Evaluation of the PIKfyve kinase inhibitor Apilimod against Hepatitis E Virus infections

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Introduction

Hepatitis E Virus (HEV) is the most common cause of viral hepatitis with over 20 million cases and up to 70,000 deaths annually. Thereby the treatment of HEV is limited to the off-label use of the nucleoside-analogue ribavirin (RBV) and PEGylated interferon-α. The phosphoinositide kinase PIKfyve plays a crucial role during various endocytotic pathways and its inhibition has already been shown to have a preventive effect on virus entry of other viruses, including Ebola virus and SARS-CoV-2. However, the importance of PIKfyve during HEV entry still remains unclear.

Aim

Here we investigated the antiviral potential of different PIKfyve inhibitors against HEV-infections *in vitro* and *in vivo*.

Method

Using a robust HEV cell culture model, we investigated the dose-dependent effect of PIKfyve kinase inhibitors. To identify which step of HEV replication cycle is affected by PIKfyve inhibition, we performed time-of-addition as well as subgenomic replicon assays. The requirement of PIKfyve during the HEV replication cycle was further validated via siRNA-mediated knockdown. In addition, we evaluated the antiviral effect of the PIKfyve kinase inhibitors in primary human hepatocytes (PHH) and in a chronic rat HEV infection model.

Importance of PIKfyve for viral infection

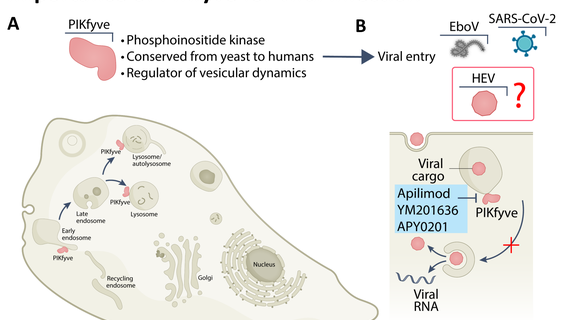

Figure 1: Targeting PIKfyve as an antiviral strategy. (**A**) The phosphoinositide kinase PIKfyve (depicted in red) mainly localizes towards endosomes and endolysosomal compartments and participates in several aspects of vesicular dynamics, thereby affecting a number of trafficking steps along the endocytic pathway. (**B**) PIKfyve as a target for viral infection. Disruption of PIKfyve kinase activity prevents prevents endocytic trafficking of endocytosed virus, preventing its escape into the cytoplasm from endosomes. Illustrations adapted from Burke et al. Nat Rev Drug Discov. 2023.

Results

Inhibition of PIKfyve impedes HEV infection in a dose-dependent manner *in vitro*

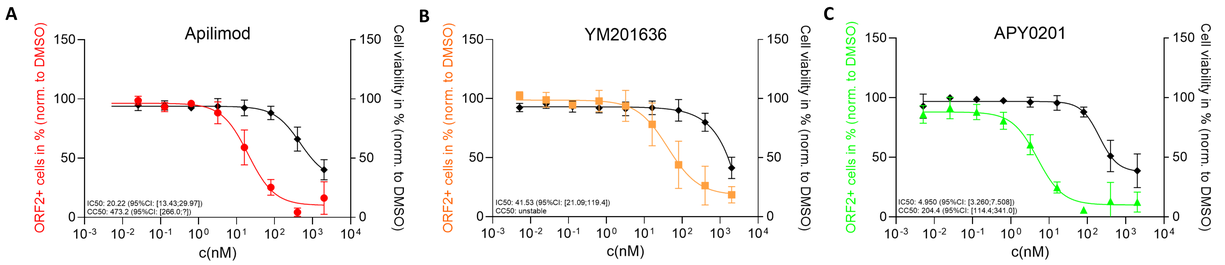

Figure 2: PIKfyve inhibition impedes HEV infection in a dose-dependent manner *in vitro*. (**A**), (**B**) and (**C**) Dose-dependent effect of the PIKfyve inhibitors Apilimod (red, (**A**)), YM201636 (orange, (**B**)) and APY0201 (green, (**C**)) on Kernow-C1 p6 HEV infections (coloured data points) and the respective cytotoxicity (black data points) in hepatoma cells. HepG2/C3A cells were inoculated with HEV for five days with different PIKfyve inhibitor or dimethyl sulfoxide (DMSO) concentrations. The relative number of HEV infected cells was determined via quantitative immunofluorescence using an HEV capsid (ORF2) antibody, while cytotoxicity was determined via MTT assay (means ± SD; n=3). Abbreviation: half-maximal inhibitory concentration (IC50), half-maximal cytotoxic concentration (CC50)

Reduced HEV infections through inhibition of PIKfyve in PHH and siRNA mediated knockdown of PIKfyve in hepatoma cells

