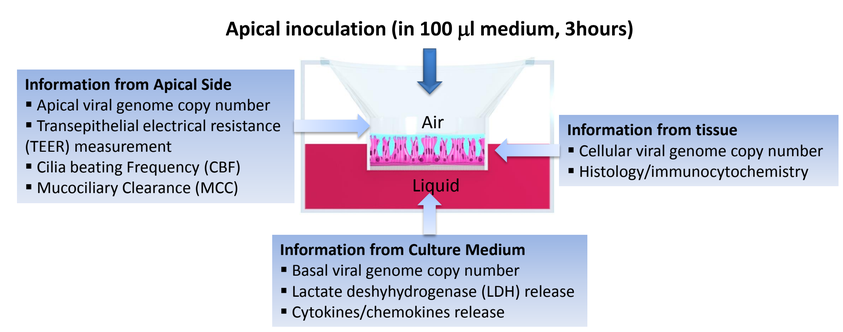
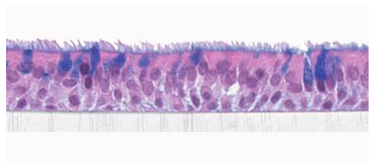
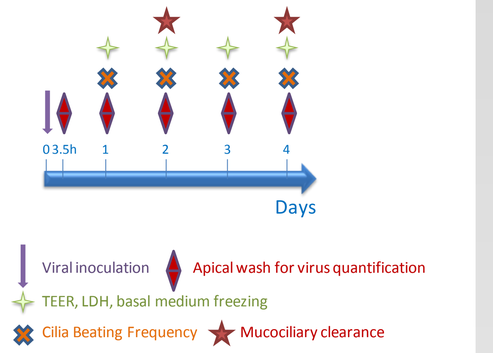
Respiratory viral infections cause mild to severe diseases worldwide, such as common cold, bronchiolitis and pneumonia and are associated with huge costs for society. To test new molecules for shortening, alleviating the diseases or to develop new therapies, relevant human models are mandatory. Interestingly, MucilAir™, a human reconstituted standardized nasal epithelia holds *in vitro* specific mechanisms to counter invaders comparable to the *in vivo* situation, such as mucus production, mucociliary clearance, and secretion of defensive molecules. Here we took advantage of this unique *in vitro* model to perform a proof of concept study designed to screen antiviral compounds.

Testing Strategy

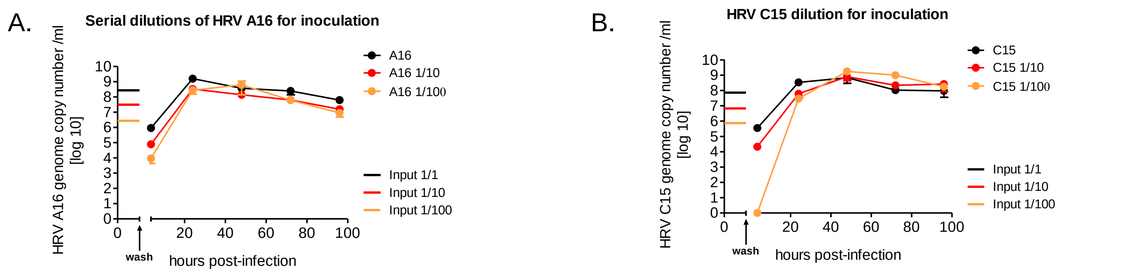




Virus	Stock concentration (prepared in MucilAir™)	Final concentration
HRV A16 (QCHRV.16)	$2x10^9$/ml	$2x10^7$/ml
HRV B14	$5.5x10^7$/ml	$5.5x10^6$/ml
HRV C15 (S07-09-09-U)	$6.8x10^7$/ml	10^7/ml
EV 68 (202)		$2x10^6$/ml
H1N1 (California/7/09)	10^{10}/ml	$1.6x10^7$/ml
H3N2 (Victoria like 9929416)		10^7/ml
Antivirals	Reference	Final concentration
Rupintrivir	sc-208317 (Santa Cruz Biotech.)	0.05-5 µM
Oseltamivir	FO26594 (Carbosynth)	0.1-10 µM

