Probing the direct effects of antiretroviral drugs on hepatitis E virus replication in cell culture models

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To the Editor,

Genotype (GT) 1 and 2 hepatitis E virus (HEV) periodically causes outbreaks in resource-limited regions. The GT2 HEV outbreak in Namibia has caused a substantial health burden in particular among pregnant women. Because Namibia has a high prevalence of HIV; a recent study by Heemelaar et al (1) specifically investigated the outcome of HEV infection in HIV infected women. Their most intriguing finding is that HIV infected women especially those receiving antiretroviral therapy had a better clinical outcome. They were less likely to develop acute liver failure, the main cause of fatality, in comparison with non-HIV infected pregnant women. The authors speculated one possible mechanistic explanation that antiretroviral agents may have a direct effect against HEV.

To probe this direct effect, we employed HEV cell culture models that are widely used for assessing anti-HEV agents (2). We tested all the eight antiretroviral drugs, which were used in those HEV infected HIV patients (1), with a range of clinically relevant concentrations. We first tested in subgenomic HEV replicon models based on the GT1 Sar55 clone and GT3 Kernow-C1/p6 clone, respectively. In these replicons, the ORF2 capsid protein was replaced by a Gaussia luciferase reporter gene for monitoring viral replication. We found that most of these agents exerted dose- and time-dependent but mild inhibition of viral replication-related luciferase activity in the human hepatic Huh7 cells harbouring the subgenomic replicons (Figure 1). However, at high concentration (10 µM), mild inhibition of cell growth was also observed, for example by treatment of tenofovir disoproxil fumarate, zidovudine, or atazanavir sulfate for 72 hours. Next, we tested these agents at 10 µM in an infectious HEV model harbouring the full-length GT3 Kernow-C1/p6 genome. Viral RNA level was quantified by qRT-PCR and normalized to a household

gene, which would specifically reflect viral replication independent of host cell growth. Overall, no major effect on HEV RNA level was observed by treating these agents for 48 hours (Figure 1). Collectively, our results suggest that antiretroviral medications are unlikely to have major direct effects in inhibiting HEV infection. This is not surprising because most of these agents specifically target the reverse transcriptase or protease of HIV, whereas the viral enzymes of HEV are very different. Nevertheless, it would be necessary to further validate in a GT2 HEV model, but such a cell culture system remains unavailable.

Hyperinflammation is a hallmark of severe acute hepatitis E. We recently demonstrated that HEV infection strongly activates NLRP3 inflammasome activation in macrophages, a key driver of pathological inflammation (3). Interestingly, nucleoside reverse transcriptase inhibitors, the major class of antiretroviral therapy, have been shown to inhibit NLRP3 inflammasome activation (4, 5). This appears in line with the second hypothesis of Heemelaar et al that antiretroviral agents may attenuate immune response to mitigate the level of hepatitis (1). However, experimental validation is required in this respect, which is certainly interesting to be perused.

