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

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Respiratory viral infections cause 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, MuclinAir™, 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.

1- Efficient growth of HRV A16 and C15

At t=0-100 ul of medium containing serial dilutions of virus particles, HRV A16 for A and C15 for B, was applied on the MuclinAir apical side for 30 mins. After washing the inoculum, a collection washing step of 30 mins was performed for each time point. 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-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 on Day 2 and 4 at 33°C. Data are mean ± SEM (n=2-4). 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 on 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. Genome copy number of viruses from the apical wash was determined by quantitative PCR using Taqman probes. B. TEER measurements were performed on 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 each day. Data are mean ± SEM (n=3).

Conclusions

1: MuclinAir™ 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 MuclinAir™.
3: HRV C15 induced transient decrease in TEER and the mucociliary clearance inhibition is 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 MuclinAir™ is a reliable and relevant tool for antiviral drug testing.