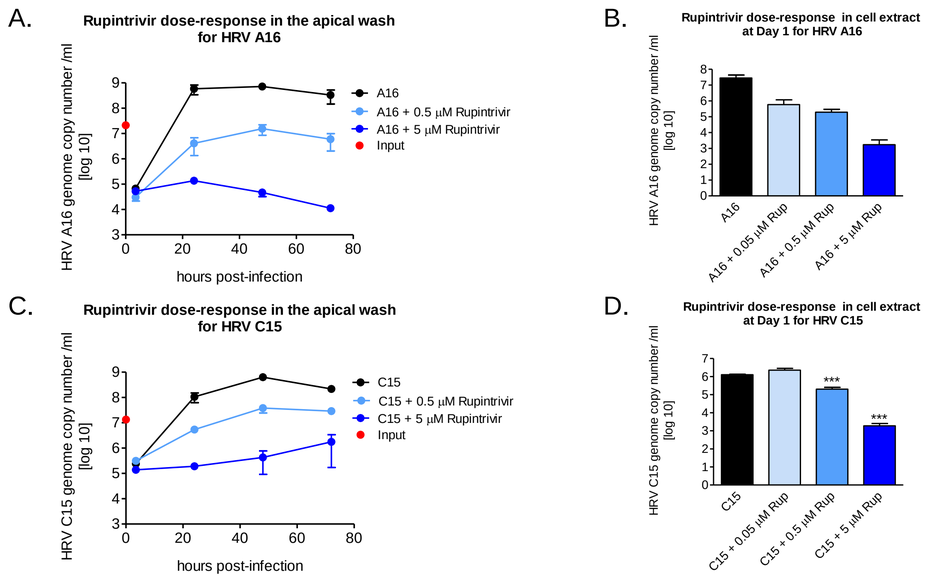


1- Efficient growth of HRV A16 and C15



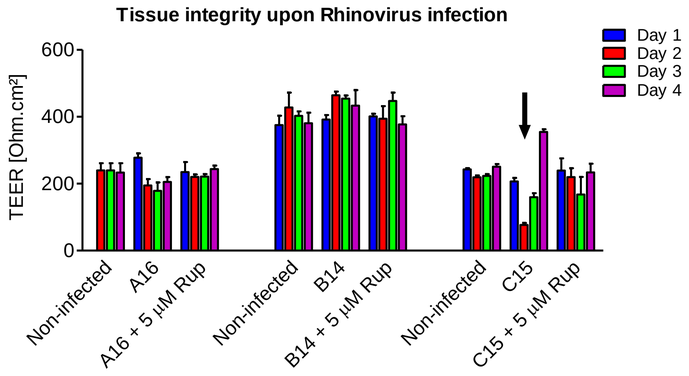
At t=0 100 µl of medium containing serial dilutions of virus particles, HRV A16 for A, C15 for B, was applied on the MucilAir apical side for 30 mins. After washing the inoculum, a collection washing step of 20 mins was performed for each time points. Genome copy number of viruses was determined by quantitative PCR using Taqman probes. Data are mean ± SEM (n=3).

2- Rupintrivir inhibits HRV A16 and C15 growth in a dose dependent manner



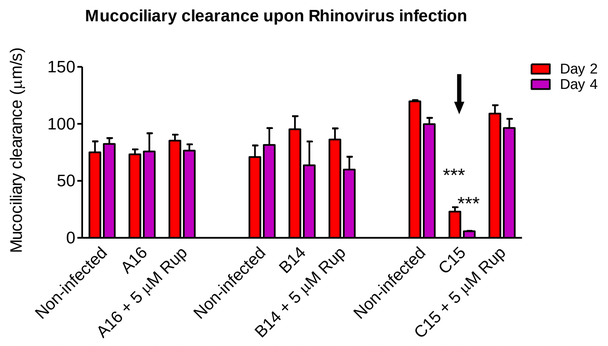
Concomitant apical inoculation of virus particles, HRV A16 for A and B, C15 for C and D, with serial dilutions of Rupintrivir in the basolateral medium. Genome copy number of viruses from the apical wash for A and C and from the tissue lysate for B and D was determined by quantitative PCR using Taqman probes. Data are mean ± SEM (n=2). Statistical comparison was done by Student's t test (*p<0.05).

3- Transient TEER diminution following HRV C15 infection is prevented by Rupintrivir



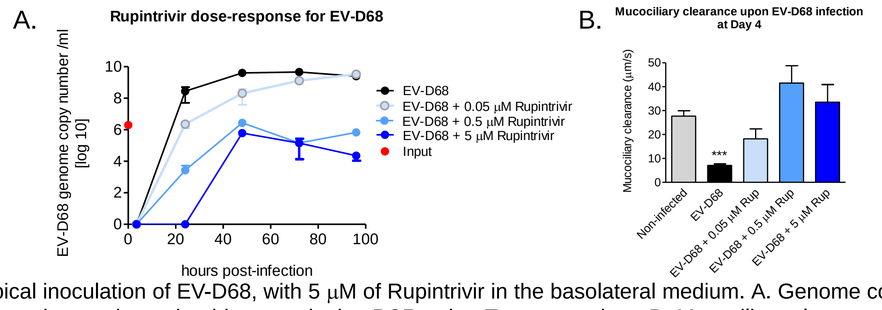
Concomitant apical inoculation of HRV A16, B14 and C15, with 5 µM of Rupintrivir in the basolateral medium. TEER measurements were performed with EVOMX volt-ohm-meter at each day. Data are mean ± SEM (n=2-6).

