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Antiviral effects of selected nucleoside analogues against human parechoviruses A1 and A3

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ABSTRACT

Parechoviruses A (HPeV, *Picornaviridae*) are neglected human pathogens that cause sepsis-like illness and severe neurological complications in infants. There are no antivirals available for the treatment of HPeV infections. We here report on cell-based assays that allow for medium-throughput antiviral screening of compound libraries against HPeV. The nucleoside viral polymerase inhibitor 2′-*C*-methylcytidine was identified as being an *in vitro* replication inhibitor of HPeV1 and HPeV3 that can serve as a reference molecule for further antiviral studies.

Parechoviruses A are being increasingly recognized as important human pathogens. Although these viruses cause typically asymptomatic/subclinical or mild respiratory or gastrointestinal disease, they may in particular in children under the age of 2 years, cause severe manifestations such as neurological complications and sepsis-like illness (Olijve et al., 2018). Similarly to enteroviruses, which also belong to the family of the *Picornaviridae*, HPeVs are very prevalent viruses. It has been estimated that 90% of children encounter infection with at least one type of HPeV by the age of 2 (de Crom et al., 2016).

There are currently 16 types of human parechoviruses, with HPeV1, HPeV3 and HPeV6 being the most prevalent in recent outbreaks reported in Australia (Cumming et al., 2015), Japan (Aizawa et al., 2016), The Netherlands (Benschop et al., 2008) and Denmark (Fischer et al., 2014). Most severe manifestations (such as neurological complications and sepsis-like illness) are attributed to infections with HPeV3 (Aizawa et al., 2017). The majority of severe manifestations of HPeV infections occur in infants below the age of 3 months (Black et al., 2018). Long-term sequelae have been reported; in a follow-up study after the HPeV3 outbreak in Australia in 2013, 50% of the observed children exhibited impaired developmental attainment (Britton et al., 2017). Despite the prevalence and clinical relevance, no specific antiviral therapy is available for the treatment of HPeV infected infants, although intravenous administration of immunoglobulins (IVIG) has been reported to result in some clinical benefit (Wildenbeest et al., 2013).

We aimed to develop a robust antiviral assay for the identification of inhibitors of HPeV1 and HPeV3 (strains commonly isolated from patients) replication. We first infected selected cell lines [human: RD, HeLa and a549; monkey: Vero A, Vero E6 and BGM; mouse: N2A] to explore whether HPeV1 and 3 can cause a cytopathic effect (CPE) and if so in which cells such CPE develops most efficiently. CPE was clearly detected in Vero A, BGM and A549 cells after HPeV1 infection. No obvious CPE was observed following HPeV3 infection in any of the cell lines tested. This is in line with a previous report where infected Vero and BGM cells were used for diagnostic purposes (Benschop et al., 2010; Westerhuis et al., 2012). Since the HPeV1 induced CPE in BGM cells was most prominent, we selected these cells for the phenotypic screening with microscopical readout.

Next, the kinetics of replication of HPeV1 and HPev3 were analyzed in BGM cells (Fig. 1A–B). Intracellular viral RNA peaked at 6 h after HPeV1 infection and at 12 h after HPeV3 infection. Extracellular RNA levels started to increase at 10 h p.i. for HPeV1 and at 16 h for HPeV3. The kinetics of HPeV1 replication was faster than that of HPeV3, but at 3 days p.i. the extracellular genome copy numbers of both viruses were comparable ($1{,}44^{\wedge}10^{7}$ and $2{,}12^{\wedge}10^{7}$ genome copies/μl respectively).

We then studied whether a panel of molecules that were earlier shown to inhibit entero/picornavirus replication, are able to inhibit the *in vitro* HPeV1/3 replication. For this panel we selected molecules with different mechanisms of action and tested their effect first in the CPE-based HPeV1 assay. Reduction in virus-induced CPE was observed when cultures were treated with nucleoside analogues 7-deaza-2′*C*-methyladenosine (7′DMA) and 2-*C*′-methylcytidine (2′CMC). These observations were confirmed by using ATPLite and qRT-PCR as readouts. Both compounds also inhibited HPeV3 replication (Table 1; Fig. 2A–C), with 2′CMC being the most effective (EC_{50} 21 μM and 4 μM

