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Selective activity of various antiviral compounds against HHV-7 infection

Ying Zhang *, Dominique Schols, Erik De Clercq

Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium

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Abstract

Human herpesvirus virus type 7 (HHV-7) is a T-lymphotropic herpesvirus which uses the CD4 receptor as main receptor to infect its target cells. Measuring the decrease of CD4 expression during HHV-7 infection is a convenient and accurate method to monitor the efficacy of antiviral agents against HHV-7 infection. Different classes of compounds, such as heparin, pentosan polysulfate (PS), dextran sulfate (DS), aurintricarboxylic acid (ATA), phosphonoformic acid (PFA), 9-(2-phosphonylmethoxyethyl)adenine (PMEA), 2-amino-7-[(1,3-dihydroxy-2-propoxy) methyl] purine (S2242), polyvinylalcohol sulfate (PVAS) and the co-polymer of vinylalcohol sulfate with acrylic acid (PAVAS), acyclovir (ACV), ganciclovir (GCV), penciclovir (PCV), brivudin (BVDU), cidofovir (HPMPC), lobucavir, (*R*)-9-[4-hydroxy-2-(hydroxymethyl)butyl]guanine] (H2G), (*R*)-9-(2-phosphonylmethoxypropyl)adenine (PMPA) and sorivudine (BVaraU), were evaluated for their anti-HHV-7 activity in the SupT1 T cell line and in purified $CD4^+$ T lymphocytes. Antiviral activity was monitored by inhibition of: (i) CD4 expression down-regulation; (ii) giant cell formation and (iii) apoptosis induction. In general, PS, DS, PVAS, PAVAS, ATA, PFA, PMEA, S2242, lobucavir and HPMPC had comparable anti-HHV-7 activity in the two cell lines, irrespective of the parameters followed to monitor antiviral activity. One of the exceptions was heparin which had an IC_{50} of 9.6 μg/ml in SupT1 cells and >250 μg/ml in $CD4^+$ T lymphocytes. The compounds PCV, GCV, H2G and PMPA showed some activity in $CD4^+$ T lymphocytes, but not in SupT1 cells. ACV, BVDU and BVaraU did not show activity in either cell system. None of the chemokines tested, such as platelet factor-4 (PF-4), eotaxin, stromal cell-derived factor 1α(SDF-1α) and RANTES, had detectable activity against HHV-7. In contrast, the HIV-1 envelope glycoprotein, gp120, and the two anti-CD4 mAbs, 13B8-2 and OKT4, were clearly active against HHV-7 infection. © 1999 Published by Elsevier Science B.V. All rights reserved.

Keywords: Human herpesvirus 7 (HHV-7); CD4 expression; Giant cells; Apoptosis

* Corresponding author. Tel.: +32-16-337341; fax: +32-16-337340.
E-mail address: ying.zhang@rega.kuleuven.ac.be (Y. Zhang)

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1. Introduction

The human herpesvirus 7 (HHV-7) is a T lymphotrophic herpesvirus which was first isolated from the peripheral blood lymphocytes of healthy individuals (Frenkel et al., 1990). It has also been isolated from the peripheral blood mononuclear cells of a patient with chronic fatigue syndrome (Berneman et al., 1992a). At present, only exanthema subitum is clearly associated with HHV-7 infection (Hidaka et al., 1994; Tanaka et al., 1994; Ueda et al., 1994; Torigoe et al., 1995), although in individual cases HHV-7 infection has been reported to be associated with febrile illness in childhood (Portolani et al., 1995; Caserta et al., 1998), pityriasis rosea (Drago et al., 1997a,b), infectious mononucleosis-like illness (Chiu et al., 1998), symptoms mimicking chronic Epstein–Barr virus infection (Kawa-Ha et al., 1993), central nervous system manifestations (Torigoe et al., 1996), hepatitis (Hashida et al., 1995) and Bell's palsy (Takasaki et al., 1998). HHV-7 is an ubiquitous human herpesvirus which infects during childhood (Wyatt et al., 1991). More than 90% of saliva samples were found to be positive for HHV-7, which suggests that the salivary gland is a major site for virus replication (Wyatt and Frenkel, 1992; Kidd et al., 1996; Yadav et al., 1997). HHV-7 was also detected in a number of tissues, such as lungs, skin, mammary glands (Kempf et al., 1998) and in the cervix of pregnant woman (Okuno et al., 1995). HHV-7 utilizes CD4 as its essential receptor to enter its target cell (Furukawa et al., 1994; Lusso et al., 1994). In vitro studies have shown that HHV-7 can interfere with human immunodeficiency virus type 1 (HIV-1) infection in $CD4^+$ T cells and mononuclear phagocytes (Lusso et al., 1994; Crowley et al., 1996), because HIV-1 also uses CD4 as its main receptor (Dalgleish et al., 1984; Klatzmann et al., 1984) to infect the cells.

Since the discovery of the chemokine receptors CXCR4 and CCR5 as the main coreceptors for HIV, numerous publications have demonstrated the importance of chemokine receptors for HIV entry (Berson et al., 1996; Feng et al., 1996; He et al., 1997). For HHV-7 no coreceptor, besides the CD4 receptor, has been described, although so far only one $CD4^+$ T cell line, the SupT1 cell line, has been found to be infectable by HHV-7 (Berneman et al., 1992b). Interestingly, HHV-7 can induce the expression of the EBV-induced genes 1 (EBI1) in $CD4^+$ T cells (Hasegawa et al., 1994). EBI 1, a putative lymphocyte-specific G protein-coupled peptide receptor, is expressed exclusively in B- and T-lymphocytes cell lines and in lymphoid tissues (Birkenbach et al., 1993). Recently, EBI 1 has been designated as CCR7 (Yoshida et al., 1997).

