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Inhibition of visna virus replication and cytopathic effect in sheep choroid plexus cell cultures by selected anti-HIV agents

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Abstract

Several anti-HIV agents were tested against visna virus replication and cytopathic effect (CPE) in sheep choroid plexus cell cultures. Sulphated polysaccharides (i.e., dextran sulphate, pentosan polysulphate and heparin) and plant lectins, which inhibit viral adsorption and fusion, were found to be 10- to 40-fold less active against visna virus than against HIV. Bicyclam derivatives were at least 250-fold less active against visna virus and the highly HIV-1 specific TIBO derivatives were without a significant inhibitory effect on visna virus at subtoxic concentrations. In contrast, several 2′,3′-dideoxynucleosides and acyclic nucleoside phosphonate analogues, which inhibit reverse transcription, were found to be very effective inhibitors of visna virus replication and viral CPE in cell culture.

Keywords: Visna virus;Antiviral agent;Anti-HIV agent

1. Introduction

Visna virus (VV) is the prototype of the lentivirus subgroup of retroviruses and is related to the human immunodeficiency virus (HIV) (Gonda et al. 1985; Sonigo et al. 1985). It causes inflammation of the central nervous system (CNS) of sheep which leads to visna, a slowly progressing neurological disease, several months or years after

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infection (Sigurdsson et al., 1960). There are important similarities between visna virus infection and the brain infection frequently caused by HIV. Most important, in the CNS, both VV and HIV replicate predominantly in macrophages, and perivascular inflammatory lesions and nodules of microglial cells are prominent, particularly in the white matter of the brain (Johnson et al., 1988). In both infections, microglia are assumed to play a role in the pathogenesis of the CNS disease, although infection of other cell types is also possible.

In sharp contrast with the multitude of studies done on HIV, few studies have focused on the effects of antiviral agents on VV infection in cell culture. However, in view of the similarities between VV and HIV, particularly with regard to the infections that they cause in the CNS, it is of interest to determine whether anti-HIV agents also have an anti-VV effect. If similar effects are seen in vitro, it may be feasible to use the visna model in sheep for in vivo evaluation of potential anti-HIV drugs, particularly with respect to their effect on the CNS infection. Also, such investigation may help to characterize the molecular relationship between VV and HIV. In a previous paper (Thormar et al., 1993) we reported the anti-VV activities of various dideoxynucleosides and acyclic nucleoside phosphonate analogues. We have now extended these studies and have evaluated a variety of different anti-HIV compounds against VV infection in cell culture in an effort to assess their comparative potency against these two viruses.

2. Materials and methods

2.1. Compounds

The following compounds were used: dextran sulphate, MW 5000 (DS-5000) (Sigma Chemical Co., St. Louis, MO, USA); pentosan polysulphate (Sigma); heparin Org 31731A, (Leo Pharmaceutical Products Ltd, Ballerup, Denmark); suramin (Bayer, Leverkusen, Germany); aurintricarboxylic acid (ATA) (Aldrich Chemical Co., Brussels, Belgium); phosphonoformic acid (PFA), (Sigma); tetrahydroimidazo-[4,5,1-*jk*][1,4]-benzodiazepin-2-(1*H*)-one and -thione (TIBO) compounds (Pauwels et al., 1990); Ro 5-3335 (7-chloro-5-(2-pyrryl)-3*H*-1,4-benzodiazepin-(2*H*)-one) (Lilly Research Laboratories, Indianapolis, IN, USA); several different plant lectins, i.e., *Listeria ovata* agglutinin (LOA), *Galanthus nivalis* agglutinin (GNA), *Narcissus pseudonarcissus* agglutinin (NPA), *Cymbidium* agglutinin (CA), *Urtica dioica* agglutinin (UDA), *Soybean* agglutinin (SBA), *Sambucus nigra* agglutinin II (SNA-II), *Bauhinia purpurea* agglutinin (BPA), *Iris reticulata* agglutinin (IRA), *Maackia amurensis* agglutinin (MAA) (Balzarini et al., 1991b, 1992); bicyclam derivatives (De Clercq et al. 1992, 1994). Acyclic nucleoside phosphonate (ANP) derivatives and 2′,3′-dideoxynucleoside (ddN) analogues were obtained as described previously (Thormar et al., 1993).