Reference

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Figure legends
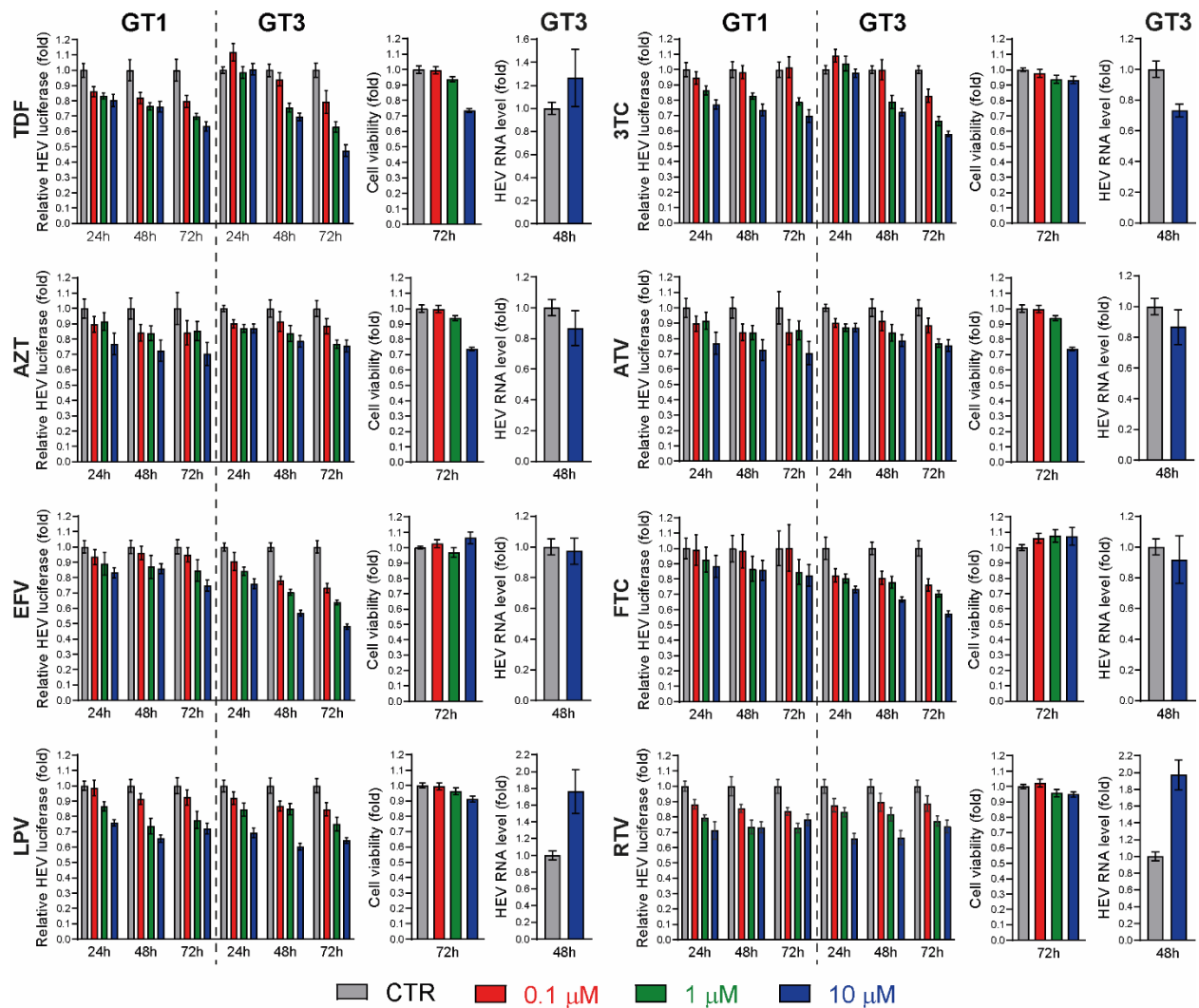
Figure 1. Profiling the effects of antiretroviral drugs on HEV infection in cell culture models. The human hepatic Huh7 cell line was used to harbour the genotype 1 Sar55 subgenomic replicon, genotype 3 Kernow-C1/p6 subgenomic replicon or infectious p6 full-length genome. The effects of antiretroviral drugs on viral replication-related luciferase activity were quantified at 24, 48 or 72 hours post-treatment. The untreated group serves as control (CTR) (set as 100%) (n = 8-11). Cell viability was measured at 72 hours post-treatment and the untreated group serves as CTR (n = 8-11). The genotype 3 p6

infectious cell model was treated with 10 µM of antiretroviral drugs for 48 hours. The effects on viral RNA was quantified by qRT-PCR and normalized to the household gene GAPDH (n = 4). Data are presented as means ± SEM. The eight antiretroviral drugs were listed as follows: TDF, tenofovir disoproxil fumarate; 3TC, lamivudine; AZT, zidovudine; ATV, atazanavir sulfate; EFV, efavirenz; FTC, emtricitabine; LPV, lopinavir; RTV, ritonavir.