Following HHV-7 infection, the SupT1 T cells (Berneman et al., 1992b), exhibit a cytopathic effect consisting of enlarged, refractile, short-lived cells and typical multinucleated giant cells (Secchiero et al., 1994). This cytopathic effect is based on two distinct mechanisms: the formation of multinucleated giant cells (syncytia), which eventually undergo necrotic lysis, and apoptosis, which predominantly seems to occur in small mononucleated cells (Secchiero et al., 1997a). We have recently shown that following HHV-7 infection there is a direct correlation between an increase in HHV-7 antigen expression, a decrease in CD4 expression and an increase in apoptosis. Using these different flow cytometric methods, several classes of antiviral compounds, chemokines and anti-CD4 mAbs have been evaluated in SupT1 T cells and purified $CD4^+$ T lymphocytes (obtained from peripheral blood). All these compounds had previously been shown to inhibit HIV infection and/or herpesvirus infection. Accurate 50% inhibitory concentration (IC_{50}) values were determined flow-cytometrically by monitoring the decrease (down-regulation) of CD4 expression. In addition, the appearance of giant cells and the induction of apoptosis in the HHV-7-infected cells were evaluated in the absence or presence of the different test compounds.

2. Materials and experimental procedures

2.1. Virus strain, cell lines and purification of $CD4^+$ T lymphocytes

The KHR strain of HHV-7 was kindly provided by Dr K. Yamanishi (Department of Mi-

crobiology, Osaka University School of Medicine, Osaka, Japan). The stock of HHV-7 was prepared as follows: HHV-7 was grown in SupT1 cells and cultured in RPMI 1640 medium (Gibco BRL, Gaithersburg, MD) with 10% heat-inactivated fetal calf serum (FCS) (Gibco BRL), 1% glutamine (Gibco BRL) and 2% gentamicin (Gibco BRL). At 7–10 days after infection, the cytopathic effect (CPE) of HHV-7 was analyzed microscopically. CD4 expression was examined by flow cytometry. When CD4 expression was decreased by more than 50%, the supernatant plus infected cell lysates were divided in aliquots and stored at −70°C. In all experiments a 1–10 dilution of the viral stock was used.

The $CD4^+$ lymphocytic SupT1 T cell line was obtained from the American Type Culture Collection (Rockville, MD). Buffy coat preparations from healthy donors were obtained from the Blood Bank in Leuven. Peripheral blood mononclear cells (PBMC) were first isolated by density gradient centrifugation over Lymphoprep™ (d = 1.077 g/ml) (Nycomed, Oslo, Norway), and then magnetic beads to CD8, CD14 and CD19 (Dynal AS, Oslo, Norway) were added to purify the $CD4^+$ T lymphocytes. A purity between 80 and 90% of $CD4^+$ T cells was obtained by this negative selection protocol. The cells were stimulated with 2 μg/ml of phytohemagglutinin (PHA) (Sigma Chemicals, Bornem, Belgium) and 25 IU/ml of IL-2 for 3 days at 37°C.

2.2. Compounds, chemokines and mAbs

Heparin (molecular weight (MW): 3000), pentosan polysulfate (PS; MW: 3100), dextran sulfate (DS; MW: 5000), aurintricarboxylic acid (ATA; MW: 422.3), and phosphonoformic acid (PFA; MW: 191.6) were purchased from Sigma Chemicals (Bornem, Belgium). Polyvinylalcohol sulfate (PVAS; MW: 40000) and its co-polymer with acrylic acid (PAVAS; MW: 20000) were kindly provided by Dr S. Görög (Chemical Works of Gedeon Richter, Budapest, Hungary). 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV; MW: 225.2) was purchased from Wellcome laboratories (Research Triangle Park, NC) and 9-(1,3-dihydroxy-2-propoxymethyl)guanine (ganciclovir, GCV; MW: 255.2) was from Syntex (Palo Alto, CA). 9-[4-hydroxy-3-(hydroxymethyl)butyl]guanine (penciclovir, PCV; MW: 253.3) was obtained from Hoechst AG (Frankfurt am Main, Germany), (*E*)-5-(2-Bromovinyl)-1-(β-D-deoxyribofuranos-1-yl)uracil (brivudin, BVDU; MW: 333.1) was synthesized at the Rega Institute for Medical Research (Leuven, Belgium). 1-β-D-Arabinofuranosyl (*E*)-5-(2-bromovinyl)uracil (sorivudine, BVaraU; MW: 349) was from Yamasa Shoyu (Choshi, Japan). (*S*)-1-(3-hydroxy-2-phosphonylmethoxypropyl) cytosine (cidofovir, HPMPC; MW: 278), 9-(2-phosphonylmethoxyethyl) adenine (PMEA; MW: 273.3), and (*R*)-9-(2-phosphonylmethoxypropyl)adenine (PMPA; MW: 296.2) were kindly provided by Gilead Sciences (Foster City, CA). 2-amino-7-[(1,3-dihydroxy-2-propoxy) methyl] purine (S2242; MW: 239) was kindly provided by Dr G. Jähne and Dr I. Winkler (Hoechst AG, Frankfurt am Main, Germany). (1*R*-1α,2β,3α)-9-[2,3-bis(hydroxymethyl) cyclobutyl]guanine (lobucavir; MW: 265.3) was kindly provided by Dr Richard Colonno (Bristol-Myers Squibb). (*R*) - 9 - [4 - hydroxy - 2 - (hydroxymethyl)butyl]guanine] (H2G; MW: 253.3) was obtained from Abbott Laboratories (courtesy of Dr Akhter Molla and Dr William Kohlbrenner). Recombinant HIV-1 gp120 was purchased from Intracell (London, UK).