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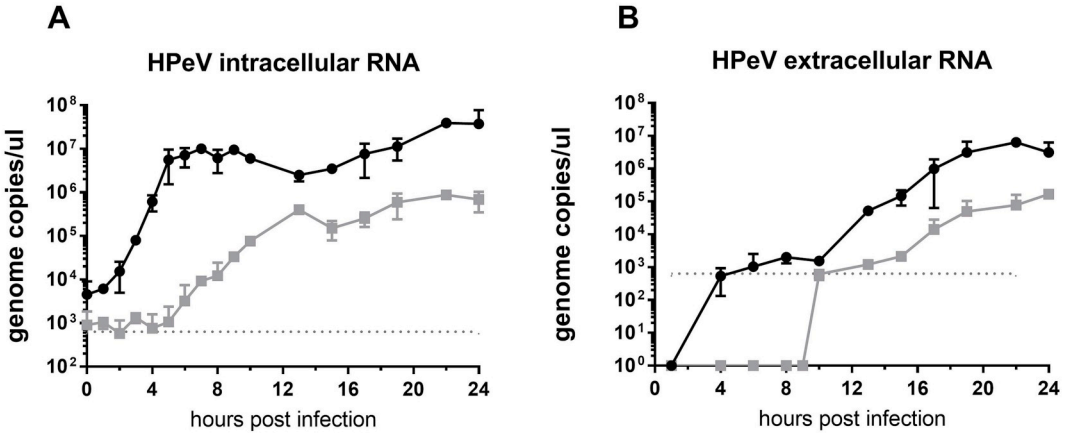

Fig. 1. Replication of HPeV1 and HPeV3 in BGM cells. BGM cells were infected with HPeV1 (●) or HPeV3 (■) at a MOI of 2 for 1 h, cells were washed with PBS to remove the inoculum and intracellular (panel A) or extracellular (panel B) viral RNA production was measured at the indicated timepoints by means of qPCR. Data are mean values for 2 independent experiments ± standard deviation.

Table 1
Effect of 2′CMC and 7′DMA on the replication of HPeV1 and HPeV3 in BGM cells.

EC50 (μM)				CC50 (μM)
	HPeV1		HPeV3	
Compound	CPE-based	qPCR	qPCR	
2′CMC	13 ± 4	21 ± 4	4 ± 1	> 100
7′DMA	37 ± 10	66 ± 4	11 ± 4	> 100

Data are mean values ± standard deviations (SD) from 3 independent experiments.

for HPeV1 and HPeV3 respectively). 2-*C*′-methyladenosine and 2-*C*′-methylguanosine resulted in a minor reduction of virus-induced CPE at the highest concentration tested (100 μM).

The enterovirus capsid binder pirodavir proved inactive against HPeV1 which is in line with the absence of a canyon structure in the capsid of HPeV, such canyon is typical for enteroviruses (Shakeel et al., 2017). The 2C-targeting enterovirus inhibitors dibucaine, fluoxetine (Ulferts et al., 2016) and N-(2-Phenylethyl)adenosine (Drenichev et al., 2016) or the enterovirus 3C protease inhibitor rupintrivir were also devoid of antiviral activity against HPeV1 and 3. Inhibitors of the host-factor PI4KIIIb (BF738735), or the host-factor OSBP (OSW-1), all of which were shown to inhibit *in vitro* enterovirus replication (Albulescu