2.2. Cells and virus

Visna virus strain K796 (Thormar et al., 1993) was used in all experiments. Monolayer cultures of sheep choroid plexus cells (SCPC) were grown in Eagle's

minimum essential medium (MEM) (Gibco, Paisley, Scotland), with 20% heat-inactivated lamb serum (Colorado Serum Co., Denver, CO, USA) and maintained in MEM with 2% lamb serum as described (Thormar et al., 1993).

2.3. Anti-viral assays and evaluation of cytotoxicity

The effects of the test compounds on VV replication and VV-induced cytopathic effect (CPE) were assayed in SCPC monolayer cultures in 96-well microtitre tissue culture plates (Nunc, Roskilde, Denmark), as described earlier (Thormar et al., 1993). Briefly, 100 μl of serial 5-fold dilutions of a test compound in maintenance medium were added to duplicate wells and the cell layers in each well were infected with 10 μl of VV at a multiplicity of infection of 0.05 $CCID_{50}$ (50% cell culture infective doses) per cell. Duplicate wells without a test compound were inoculated in the same way and served as controls. After incubation at 37°C for 6 days the culture fluids of infected cultures were harvested for determination of virus yield. The monolayers were fixed in ethanol, stained with a 2% Giemsa solution and the CPE evaluated by microscopic examination and counting of virus-induced multinucleated syncytia. The inhibitory effect of a test compound on the CPE was expressed as the concentration which caused a 50% reduction in syncytium formation as compared with control cultures without a test compound added (50% effective concentration (EC_{50})). The cytotoxicity of a test compound was evaluated by light microscopy of fixed and Giemsa stained duplicate SCPC monolayers incubated for 6 days with dilutions of the compound, but without virus. The minimum cytotoxic concentration (MCC) was determined as the lowest concentration of a compound causing changes in cell monolayer morphology visible by microscopic examination.

2.4. Determination of virus yield

End-point titrations were done by inoculation of 10-fold dilutions of fluid into SCPC monolayers in quadruplicate wells (Thormar et al., 1993). The CPE was read at 14 days after inoculation and virus titres ($\log_{10}$ $CCID_{50}$ per ml) were calculated by the method of Reed and Muench (1938).

3. Results

3.1. Polyanionic compounds

The sulphated polysaccharides, DS-5000, pentosan polysulphate and heparin, which are known to inhibit HIV adsorption to host cells (De Clercq, 1993), showed an inhibitory effect on VV CPE with EC_{50} values ranging from 3.6 to 18 μg/ml (Table 1). The activity was markedly less than against HIV-1 in MT-4 cells (Baba et al., 1988a,b). Other polyanionic substances, i.e., suramin and ATA, showed an EC_{50} of 18 μg/ml, but were more cytotoxic than the polysaccharides with an MCC of 200 μg/ml.

3.2. Plant lectins

Four mannose-specific (LOA, GNA, NPA, CA), one $(GlcNAc)_n$-specific (UDA), 4 GalNAc/Gal-specific (SNA-II, BPA, IRA, SBA) and one Neu5Ac (Gal/GalNAc)-specific (MAA) lectins (Balzarini et al., 1992) were tested for their inhibitory effect on VV. All of the mannose- and $(Glc/NAc)_n$-specific lectins showed a significant inhibition of viral CPE with EC_{50} values varying from 2 to 12 $\mu g/ml$ (Table 2). These lectins were not toxic to the cells in concentrations ranging from 20 to 100 $\mu g/ml$ or greater. All of the other lectins were cytotoxic at the lowest concentrations tested (8 $\mu g/ml$), except for SBA which had no antiviral or visible cytotoxic effects at the highest concentration tested, i.e., 100 $\mu g/ml$. These results are similar to those obtained for HIV-1 (Balzarini et al., 1991b, 1992), except that the EC_{50} values of mannose and $(Glc/NAc)_n$-specific lectins were 10- to 30-fold higher for VV than for HIV-1.