The chemokines platelet factor-4 (PF-4), eotaxin, stromal cell-derived factor-1α(SDF-1α) and regulated on activation normal T cell expressed and secreted (RANTES) were purchased from PeproTech (London, UK).

To test the antiviral activity of anti-CD4 mAbs 13B8-2 (Immunotech, S.A. Marseille, France) and OKT4 (Ortho Diagnostic, Beerse, Belgium) were used. The Leu3a mAb labelled with phycoerythrin (PE) or fluorescein isothiocyanate-conjugated (FITC) (Becton Dickinson, Erembodegem, Belgium) was used for the flow cytometric analysis of CD4 expression. A specific mouse monoclonal antibody to HHV-7 (RK-4) (Advanced Biotechnologies, Columbia, MD) detecting an early/late HHV-7 protein was used to evaluate HHV-7 antigen expression.

2.3. Antiviral assays and flow cytometric analysis

In SupT1 cell assay, fivefold serial dilutions of the test compounds were added in 500 μl culture medium in 24-well flat bottom plates (Falcon), then 2×10^5 SupT1 cells were added in 400 μl culture medium, and finally 100 μl of the HHV-7 stock diluted one to ten was added to each well. HHV-7-infected and mock-infected SupT1 cells were cultured in a final volume of 1 ml medium in every plate in the absence of the compounds. On day 5, half of the medium and cells were replaced and fresh medium but no new compound was added. This procedure was repeated every 2 or 3 days. In the purified $CD4^+$ T lymphocytes assay, fivefold serial dilutions of the test compounds were added in 250 μl culture medium in 48-well flat bottom plates (Falcon), then 1×10^6 cells were added in 200 μl cell culture medium with IL-2, and finally 50 μl HHV-7 stock was added to each well. HHV-7-infected and mock-infected purified $CD4^+$ T lymphocytes were cultured in a final volume of 500 μl with 25 U/ml IL-2 in every plate in the absence of the compounds. At day 3, 500 μl fresh culture medium with 25 U/ml IL-2 was added but without additional compound.

To monitor the decrease in CD4 expression, 1×10^6 cells were centrifuged, washed twice with phosphate-buffered saline (PBS) containing 2% FCS, and 10 μl of Leu3a-PE mAb were added. After a 20 min incubation period at 4°C, the cells were washed again and fixed in 1% formaldehyde solution in PBS. To monitor the increase in HHV-7 antigen expression during the infection in SupT1 cell line, 1×10^6 cells were centrifuged, washed with PBS containing 2% FCS, and then fixed in 2% formaldehyde solution in PBS. After 15 min incubation at room temperature, the cells were pelleted and 500 μl 0.2% Triton X-100 in PBS was added for 2 min at 4°C. Then the cells were washed and 1 μl anti-HHV-7 mAb (RK-4) was added for 30 min; then the cells were washed again and incubated with fluorescein isothiocyanate-conjugated goat-anti-mouse antibody (GaM-IgG-FITC) (Caltag Laboratories) for 30 min, washed and analyzed by flow cytometry. To test the increase in HHV-7 antigen expression following infection of the purified $CD4^+$ T lymphocytes a slightly different staining protocol was followed. Briefly, 1×10^6 cells were washed with cold PBSA (PBS with 2% new calf serum and 0.1% NaN_3) and then once with PBS. Then the cells were resuspended and 2% formaldehyde solution in PBS was added. After a 60 min incubation, the cells were pelleted and 1 ml 0.2% Tween-20 in PBS was added. After 15 min, the cells were centrifuged and resuspended in 10 μl human serum and 1 μl RK-4 mAb was added for 30 min; then the cells were washed and incubated with GaM-IgG-FITC for 30 min. After washing, 10 μl of Leu3a-PE mAb was added to the cell pellet, washed again, and analyzed by flow cytometry.

The annexin-V-FLUOS staining kit was purchased from Boehringer Mannheim (Brussels, Belgium) and used to analyze the occurrence of apoptosis during the HHV-7 infection, as described previously (Koopman et al., 1994; Vermes et al., 1995). Briefly, 1×10^6 SupT1 cells were collected and washed once with PBS. Then, 100 μl of binding buffer containing Annexin-V-FITC and propidium iodide (PI) were added. After an incubation for 15 min at room temperature in the dark, 500 μl of binding buffer was added and the samples were analyzed by flow cytometry. Dead cells and debris were eliminated by appropriate FCS/SSC gating (as shown in Fig. 1 Panel C and D).

A FACScan™ (Becton Dickinson Immunocytometry Systems, San Jose, CA) flow cytometer was used to analyze the cells with CellQuest™ software (Becton Dickinson) on an Apple Macintosh computer.

3. Results

The SupT1 cells were already described to be very sensitive to HHV-7 infection (Berneman et al., 1992b) and a comparison between light microscopic pictures and flow cytometric data are shown in Fig. 1. In Panel A and Panel C are the uninfected SupT1 cells and in Panel B and Panel D are the HHV-7-infected SupT1 cells shown 10 days after infection. As forward scatter (FSC) is a parameter of the cell size, the region 1 (R1) in

Panel C only eliminates dead cells and cell debris. The percentages (%) of viable cells in the uninfected cells were 93.6% and in the HHV-7-infected cells 50.6%. Besides the FSC, the side scatter (SSC) is a parameter of cell granularity and cell size. Region 2 (R2) will eliminate the cells with the normal size and these cells are then defined as giant cells. The percentage of giant cells in uninfected SupT1 cells was 1.7% (Panel C) and increased in the HHV-7-infected SupT1 cells (Panel D) to 29.5%.