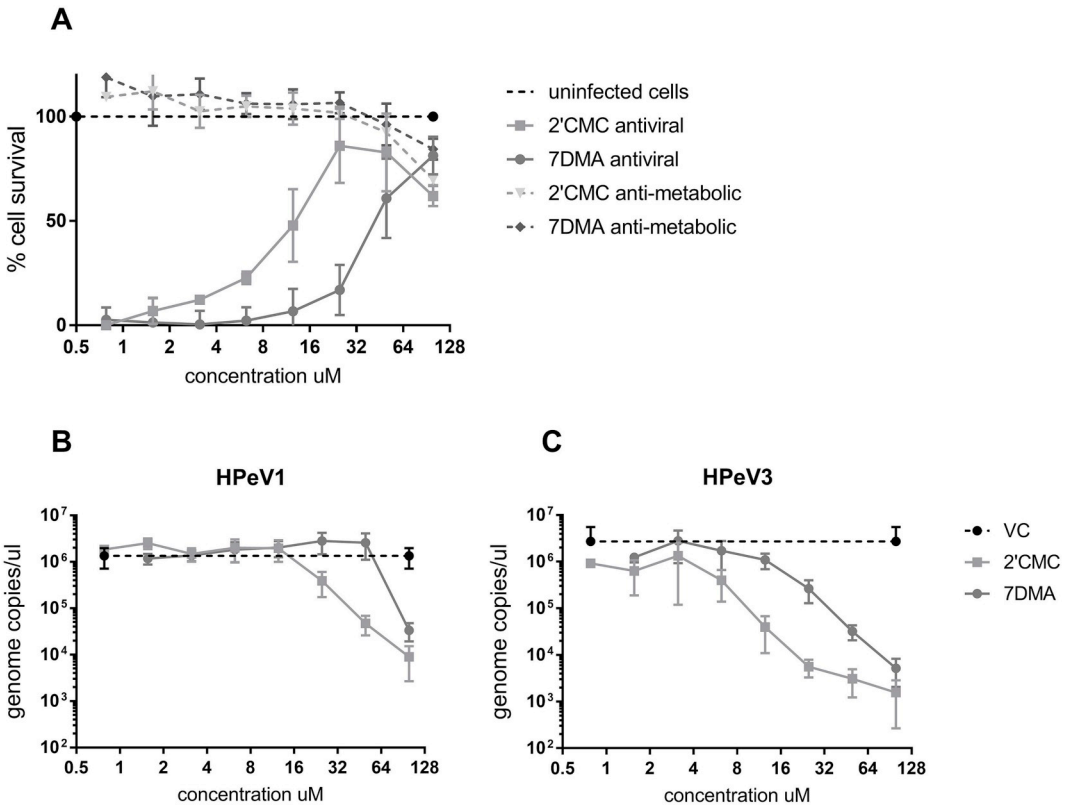

Fig. 2. *In vitro* antiviral effect of 2′CMC and 7′DMA against HPeV1 and HPeV3. Antiviral effect and effect on uninfected cells of 2′CMC and 7′DMA as assessed in a CPE-based assay with ATPLite readout (A). Inhibition of viral RNA production by 2′CMC and 7′DMA was assessed for HPeV1 (B) and HPeV3 (C). Data are mean values for 3 independent experiments ± standard deviation.

et al., 2015) did not elicit any activity against either HPeV1 or HPeV3. Recently the *in vitro* antiviral activity of itraconazole [(ITZ) another OSBP-targeting compound] was reported against HPeV3 but not HPeV1 (Rhoden et al., 2018). We obtained similar results in our assays where ITZ was found to inhibit HPeV3 (EC_{90} of 0,5 ± 0,2 μM) but not HPeV1 replication. The fact that OSW-1 is, in contrast to ITZ, not active against HPeV3, indicates that the anti-HPeV3 activity is possibly not mediated through OSBP. Following validation of the screening assays with the above-mentioned reference compounds, we performed a screening of ~2000 compounds of the diverse set and oncology drug set from the National Cancer Institute library (NCI, USA). BGM cells were seeded in 96-well plates and the next day serial dilutions of the compounds were added to the cells and cultures were infected with HPeV1. CPE-based readout was performed at day 3 post infection. However, none of the hits identified in this assay resulted in more than 1-1,5 $\log_{10}$ inhibition of viral RNA production at a non-toxic concentrations and were thus not further considered.

In conclusion we established and validated *in vitro* assays that will allow the identification of inhibitors of human parechovirus replication. Typical inhibitors of enterovirus replication (such as capsid binders, 2C or 3C targeting compounds) were found not to inhibit parechovirus replication. Given the medical need it will be important to develop either specific parechovirus inhibitors or to further consider the development of broad-spectrum RNA virus inhibitors that can also be used to treat infections with human parechoviruses.

Declarations of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.antiviral.2018.12.009.

References

Aizawa, Y., Izumita, R., Saitoh, A., 2017. Human parechovirus type 3 infection: an emerging infection in neonates and young infants. J. Infect. Chemother. 23, 419–426. https://doi.org/10.1016/J.JIAC.2017.04.009.

Aizawa, Y., Suzuki, Y., Watanabe, K., Oishi, T., Saitoh, A., 2016. Clinical utility of serum samples for human parechovirus type 3 infection in neonates and young infants: the 2014 epidemic in Japan. J. Infect. 72, 223–232. https://doi.org/10.1016/J.JINF.2015.10.010.

Albulescu, L., Strating, J.R.P.M., Thibaut, H.J., van der Linden, L., Shair, M.D., Neyts, J., van Kuppeveld, F.J.M., 2015. Broad-range inhibition of enterovirus replication by OSW-1, a natural compound targeting OSBP. Antivir. Res. https://doi.org/10.1016/j.antiviral.2015.02.013.

Benschop, K., Minnaar, R., Koen, G., van Eijk, H., Dijkman, K., Westerhuis, B., Molenkamp, R., Wolthers, K., 2010. Detection of human enterovirus and human parechovirus (HPeV) genotypes from clinical stool samples: polymerase chain reaction and direct molecular typing, culture characteristics, and serotyping. Diagn. Microbiol. Infect. Dis. 68, 166–173. https://doi.org/10.1016/J.DIAGMICROBIO.2010.05.016.