3.3. Inhibitors of reverse transcriptase (RT)

Three different types of RT inhibitors were tested, i.e., ddN, ANP analogues and TIBO compounds. The virus yields in SCPC cultures incubated with varying concentrations of ANP and ddN were determined by end-point titration and $\log_{10}$ of virus yields plotted against $\log_{10}$ of compound concentration. Fig. 1 shows the $\log_{10}$ of virus yields as a percentage of the virus titre in untreated control cultures ($10^{7.5}$ $CCID_{50}$ per ml). The reduction in virus yield as a function of compound concentration varied considerably among the compounds. For example, the ANP derivatives (*S*)-HPMPC and (*S*)-PMPA caused a steep reduction in yield with increasing concentrations, i.e., about a 3000-fold reduction for each 10-fold increase in concentration, whereas the other ANP derivatives showed a 100- to 300-fold reduction. 3′-Azido-2′,3′-dideoxythymidine (AZT) showed by far the lowest concentration response with only about a 5-fold reduction in virus yield for a 10-fold increase in drug concentration.

Table 1
Inhibitory effect of polyanionic compounds on visna virus cytopathic effect (CPE) in SCPC cultures and toxic effect on cell monolayers

Compound	EC_{50} [a] ($\mu g/ml$)	MCC [b] ($\mu g/ml$)
Dextran sulphate 5000	3.6 (0.3) [c]	> 200
Pentosan polysulphate	8 (0.2)	> 200
Heparin	18 (0.6)	> 200
Suramin	18 (40)	200
Aurintricarboxylic acid	18 (2)	200

Note: The data represent average values for 3 separate experiments.
[a] EC_{50}: concentration of compound causing a 50% reduction in syncytium formation.
[b] MCC: minimal cytotoxic concentration or the lowest concentration of compound causing morphologically visible changes in cell monolayers.
[c] In parentheses: EC_{50} values for HIV-1 in MT-4 cells (Baba et al., 1988b; De Clercq, 1989).

Table 2
Inhibitory effect of plant lectins on visna virus CPE in SCPC cultures and toxic effect on cell monolayers

Compound [a]	EC_{50} (μg/ml)	Specificity	MCC (μg/ml)
LOA	9 (0.3) [b]	D-Mannose	40
GNA	12 (0.4)	D-Mannose	> 100
NPA	8 (0.6)	D-Mannose	100
CA	2 (0.08)	D-Mannose	20
UDA	9 (0.9)	$(GlcNAc)_n$	20
SBA	> 100 (> 100)	GalNAc/GAL	> 100

Note: The data represent average values for 3 separate experiments.
[a] Abbreviations as in Balzarini et al. (1992).
[b] In parentheses: EC_{50} values for HIV-1 in MT-4 cells (Balzarini et al., 1992).

The compound concentrations which caused a 90% reduction in VV yield (IC_{90} values) were estimated from the graphic plots for the 11 compounds tested (Fig. 1, horizontal line). 2',3'-Dideoxycytidine (ddCyd) had by far the lowest and AZT the highest IC_{90}. Of the ANP derivatives, (*S*)-PMPA, (*S*)-PMPDAP and (*S*)-HPMPC had the highest IC_{90} values, i.e., were least active against VV, whereas the remaining derivatives all showed similar inhibitory activities with IC_{90} values varying from 0.14 to 0.5 μM. The IC_{90}s for the ddN and ANP compounds showed a good correlation with the corresponding EC_{50} values previously reported (Thormar et al., 1993).

Two TIBO derivatives were tested for anti-VV activities. One derivative (R 82150) had an EC_{50} of 62 μM which was slightly lower than the MCC of 140 μM. The other derivative (R 89439) had no inhibitory effect on VV at the minimum cytotoxic concentration (28 μM). PFA, a non-competitive inhibitor of RT, was also tested for its