Triton X-100 was used to detect the appearance of intracellular viral antigens with a HHV-7 Ag-specific mAb, RK-4. Uninfected and HHV-7 infected SupT1 cells at day 7 were stained with RK-4/GAM-FITC and Leu-3a PE. As shown in Fig. 2A, the uninfected SupT1 cells were 95.4% $CD4^+RK\text{-}4^-$. Following HHV-7 infection the CD4 expression decreased to 50.7% and the % of $CD4^+RK\text{-}4^+$ cells increased from 0.7 to 9.6%, $CD4^-RK\text{-}4^+$ cells increased from 0.1 to 42.3%, and $CD4^-RK\text{-}4^-$ increased from 3.8 to 13.3%.

The correlation between the decrease in CD4 expression and increase in HHV-7 antigen expression was also studied in the purified $CD4^+$ T lymphocytes. As shown in Fig. 3A, the uninfected purified $CD4^+$ T lymphocytes were 85.7% $CD4^+$. At day 7 post infection, the percentage of $CD4^+$ cells decreased to 35.1% and the HHV-7 Ag^+ cells increased to 45.5% (Fig. 3 B).

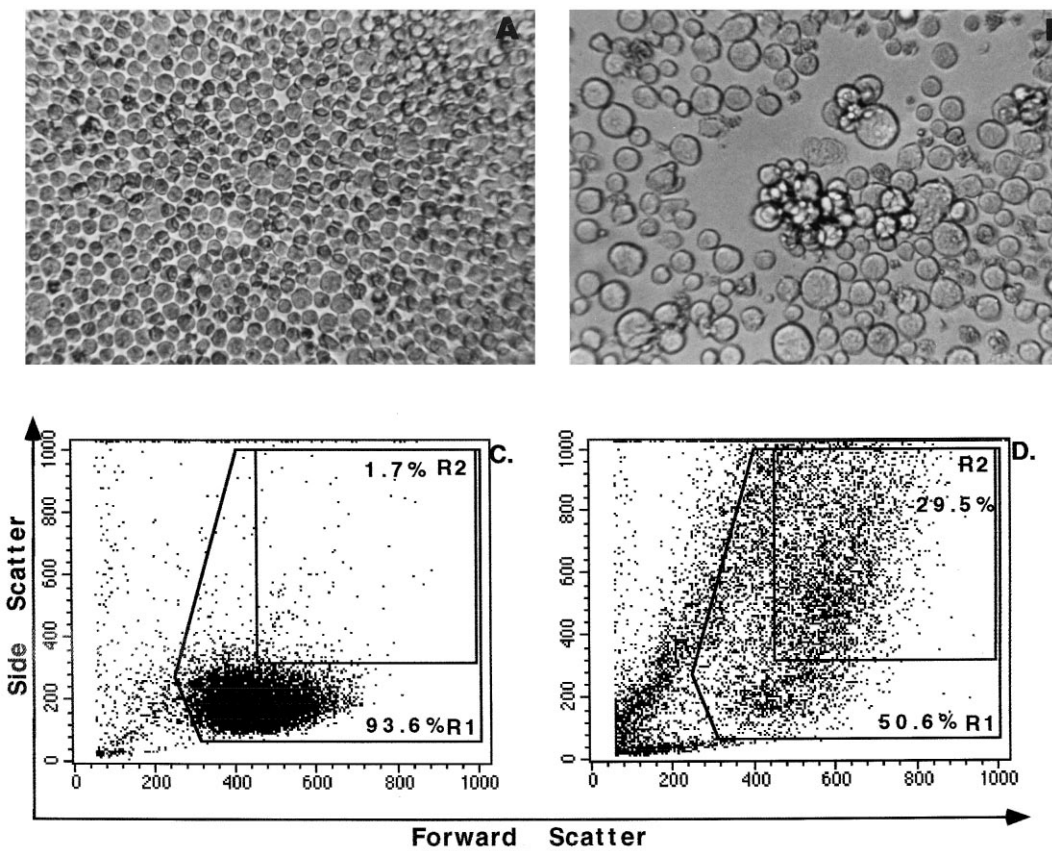

Fig. 1. Microscopic appearance and FSC/SSC dot plot of uninfected and HHV-7 infected SupT1 cells. A: Microscopic picture of uninfected SupT1 cells. B: Microscopic picture of HHV-7-infected SupT1 cells at day 10 post-infection. C: Dot plot of uninfected SupT1 cells. The percentage of viable cells in R1 and of giant cells in R2 of the uninfected SupT1 cells were 93.6 and 1.7%, respectively. D: Dot plot of HHV-7-infected SupT1 cells at day 10 post-infection. The percentage of cells in R1 and R2 were 50.6 and 29.5%.