Benschop, K., Thomas, X., Serpenti, C., Molenkamp, R., Wolthers, K., 2008. High prevalence of human Parechovirus (HPeV) genotypes in the Amsterdam region and identification of specific HPeV variants by direct genotyping of stool samples. J. Clin. Microbiol. 46, 3965–3970. https://doi.org/10.1128/JCM.01379-08.

Black, S., Bradley, C., Lai, F.Y., Shenoy, S., Bandi, S., Allen, D.J., Tang, J.W., 2018. Comparing the clinical severity of disease caused by enteroviruses and human parechoviruses in neonates and infants. Pediatr. Infect. Dis. J. 1. https://doi.org/10.1097/INF.0000000000002145.

Britton, P.N., Khandaker, G., Khatami, A., Teutsch, S., Francis, S., McMullan, B.J., Jones, C.A., 2017. High prevalence of developmental concern amongst infants at 12 months following hospitalised parechovirus infection. J. Paediatr. Child Health. https://doi.org/10.1111/jpc.13728.

Cumming, G., Khatami, A., McMullan, B.J., Musto, J., Leung, K., Nguyen, O., Ferson, M.J., Papadakis, G., Sheppeard, V., 2015. Parechovirus genotype 3 outbreak among infants, New south Wales, Australia, 2013–2014. Emerg. Infect. Dis. 21. https://doi.org/10.3201/eid2107.141149.

de Crom, S.C.M., Rossen, J.W.A., van Furth, A.M., Obihara, C.C., 2016. Enterovirus and parechovirus infection in children: a brief overview. Eur. J. Pediatr. 175, 1023–1029. https://doi.org/10.1007/s00431-016-2725-7.

Drenichev, M.S., Oslovsky, V.E., Sun, L., Tijsma, A., Kurochkin, N.N., Tararov, V.I., Chizhov, A.O., Neyts, J., Pannecouque, C., Leyssen, P., Mikhailov, S.N., 2016. Modification of the length and structure of the linker of N6-benzyladenosine modulates its selective antiviral activity against enterovirus 71. Eur. J. Med. Chem. 111, 84–94. https://doi.org/10.1016/J.EJMECH.2016.01.036.

Fischer, T.K., Midgley, S., Dalgaard, C., Nielsen, A.Y., 2014. Human parechovirus infection, Denmark. Emerg. Infect. Dis. 20, 83–87. https://doi.org/10.3201/eid2001.130569.

Olijve, L., Jennings, L., Walls, T., 2018. Human parechovirus: an increasingly recognized cause of sepsis-like illness in young infants. Clin. Microbiol. Rev. 31 e00047-17. https://doi.org/10.1128/CMR.00047-17.

Rhoden, E., Nix, W.A., Weldon, W.C., Selvarangan, R., 2018. Antifungal azoles itraconazole and posaconazole exhibit potent in vitro antiviral activity against clinical isolates of parechovirus A3 (Picornaviridae). Antivir. Res. 149, 75–77. https://doi.org/10.1016/J.ANTIVIRAL.2017.11.011.

Shakeel, S., Dykeman, E.C., White, S.J., Ora, A., Cockburn, J.J.B., Butcher, S.J., Stockley, P.G., Twarock, R., 2017. Genomic RNA folding mediates assembly of human parechovirus. Nat. Commun. 8, 5. https://doi.org/10.1038/s41467-016-0011-z.

Ulferts, R., de Boer, S.M., van der Linden, L., Bauer, L., Lyoo, H.R., Maté, M.J., Lichière, J., Canard, B., Lelieveld, D., Omta, W., Egan, D., Coutard, B., van Kuppeveld, F.J.M., 2016. Screening of a library of FDA-approved drugs identifies several enterovirus replication inhibitors that target viral protein 2C. Antimicrob. Agents Chemother. 60, 2627–2638. https://doi.org/10.1128/AAC.02182-15.

Westerhuis, B.M., Koen, G., Wildenbeest, J.G., Pajkrt, D., de Jong, M.D., Benschop, K.S.M., Wolthers, K.C., 2012. Specific cell tropism and neutralization of human parechovirus types 1 and 3: implications for pathogenesis and therapy development. J. Gen. Virol. 93, 2363–2370. https://doi.org/10.1099/vir.0.043323-0.

Wildenbeest, J.G., Wolthers, K.C., Straver, B., Pajkrt, D., 2013. Successful IVIG treatment of human parechovirus-associated dilated cardiomyopathy in an infant. Pediatrics 132.