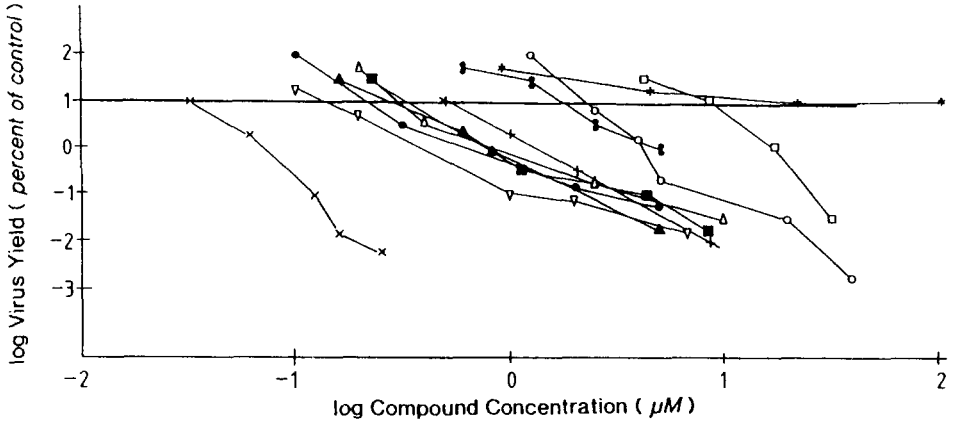

Fig. 1. Concentration–response curves for the inhibitory effects of various ANP and ddN derivatives on visna virus yield. The horizontal line represents 90% reduction in virus yield compared to untreated control cultures (100% yield, i.e., $10^{7.5}$ $CCID_{50}$/ml). △, PMEA; ▲, PMEDAP; □, (*S*)-PMPA; ■, (*R*)-PMPA; ●, (*S*)-PMPDAP; +, (*R*)-PMPDAP; ▽, (*S*)-FPMPA; ●, (*S*)-HPMPA; ○, (*S*)-HPMPC; ×, ddCyd; *, AZT. Each point represents the mean of two separate experiments.

Table 3
Inhibitory effect of bicyclams on visna virus CPE and replication in SCPC cultures and toxic effect on cell monolayers

Compound	EC_{50} [a] (μg/ml)	IC_{90} [b] (μg/ml)	MCC (μg/ml)
JM 1498	> 1000	> 1000	1000
JM 1657	40	70	200
JM 2763	60	50	200
JM 2936	> 400	> 400	> 400
JM 2987	200	> 200	> 200

Note: The data represent average values for 2 separate experiments.
[a] EC_{50}: the concentration causing a 50% reduction in syncytium formation.
[b] IC_{90}: the concentration causing a 90% reduction in virus yield.

inhibitory effect on VV CPE. An EC_{50} of about 100 μM was found, whereas the MCC for PFA exceeded 1.6 mM.

3.4. Bicyclams

Five bicyclam derivatives, all of which are very active inhibitors of HIV infection in vitro, were tested against VV. The EC_{50}s for virus CPE and IC_{90}s for virus yield are shown in Table 3. Two of the compounds, JM 1657 and JM 2763, showed an inhibitory effect at concentrations only slightly lower than the MCC. There was good agreement between the inhibition of viral replication and inhibition of syncytium formation.

3.5. Ro 5-3335

An anti-HIV compound of a different type, i.e., the transactivation inhibitor Ro 5-3335 was also tested against VV. It was not effective at subtoxic concentrations.

4. Discussion

The anti-HIV compounds tested in this study could be divided into three groups with respect to their anti-VV activities tested under the standardized experimental conditions. First, sulphated polysaccharides and plant lectins, which are thought to interfere with viral adsorption and fusion (Baba et al., 1990; Balzarini et al., 1992), showed a significant inhibitory effect on visna virus CPE well below the minimum cytotoxic concentration. However, the EC_{50}s for the sulphated polysaccharides, i.e., DS-5000, pentosan polysulphate and heparin were 12- to 40-fold higher for VV than for HIV-1 as reported by Baba et al. (1988a), but similar to or lower than EC_{50} values for a number of other enveloped viruses, i.e., herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2), cytomegalovirus and vesicular stomatitis virus (Baba et al., 1988b, 1990). Similarly, the inhibitory effect of certain plant lectins on VV correlated with their effect on HIV as reported by Balzarini et al. (1991b, 1992). Thus, mannose-specific and

$(GlcNAc)_n$-specific lectins were inhibitory to VV syncytium formation, but only at a 10- to 30-fold higher concentration than required to inhibit the HIV-1 and HIV-2 cytopathic effect. On the other hand, they were more inhibitory to VV than to Moloney murine sarcoma virus (MSV) and HSV-1 (Balzarini et al., 1992). Lectins specific for GalNAc/Gal and Neu5Ac (Gal/GalNAc) were not inhibitory to VV at a subtoxic concentration. This is in accordance with their lack of effect on HIV. However, most of these lectins are active against HSV-1 and HSV-2 at subtoxic concentrations (Balzarini et al., 1992). The variability in the inhibitory activity of sulphated polysaccharides and lectins is not surprising in view of the differences in envelope glycoproteins and cellular receptors of the different viruses. However, lectins with different specificities affected VV and other lentiviruses in a similar way, but differently from HSV.