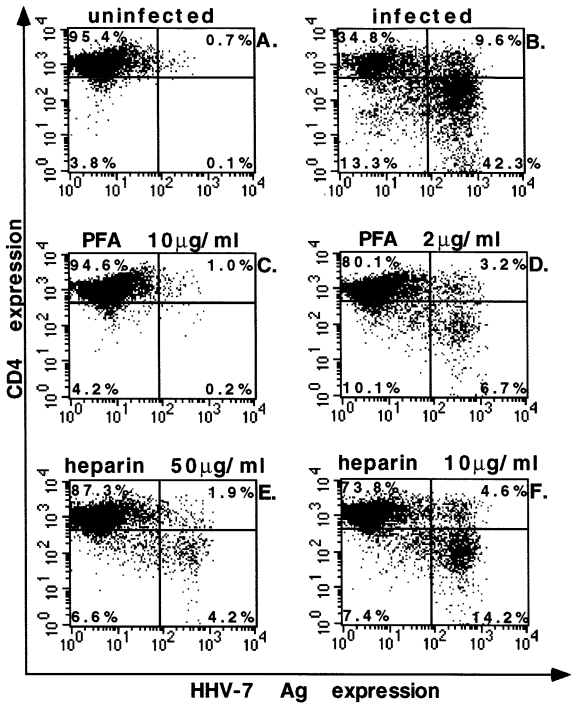

Fig. 2. Inhibitory effect of PFA and heparin on HHV-7 replication in the SupT1 cell line. A: mock-infected SupT1 cells. B: HHV-7-infected SupT1 cells at day 7 post-infection. C: HHV-7 infected SupT1 cells treated with PFA at 10 μg/ml. D: HHV-7 infected SupT1 cells treated with PFA at 2 μg/ml. E: HHV-7-infected SupT1 cells treated with heparin at 50 μg/ml. F: HHV-7-infected SupT1 cells treated with heparin at 10 μg/ml. The cells were stained with RK-4 GaM-IgG-FITC (*X*-axis) and Leu3a-PE (*Y*-axis) and the percentages of fluorescent cells are indicated in each quadrant.

The inhibitory effects of PFA and heparin were first evaluated in the SupT1 cell line. The cells were stained with Leu3a-PE (*Y*-axis) and with RK-4 GaM-IgG-FITC to detect HHV-7 Ag intracellularly (*X*-axis). As can be seen in Fig. 2 C, PFA at 10 μg/ml completely inhibited HHV-7 antigen expression and the decrease in CD4 expression. With PFA at 2 μg/ml, 80.1% of the cells were still $CD4^+$ and HHV-7 antigen expression reduced to 9.9% (Fig. 2 D). Heparin was less potent than PFA in inhibiting HHV-7 infection in SupT1 cells. At 50 μg/ml, 87.3% cells were $CD4^+$ and HHV-7 antigen expression was suppressed to 4.2% (Fig. 2 E). With heparin at 10 μg/ml, 73.8% cells still were $CD4^+$ and HHV-7 antigen expression was reduced to 18.8% (Fig. 2 F).

PFA and heparin were then evaluated for their anti-HHV-7 activity in purified $CD4^+$ T lymphocytes. As can be seen in Fig. 3 C, heparin was not able to inhibit HHV-7 antigen expression even at 250 μg/ml. In contrast, PFA showed potent activity at 10 μg/ml, 69.2% of the cells were still $CD4^+$ and HHV-7 antigen negative and the HHV-7 antigen expression increased to 15.4% (Fig. 3 D).

As the decrease in CD4 expression is correlated with HHV-7 antigen expression and thus viral replication, we further used CD4 expression as a parameter for the antiviral evaluation of the compounds. In addition, the number of giant cells and the induction of apoptosis in SupT1 cells following HHV-7 infection were also used as parameters to calculate the 50% inhibitory concentration (IC_{50}) values of the test compounds. As shown in Table 1, the IC_{50} values for dextran sulfate (DS) that were calculated from the three parameters (CD4 expression, giant cells and apoptosis) were 2.0, 1.5 and 2.6 μg/ml, respectively, and the corresponding IC_{50} values for pentosan sulfate (PS) were 1.2, 1.0 and 1.0 μg/ml, respectively. The two sulfated polymers PVAS and PAVAS had IC_{50} values between 1.1 and 2.3 μg/ml. Heparin, as compared to DS and PS, was less active, its IC_{50} was between 5.8 and 14.3 μg/ml. Aurintricarboxylic acid (ATA) inhibited HHV-7 infection at an IC_{50} value of between 2.8 and 7.1 μg/ml. The acyclic nucleoside phosphonates HPMPC and PMEA had IC_{50} values of 11.7, 17.5, >90 and 3.6 μg/ml; and 3.4 and 11.1 μg/ml respectively, but PMPA had no activity. S2242 had an IC_{50} of about 0.0001 μg/ml and PFA had an IC_{50} of about 2.5 μg/ml. The deoxyguanine nucleoside analog, lobucavir, had an IC_{50} of 4.7 and 3.4 μg/ml in inhibiting CD4 decrease and giant cell formation, but did not inhibit HHV-7-induced apoptosis in SupT1 cells. The nucleoside analogues, ACV, GCV, PCV, BVDU, BVaraU and H2G did not show significant activity against HHV-7 infection.

Also HIV-1 gp120 was evaluated for its inhibitory effect on HHV-7 replication in the SupT1 cells, and its IC_{50} values were between 0.1 and 0.6 μg/ml. The two anti-CD4 mAbs 13B8-2 and OKT-4 inhibited HHV-7 infection at an IC_{50} be-

tween 0.01 and 0.07 μg/ml. The chemokines PF-4, eotaxin, SDF-1α and RANTES had no anti-HHV-7 activity at a concentration up to 1 μg/ml.

The 50% cytoxic concentration (CC_{50}), monitored by the propidium iodide exclusion method, was defined as the concentration of the test compound required to inhibit cell growth by 50%. No toxicity was noted for heparin, ACV, PMPA and BVaraU at concentrations up to 250 μg/ml. For DS, PFA, GCV and H2G the CC_{50} values were above 100 μg/ml. The CC_{50} values of PAVAS, ATA, GCV and HPMPC were between 50 and 100 μg/ml, whereas PVAS, PMEA, BVDU, lobucavir and S2242 had CC_{50} values of 26.3, 43.1, 20, 15 and 0.051 μg/ml, respectively.

