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Spectrum of antiviral activity of *o*-(acetoxyphenyl)hept-2-ynyl sulphide (APHS)

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Abstract

Since some antiviral drugs have a broad spectrum of action, the aim of this study was to assess whether *o*-(acetoxyphenyl)hept-2-ynyl sulphide (APHS), a recently described inhibitor of human immunodeficiency virus type 1 (HIV-1) replication, has an effect on the replication of other retroviruses, (−) and (+) RNA viruses and DNA viruses. APHS did not affect the replication of feline immunodeficiency virus, HIV-2 and a HIV-1 strain resistant to non-nucleoside reverse transcriptase inhibitors (NNRTI). APHS could also not inhibit the replication of the RNA viruses, respiratory syncytium virus or mouse hepatitis virus. In contrast, APHS did inhibit the replication of wild-type herpes simplex virus type 1 (HSV-1) as well as acyclovir-resistant HSV-1 and HSV-2 mutant. These results suggest that APHS is a NNRTI of HIV-1 replication, but not HIV-2 replication, and that APHS is an inhibitor of both HSV-1 and HSV-2 replication.

Keywords: APHS; Antiviral; Antiherpetic; HIV; HSV; Drug resistance

1. Introduction

Some antiviral compounds have a broad spectrum of action while others are more specific [1]. The pyrophosphate analogue phosfonoformic acid (foscarnet, PFA) is a broad-spectrum DP inhibitor that is active against several DNA and RNA viruses, including herpes simplex virus type 1 (HSV-1) and human immunodeficiency virus type 1 (HIV-L) and acts by interfering with the exchange of pyrophosphate from deoxynucleoside triphosphate during viral replication by binding to a site on the HSV-1 DP or HIV-1 RT [2,3]. Acyclic nucleoside phosphonates are chain terminators which are active against HIV, feline immunodeficiency virus (FIV), HSV, adenovirus, papillomaviruses and hepatitis B virus [4–6]. Receptor antagonists such as bicyclams inhibit cell-virus fusion and can inhibit HIV, simian immunodeficiency virus (SIV) and FIV replication [1]. Most of the non-nucleoside reverse transcriptase inhibitors (NNRTI) are very specific; for instance they inhibit the replication of HIV-1, but not that of HIV-2, SIV or FIV [7].

We have previously found that the non-steroidal anti-inflammatory drug *o*-(acetoxyphenyl)hept-2-ynyl sulphide (APHS) can inhibit HIV-1 replication by interfering with the reverse transcription process [8]. The aim of this study was to determine the antiviral specificity of APHS against viruses representative of different classes. HIV-1, HIV-2 and FIV were selected from the retroviridae family. Retroviruses are RNA viruses, which possess a reverse transcriptase (RT) enzyme, which is a RNA- and DNA-dependent DNA polymerase (DP) that transcribes the viral RNA into proviral DNA [9–12]. This proviral DNA can be inserted into the cellular genome by the viral integrase. Once the virus is integrated into the cellular genome it can stay latent for many years until the cell is stimulated and viral transcription starts.

Respiratory syncytium virus (RSV) is a negative (−) stranded RNA virus, which belongs to the family paramyxoviridae [13]. The RSV core contains an RNA replicase,

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which is an RNA-dependent RNA polymerase. When released into the cell cytoplasm, this RNA polymerase makes a complementary copy of the genome, which is (+) stranded. This complementary strand acts as a template for genome synthesis. Simultaneously, a series of (+) strands are produced, which act as mRNAs.

Mouse hepatitis virus (MHV) is a positive (+) stranded RNA virus, which belongs to the family coronaviridae [14]. The MHV genome serves as an mRNA for a polyprotein from which, by proteolytic cleavages, the various subunits of the viral RNA-dependent RNA polymerase are derived. The coronaviral RNA polymerase directs the synthesis of both genome-length and subgenomic negative-stranded RNAs, which in turn serve as templates for the synthesis of genomic RNA and subgenomic mRNAs, respectively.

HSV-1 is a double-stranded DNA virus, which belongs to the family herpesviridae and subfamily α-herpesviridae [15]. After virus entry into the cytoplasm, the nucleocapsid is transported to the nuclear pores where the viral DNA is released into the nucleus. The HSV-1 DNA forms a circular molecule, which acts as the template for replication. Early gene expression results in the expression of enzymes involved in nucleic acid metabolism such as thymidine kinase (TK) and proteins essential for DNA synthesis such as DNA replicase, which is a DNA-dependent DP. The DP catalyses the viral DNA synthesis. New progeny virus DNA is synthesised off the DNA genome of the input parental virus. New progeny DNA acts as a template for the synthesis of more genomes for new virus particles and for transcription of late virus mRNA that encode mainly viral structural proteins.