4- Arrest of mucociliary clearance caused by HRV C15 infection is prevented by Rupintrivir



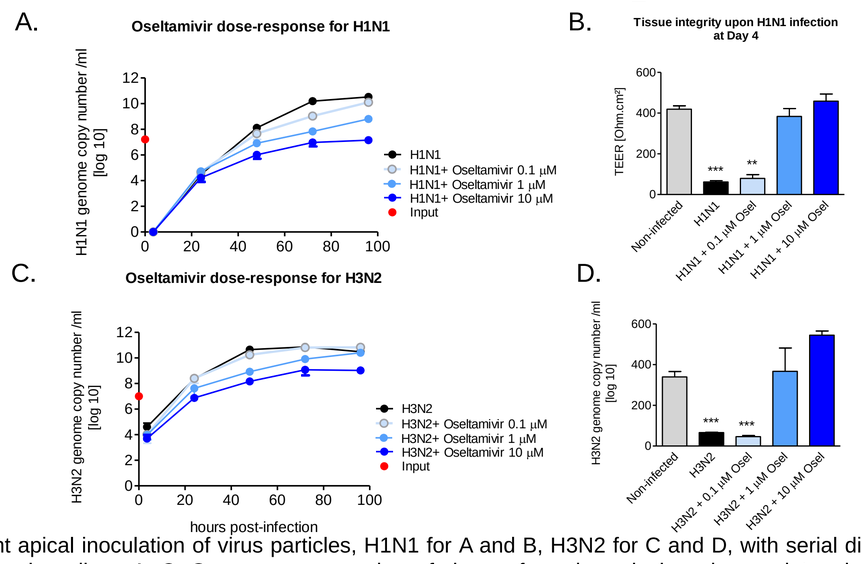
Concomitant apical inoculation of HRV A16, B14 and C15, with 5 µM of Rupintrivir in the basolateral medium. Mucociliary clearance measurements were performed at Day 2 and 4 at 33ºC. Data are mean ± SEM (n=2-6). Statistical comparison was done by Student's t test (*p<0.05).

5- Rupintrivir prevents EV-D68 induced impairment of the clearance



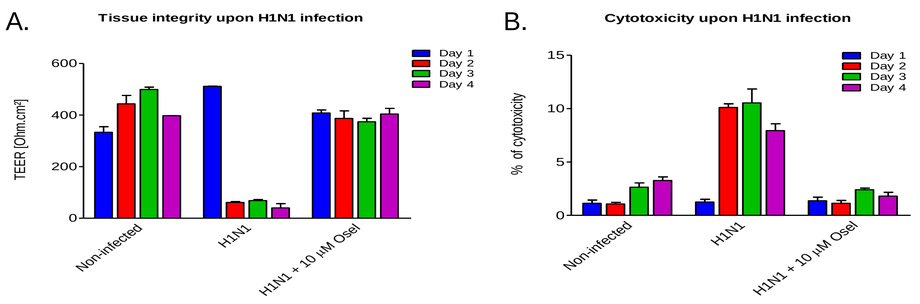
Concomitant apical inoculation of EV-D68, with 5 µM of Rupintrivir in the basolateral medium. A. Genome copy number of viruses from the apical wash was determined by quantitative PCR using Taqman probes. B. Mucociliary clearance measurements were performed at Day 4 at RT. Data are mean ± SEM (n=3). Statistical comparison was done by Student's t test (*p<0.05).

6- Oseltamivir inhibits H1N1 and H3N2 replication and the loss of TEER in a dose dependent manner



Concomitant apical inoculation of virus particles, H1N1 for A and B, H3N2 for C and D, with serial dilutions of Oseltamivir in the basolateral medium. A, C. Genome copy number of viruses from the apical wash was determined by quantitative PCR using Taqman probes. B, D. TEER measurements were performed at Day 4. Data are mean ± SEM (n=3). Statistical comparison was done by Student's t test (*p<0.05).

7- Loss of tissue integrity and cytotoxicity induced by H1N1 infection is prevented by Oseltamivir – Time course



Concomitant apical inoculation of H1N1 with 10 µM of Oseltamivir in the basolateral medium. TEER measurements were performed with EVOMX (A.) and LDH release measurements (B.) were performed at each day. Data are mean ± SEM (n=3).

Conclusions

1: MucilAir™ amplifies the Picorna and Influenza A viruses at high rate.
2: The replication of the HRV A16, B14, C15 and EV-D68 is inhibited by Rupintrivir in a dose dependent manner in the MucilAir™.
3: HRV C15 induced transient decrease in TEER and the mucociliary clearance inhibition are prevented by Rupintrivir treatment.
4: EV-D68 induced impairment of MCC is prevented by Rupintrivir.
5: Oseltamivir inhibits dose dependent manner the replication of H1N1 and H3N2.
6: H1N1 and H3N2 cause tissue damage monitored by TEER and cytotoxicity measurement, which is prevented by Oseltamivir.

Altogether, these results demonstrate that MucilAir™ is a reliable and relevant tool for antiviral drug testing.