In purified $CD4^+$ T lymphocytes the percentages of $CD4^+$ cells were obtained by staining with a mAb (Leu3a). Also the percentages of giant cells were obtained by gating on the FSC and SSC by flow cytometry as explained in Fig. 1. From these percentages the IC_{50} values were calculated (Table 2). The IC_{50} values of DS calculated from CD4 expression and giant cells were 7.3 and 7.2 μg/ml, respectively; and the IC_{50} values of PS were 5.2 and 2.6 μg/ml, respectively. The IC_{50} values of PVAS and PAVAS were between 3.4 and 9.3 μg/ml and for ATA between 4.5 and 17.7 μg/ml. S2242 had an IC_{50} of 0.0029–0.0096 μg/ml; and PFA had an IC_{50} of between 3.9 and 7.6 μg/ml, respectively. PMEA and HPMPC had an IC_{50} of 5.9, 8.4; and 0.4 and 1.0 μg/ml, respectively. PMPA was less active. GCV and lobucavir had an IC_{50} of 25.4, 10.7; and 1.2 and 4.5 μg/ml, respectively. PCV and H2G had IC_{50} values of 41.5 and 156.3 μg/ml, respectively, when evaluated for CD4 expression, but IC_{50} values were >230 μg/ml when based on giant cell scores. Heparin, ACV, BVDU and BVaraU did not show activity against HHV-7 infection up to a concentration of 250 μg/ml. Because HIV-1 gp120 and the two anti-CD4 mAbs, 13B8-2 and OKT4, directly interfere with binding of the CD4 mAb, only the percentages of giant cells were used to calculate their IC_{50} values. The IC_{50} values for HIV-1 gp120, 13B8-2 and OKT4 were 0.6, 0.07 and 0.12 μg/ml, respectively.

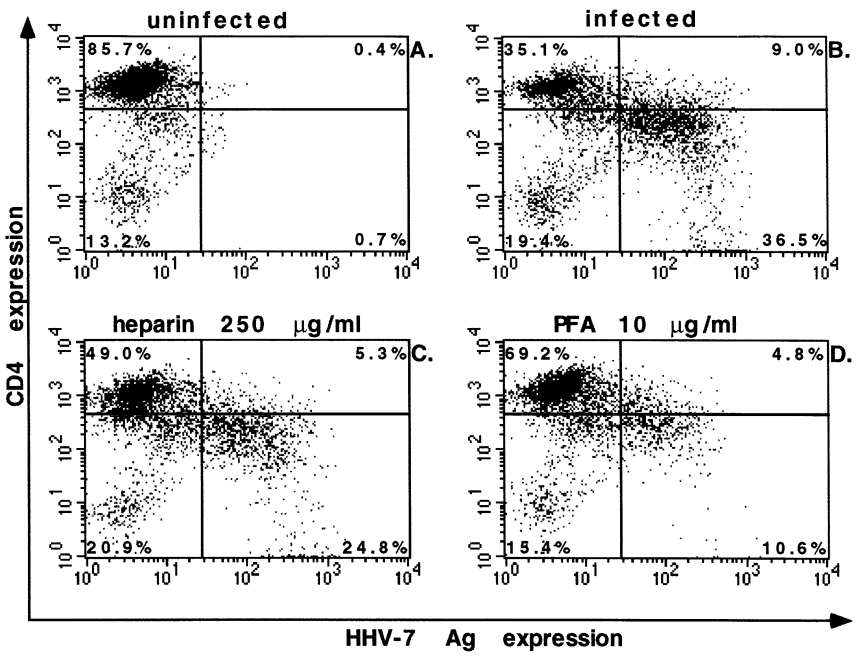

Fig. 3. Inhibitory effect of PFA and heparin on HHV-7 replication in purified $CD4^+$ T lymphocytes. A: mock-infected $CD4^+$ T lymphocytes. B: HHV-7-infected $CD4^+$ T lymphocytes at day 7 post-infection. C: HHV-7 infected $CD4^+$ T lymphocytes treated with heparin at 250 μg/ml. D: HHV-7 infected $CD4^+$ T lymphocytes treated with PFA at 10 μg/ml.

Table 1
Inhibitory effects of antiviral compounds, chemokines, gp120 and anti-CD4 mAbs on the replication of HHV-7 in the SupT1 cell line

	IC_{50} (μg/ml)[a]			CC_{50} (μg/ml)[b]
	CD4 expression	Giant cells	Apoptosis	
Antiviral compounds				
Heparin	9.6	5.8	14.3	>250
PS	1.2	1.0	1.0	46.0
DS	2.0	1.5	2.6	143
PVAS	1.1	1.4	2.1	26
PAVAS	1.3	2.3	1.7	77
ATA	4.7	2.8	7.1	52
PFA	2.9	2.3	2.4	134
S2242	0.0001	0.0001	0.0002	0.051
PMEA	3.6	3.4	11.1	43
HPMPC	11.7	17.5	>90	90
PMPA	>250	>250	>250	>250
PCV	>170	>170	>170	170
GCV	>50	>50	>50	50
H2G	>107	>107	>107	107
Lobucavir	4.7	3.4	>15	15
ACV	>250	>250	>250	>250
BVDU	>20	>20	>20	20
BVaraU	>250	>250	>250	>250
Chemokines				
PF-4	>1	>1	n.d.[c]	>1
eotaxin	>1	>1	n.d.	>1
SDF-1α	>1	>1	n.d.	>1
RANTES	>1	>1	n.d.	>1
Peptide				
HIV-1 gp120	0.4	0.1	0.6	>10
Anti-CD4 mAbs				
13B8-2	0.01	0.01	0.02	>10
OKT4	0.03	0.07	0.05	>10

[a] IC_{50}: 50% inhibitory concentration was defined as the concentration of the test compound required to: inhibit the decrease in CD4 receptor expression; the formation of giant cells or; HHV-7 induced apoptosis by 50%, as evaluated in SupT1 cells on day 7–9 after HHV-7 infection.