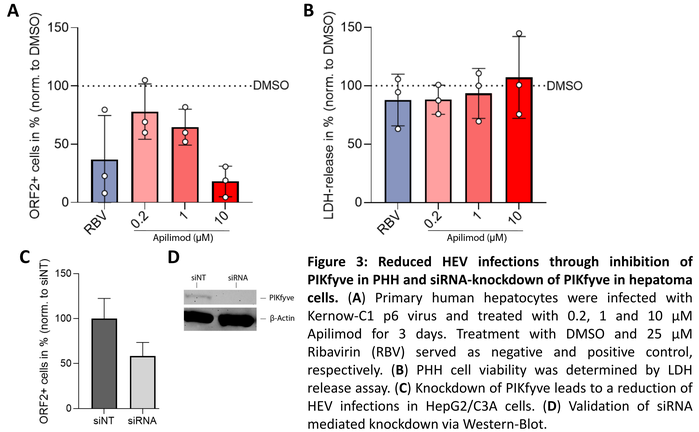

Figure 3: Reduced HEV infections through inhibition of PIKfyve in PHH and siRNA-knockdown of PIKfyve in hepatoma cells. (**A**) Primary human hepatocytes were infected with Kernow-C1 p6 virus and treated with 0.2, 1 and 10 µM Apilimod for 3 days. Treatment with DMSO and 25 µM Ribavirin (RBV) served as negative and positive control, respectively. (**B**) PHH cell viability was determined by LDH release assay. (**C**) Knockdown of PIKfyve leads to a reduction of HEV infections in HepG2/C3A cells. (**D**) Validation of siRNA mediated knockdown via Western-Blot.

Apilimod lowers HEV infectivity *in vivo*

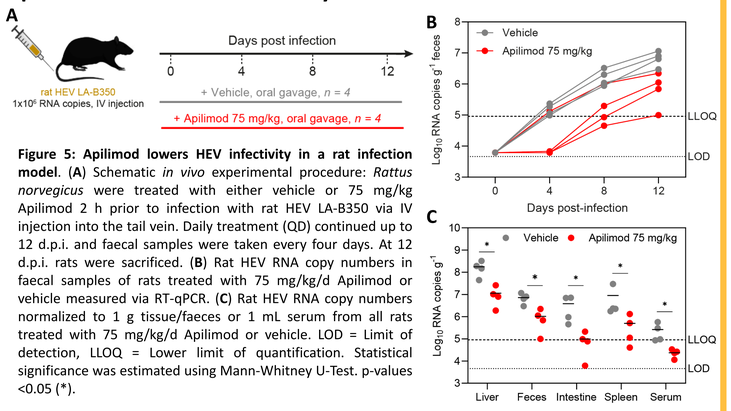

Figure 5: Apilimod lowers HEV infectivity in a rat infection model. (**A**) Schematic *in vivo* experimental procedure: *Rattus norvegicus* were treated with either vehicle or 75 mg/kg Apilimod 2 h prior to infection with rat HEV LA-B350 via IV injection into the tail vein. Daily treatment (QD) continued up to 12 d.p.i. and faecal samples were taken every four days. At 12 d.p.i. rats were sacrificed. (**B**) Rat HEV RNA copy numbers in faecal samples of rats treated with 75 mg/kg/d Apilimod or vehicle measured via RT-qPCR. (**C**) Rat HEV RNA copy numbers normalized to 1 g tissue/faeces or 1 mL serum from all rats treated with 75 mg/kg/d Apilimod or vehicle. LOD = Limit of detection, LLOQ = Lower limit of quantification. Statistical significance was estimated using Mann-Whitney U-Test. p-values <0.05 (*).

PIKfyve kinase inhibition impedes HEV entry

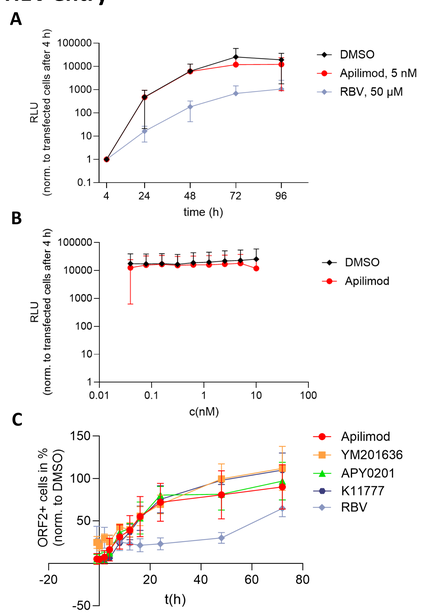

Figure 4: PIKfyve kinase inhibition impedes HEV entry. (**A**) and (**B**) Impact of the PIKfyve kinase inhibitor Apilimod on the replication of HEV subgenomic reporter replicon based on the Kernow-C1 p6 strain. Depicted are normalized relative light units (RLU) measured 4, 24, 48 and 72 hours post electroporation (h.p.e.) upon treatment with 5 nM Apilimod (**A**) or upon treatment with increasing dosages of Apilimod 72 h.p.e. (**B**). 50 µM Ribavirin (RBV) and DMSO were employed as positive and negative controls, respectively (means ± SD; n = 3). (**C**) Time of drug addition assay. Hepatoma HepG2/C3A cells were inoculated with the HEV Kernow-C1 p6 strain at 0 h and treated with DMSO, Apilimod (100 nM), YM201636 (100 nM), APY0201 (100 nM), K11777 (100 nM) or RBV (25 µM) for 1 h (-1) prior to, during (0) and 2, 4, 8, 12, 16, 24, 48 and 72 hours after infection until fixation at 96 hours post infection. At 8 h after infection, inoculum was removed, and replenished with fresh medium containing drugs after several washes with PBS. The broad-spectrum RNA virus inhibitor RBV served as positive control for HEV replication and the broad-spectrum cathepsin inhibitor K11777 served as positive control for HEV entry (means ± SD; n = 3).

Conclusions

- PIKfyve kinase inhibition efficiently inhibits HEV infection *in vitro*
- PIKfyve plays a role during HEV entry
- PIKfyve inhibition impedes HEV infection in a rat infection model
- Targeting PIKfyve kinase activity might guide novel antiviral strategies against HEV infections especially since Apilimod has been well tolerated in Phase II clinical trials

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