2. Materials and methods

2.1. Cells

Donor peripheral blood mononuclear cells (PBMC) were isolated from heparinised blood from HIV-1, HIV-2, and hepatitis B-seronegative donors and obtained on Ficoll-Isopaque density gradients. To prepare a PBMC mixed batch, PBMC isolated from six donors were pooled together in RPMI 1640 medium (Gibco, Invitrogen, Paisley, Scotland) supplemented with 10% dimethyl sulphoxide (DMSO; Merck, Darmstadt, Germany), 20% foetal calf serum (FCS; Invitrogen) and 10 μg/ml gentamicin (Invitrogen) and frozen at −140 °C. Cells were thawed and cultured for 4 days prior to the experiment in RPMI 1640 medium supplemented with 10% FCS, 10 μg/ml gentamicin and 2 μg/ml lectin from *Phaseolus vulgaris* (PHA, Sigma Chemie, Zwijndrecht, The Netherlands) at 37 °C and 5% CO_2.

Crandell feline kidney (CrFK) cells were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Invitrogen, Paisley, Scotland), supplemented with 5% foetal calf serum (FCS; Invitrogen) and antibiotics. Primary feline thymocytes were isolated from specific-pathogen-free cats (Harlan, Zeist, The Netherlands), stimulated with concanavalin A at 2.5 μg/ml and cultured in RPMI 1640 medium containing 10% FCS supplemented with 100 IU/ml of recombinant IL-2 (Roche Diagnostics GmbH, Mannheim, Germany). Human epithelial (HEp-2) cells were cultured as monolayers in Iscove's modified Dulbecco's medium (IMDM; Invitrogen), supplemented with 5% FCS and 10 μg/ml gentamicin (Invitrogen) and maintained at 37 °C and 5% CO_2. Mouse L cells were cultured in DMEM containing 10% FCS, 100 IU/ml penicillin and 100 μg/ml streptomycin (DMEM/10).

Human foreskin fibroblasts (HFF) were cultured as monolayers in IMDM supplemented with 10% FCS and 10 μg/ml gentamicin and maintained at 37 °C and 5% CO_2. The HSV inducible reporter cell line baby hamster kidney (BHKICP6LacZ-5) cells [16] were obtained as frozen aliquots of 7.5×10^5 cells/vial from Diagnostic Hybrids, Inc., Athens, OH. BHKICP6LacZ-5 cells contain the *Escherichia coli lacZ* gene placed behind the inducible HSV-1 early promoter ICP6. The *lacZ* gene encodes β-galactosidase. Upon HSV-1 or HSV-2 infection there is induction of β-galactosidase activity. There is no constitutive expression from this promoter in uninfected cells, activation of the promoter appears to be specific for HSV and expression from the promoter occurs within hours after infection. Cells were thawed immediately before usage in minimal essential medium (MEM; Invitrogen) containing 7% FCS and 10 μg/ml gentamicin.

2.2. Virus

The following reagents were obtained from NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: HIV-$1_{Ba\text{-}L}$ (subtype B) strain from Dr. Suzanne Gartner, Dr. Mikulas Popovic and Dr. Robert Gallo; HIV-$2_{CDC310319}$ (subtype B isolate) strain from Dr. Stefan Wiktor and Dr. Mark Rayfield [17]; and NNRTI-resistant HIV-1_{IIIB} (A17 variant) strain from Dr. Emilio Emini [18]. HIV-$1_{Ba\text{-}L}$ and HIV-$2_{CDC310319}$ were propagated and titrated in PBMC. HIV-1_{IIIB} A17 was propagated in the H9 cell line and titrated in PBMC. HIV-1_{IIIB} A17 contains the mutations K103N and Y181C in the viral RT domain. A virus stock of the Dutch isolate FIV UT-113 was prepared from a culture of persistently infected CrFK cells. RSV serotype A was propagated and titrated in HEp-2 cells. RSV stock contained 2.4×10^7 PFU (plaque-forming units)/ml. MHV-A59 was propagated in Moloney sarcoma virus-transformed Sac(−) cells and plaque titrated on mouse L cells as described previously [19]. The MHV stock contained 5.3×10^8 PFU/ml.

The HSV-1 strain KOS and the KOS-derived acyclovir (ACV)-resistant HSV-1 strains PAA^{r}5, F891C, PFA^{r}2, and PFA^{r}5, which contain mutations in the DP gene [20–22] were kindly provided by Dr. D.M. Coen (Harvard Medical School, Boston, MA, USA). The HSV-1 ACV-sensitive strain McIntyre was generously provided from Dr. A. Linde (Swedish Institute for Infectious Disease Control, Solna, Sweden). The ACV-resistant HSV-1 clinical isolate 98.14742-PE/1, which contains mutations in the TK gene [23], was a gift of Dr.