[b] CC_{50}: 50% inhibitory concentration was defined as the concentration of the test compound inhibiting the viability of the SupT1 cells, as determined by propidium iodide exclusion.

[c] n.d., not done.

No toxicity was found for heparin, PS, DS, ACV, PMPA and BVaraU at concentrations up to 250 μg/ml. For ATA, PFA and H2G the CC_{50} values were above 100 μg/ml. The CC_{50} values of PVAS, PAVAS, PMEA, BVDU and GCV were between 50 and 100 μg/ml, whereas HPMPC, lobucavir and S2242 had CC_{50} values of 44.0, 6.4 and 1.3 μg/ml, respectively.

4. Discussion

The sulfated polysaccharides, such as DS and PS, are known to be highly potent inhibitors of HIV replication in vitro (Baba et al., 1988), and this anti-HIV activity has been attributed to the inhibition of virus adsorption to the cells (Schols et al., 1990b). Sulfated polymers, such as PVAS

and PAVAS, have been described as potent and selective inhibitors of various enveloped viruses, including herpes simplex virus (HSV-1, HSV-2), cytomegalovirus (CMV), vesicular stomatitis virus, respiratory syncytial virus, and toga-, arena- and retroviruses (Schols et al., 1990a).

Table 2
Inhibitory effects of antiviral compounds, chemokines, gp120 and anti-CD4 mAbs on the replication of HHV-7 in purified CD4+ T lymphocytes

	IC_{50} (μg/ml)[a]		CC_{50} (μg/ml)[b]
	CD4 expression	Giant cells	
Antiviral compounds			
Heparin	>250	>250	>250
PS	5.2	2.6	>250
DS	7.3	7.2	>250
PVAS	6.3	9.3	86
PAVAS	3.4	6.0	55
ATA	17.7	4.5	100
PFA	3.9	7.6	132
S2242	0.0029	0.0096	1.3
PMEA	5.9	8.4	54
HPMPC	0.4	1.0	44
PMPA	102.4	84.0	>250
PCV	41.5	>250	>250
GCV	25.4	10.7	61
H2G	156.3	>230	230
Lobucavir	1.2	4.5	6.4
ACV	>250	>250	>250
BVDU	>90	>90	90
BvaraU	>250	>250	>250
Peptide			
HIV-1 gp120	n.a.[c]	0.6	>10
Anti-CD4 mAbs			
13B8-2	n.a.	0.07	>10
OKT4	n.a.	0.12	>10

[a] IC_{50}: 50% inhibitory concentration was defined as the concentration of the test compound required to inhibit: the decrease in CD4 receptor expression; and the formation of giant cells, by 50%, as evaluated on day 7 after HHV-7 infection.

[b] CC_{50}: see footnote to Table 1

[c] n.a., not applicable because of the interference of HIV-1 gp120 and anti-CD4 mAbs with the binding of the Leu-3a mAb.

Here, we demonstrate for the first time that the sulfated polymers and polysaccharides are also active against HHV-7. It has been shown recently that heparin is able to inhibit HHV-7 infection in the SupT1 cell line (Secchiero et al., 1997b). It was also demonstrated that heparan sulfate proteoglycans may play an important role in HHV-7 cell surface membrane interactions. However, the CD4+ T cell lines MT-4 and CEM/X174, that also strongly express heparan sulfate (even more than SupT1 cells) became not infected with HHV-7 (data not shown). Also HHV-7 was not able to infect many other CD4+ cell lines as described by others (Berneman et al., 1992a). Although we have found that heparin is active against HHV-7 infection in the SupT1 cells (IC_{50}: 6–14 μg/ml), it has almost no activity against HHV-7 infection in purified CD4+ T lymphocytes (IC_{50}: >250 μg/ml). This may be related to the fact that heparan sulfate is less efficiently expressed in peripheral blood mononuclear cells (PBMC). Alternatively, it may suggest that heparin-like molecules do not play an important role in the binding of HHV-7 to the cell surface and that other or additional factors may be involved in the entry process of HHV-7. The anionic polymer aurintricarboxylic acid (ATA) inhibits HIV-1 replication in vitro, it inhibits attachment of HIV virions and it selectively prevents the binding of the OKT4A/Leu-3a mAb to the CD4 receptor (Schols et al., 1989). Although ATA specifically interacts with the CD4 cell receptor (Schols et al., 1989), it also prevents the binding of mAbs to gp120 expressed on HIV-infected cells (Cushman et al., 1991). Here we show that ATA is also active against HHV-7 infection, probably due to its interference with the CD4 receptor.

It has already been described that the nucleoside analogue, S2242, possesses marked activity against herpesvirus infections, such as HSV-1, HSV-2 and CMV (Neyts et al., 1994), HHV-6, -7 and -8 (Reymen et al., 1995; Neyts and De Clercq, 1997; Takahashi et al., 1997). The pyrophosphate analogue, PFA, which also has been shown to be active against HHV-6 infection (Åkesson-Johansson et al., 1990; Reymen et al., 1995), was also shown to be active against HHV-7 infection (IC_{50}: 1–10 μg/ml) when evaluated in CBMC (Black et al., 1997; Takahashi et al., 1997;

Yoshida et al., 1998). When PFA was evaluated (in this study) in two different cell types with three different parameters, the IC_{50} values obtained were very comparable (IC_{50}: 2–8 μg/ml). Acyclic nucleoside phosphonate analogues, such as HPMPC, have been shown to be active against herpesviruses (HSV-1 and -2, VZV, CMV, EBV, HHV-6, -7 and -8), and polyoma-, papilloma-, adeno-, and poxvirus infections (Reymen et al., 1995; De Clercq, 1997; Neyts and De Clercq, 1997; Takahashi et al., 1997; Yoshida et al., 1998). PMEA, is active against herpesviruses (HSV-1 and -2, CMV, HHV- 6 and -7), HBV and retrovirus (HIV-1 and -2, SIV and FIV) infections (Reymen et al., 1995; De Clercq, 1997; Takahashi et al., 1997). Both HPMPC and PMEA proved also active against HHV-7 infection. PMPA, also an acyclic nucleoside phosphonate analogue, with marked activity against hepadnaviruses and retroviruses (De Clercq, 1997) and H2G, an acyclic guanosine analogue, with activity against HSV-1 and -2, VZV and HHV-6 (Åkesson-Johansson et al., 1990; Abele et al., 1991) were essentially inactive against HHV-7 infection in SupT1 cells and purified $CD4^+$ T lymphocytes. Lobucavir, a deoxyguanine nucleoside analogue which was previously shown to inhibit HBV, HSV-1 and -2, VZV, CMV and HIV infection (Field et al., 1990; Norbeck et al., 1990; Innaimo et al., 1997; Tenney et al., 1997), has distinct activity against HHV-7 infection. Although BVDU was found to be weakly active against HHV-7 in CBMC (Takahashi et al., 1997), it was not active in our assay systems (Tables 1 and 2). BVDU was also not active against HHV-6 in HSB-2 (Reymen et al., 1995) and only weakly active against HHV-6 in CBMC (Takahashi et al., 1997). BVaraU was not active against HHV-6 (Reymen et al., 1995) and HHV-7 (Takahashi et al., 1997), and was also not active in our assays. Also, ACV was not active against HHV-7. GCV showed activity against HHV-6 (IC_{50}: 0.4–1.8 μg/ml) in CBMC (Takahashi et al., 1997; Yoshida et al., 1998), although no activity was seen when GCV was evaluated in HSB-2 cells (IC_{50}: >7 μg/ml) (Reymen et al., 1995). GCV had some activity against HHV-7 (IC_{50}: 4.6–7.5 μg/ml) in CBMC (Takahashi et al., 1997; Yoshida et al., 1998). Also in our study GCV had some activity against HHV-7 in purified $CD4^+$ lymphocytes (Table 2). PCV was not active against HHV-6 (Reymen et al., 1995), but showed some activity against HHV-7 in CBMC when used at a concentration of 50 μg/ml (Black et al., 1997). We also found some activity (when based on restoration of CD4 expression) with PCV in purified $CD4^+$ lymphocytes (Table 2), but not in SupT1 cells.

The HIV-1 glycoprotein gp120 and the mAb OKT4 also exert a dose-dependent inhibitory effect on HHV-7 infection (Lusso et al., 1994). The anti-CD4 mAb 13B8-2 which is specific for the CDR3-like region of the CD4 molecule inhibits HIV-1 infection (Benkirane et al., 1995) and here it was demonstrated to be active against HHV-7. The CD4 ligand, HIV-1 gp120, and the two CD4 mAbs, OKT4 and 13B8-2, proved equally active against HHV-7 infection in SupT1 cells as well as purified $CD4^+$ T lymphocytes.

Chemokines are chemotactic cytokines, which are classified as CC or CXC, depending on the positioning of the conserved cysteine residues. Chemokine receptors are important co-receptors for HIV entry (Cocchi et al., 1995; Feng et al., 1996). The natural ligand for CXCR4, SDF-1α, has been shown to inhibit infection of the cells by T-tropic HIV-1 strains (Bleul et al., 1996; Oberlin et al., 1996). Although SupT1 cells are CXCR-4 positive (Schols et al., 1997), the chemokine SDF-1α has no measurable anti-HHV-7 activity in these cells. The CC-chemokine RANTES (a natural ligand for CCR5) which blocks M-tropic HIV-1 strains (Cocchi et al., 1995), also has no anti-HHV-7 activity. Also, PF-4 which is a platelet-released chemokine shown to interact with heparan sulfate on the cell membrane (Stringer and Gallagher, 1997) has no anti-HHV-7 activity, again suggesting that heparan sulfate proteoglycans are not directly involved in HHV-7 binding and entry. The CC-chemokine eotaxin (a specific ligand for CCR3), which has been shown to be a co-receptor for certain HIV-1 strains (He et al., 1997), also has no anti-HHV-7 activity. Thus, none of the chemokines tested so far displayed an inhibitory activity against HHV-7 infection in SupT1 cells.

In conclusion, this study demonstrates that the sulfated polysaccharides (DS and PS), sulfated polymers (PVAS and PAVAS), aurintricarboxylic acid (ATA), the nucleoside analogue S2242, the pyrophosphate analogue PFA, the acyclic nucleoside phosphonate analogues HPMPC and PMEA, and the deoxyguanine nucleoside analogue lobucavir possess potent activity against HHV-7 infection in SupT1 cells and purified $CD4^+$ T lymphocytes. PMPA (also an acyclic nucleoside phosphonate), and the viral thymidine kinase-dependent compounds, PCV, H2G, ACV, BVDU and BVaraU, had essentially no activity against HHV-7 infection in both in vitro cell systems. Although heparin has activity against HHV-7 infection in the SupT1 cells, it did not show activity against HHV-7 infection in the purified $CD4^+$ T lymphocytes. This suggests that heparan sulfate proteoglycan may not play an important role in the adsorption of HHV-7 to cell surface proteoglycans. HIV-1 gp120 and two anti-CD4 mAbs were potent inhibitors of HHV-7 infection, whereas no activity was noted with any of the chemokines tested.

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