A second group of anti-HIV compounds showing a very high inhibitory effect on HIV had negligible or no effect on VV. This group comprises the TIBO derivatives which are non-nucleoside RT inhibitors with a high anti-HIV-1 specificity (Debyser et al., 1992). The one derivative (R 82150) which showed a slight inhibitory activity against VV at subtoxic concentration was 2000- to 3000-fold less active against VV than against HIV-1 (Pauwels et al., 1990).

The bicyclams which are highly potent inhibitors of HIV-1 and HIV-2 (De Clercq et al., 1992) were also in this group. The bicyclam derivative JM 2987 which has the highest anti-HIV activity had no effect on VV. The two bicyclam derivatives (JM 1657 and JM 2763) which showed anti-VV activities slightly below cytotoxic concentrations were 250- to 500-fold less inhibitory against VV than against HIV-1. Capsid proteins involved in the uncoating of HIV in host cells are thought to be the target for the anti-HIV action of bicyclams (De Clercq et al., 1992, 1994). Differences in the composition of the capsid proteins of HIV and VV may be the reason for the lack of anti-VV effect of these compounds.

Similarly, the lack of anti-VV effect of the anti-HIV compound Ro 5-3335, which inhibits the function of the tat protein of HIV-1 (Hsu et al., 1991; Witvrouw et al., 1992) is possibly due to differences in the tat proteins involved in the replication of VV and HIV. It has been reported that ovine lentivirus tat genes show little homology to the tat gene of HIV (Querat et al., 1990).

A third group of compounds showed comparable anti-HIV and anti-VV activities, with the inhibitory effect on VV in some cases even exceeding that on HIV under the experimental conditions (Thormar et al., 1993). The ddN and ANP analogues are in this group. They are broad-spectrum anti-retroviral agents which in their triphosphorylated form act as chain terminators of the RT reaction (Balzarini et al., 1991a; De Clercq, 1993). Of the ddN analogues, ddCyd was the most active inhibitor of VV replication. AZT, on the other hand, was the least active, probably because of an inability of SCP cells to efficiently phosphorylate the compound (Thormar et al., 1993). The anti-VV activity of the ANP analogues showed a good correlation with their anti-HIV activities (Balzarini and De Clercq, in press; Balzarini et al., 1993). The prototype ANP compound PMEA (9-(2-phosphonomethoxyethyl)adenine) has recently been tested in vivo in VV-infected lambs, where it showed a marked inhibitory effect on virus replication and inflammatory lesions in the brain (Thormar et al., in press).

Apparently, the mechanism of action of ATA and suramin on HIV is inhibition of

virus adsorption (De Clercq, 1989). Both compounds showed a significant anti-VV activity, although ATA was somewhat less active against VV than against HIV-1. PFA was also inhibitory to VV with an EC_{50} comparable to that found by Sundquist and Larner (1977). It was, however, much less inhibitory to VV than were the ANP analogues, which is in agreement with the inhibitory effect of these compounds on HIV-1 (De Clercq, 1991).

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References

Baba, M., Nakajima, M., Schols, D., Pauwels, R., Balzarini, J. and De Clercq, E. (1988a) Pentosan polysulfate, a sulfated oligosaccharide, is a potent and selective anti-HIV agent in vitro. Antiviral Res. 9, 335–343.

Baba, M., Snoeck, R., Pauwels, R. and De Clercq, E. (1988b) Sulfated polysaccharides are potent and selective inhibitors of various enveloped viruses, including herpes simplex virus, cytomegalovirus, vesicular stomatitits virus and human immunodeficiency virus. Antimicrob. Agents Chemother. 32, 1742–1745.

Baba, M., De Clercq, E., Schols, D., Pauwels, R., Snoeck, R., van Boeckel, C., van Dedem, G., Kraaijeveld, N., Hobbelen, P., Ottenheijm, H. and Den Hollander, F. (1990) Novel sulfated polysaccharides: dissociation of anti-human immunodeficiency virus activity from antithrombin activity. J. Infect. Dis. 161, 208–213.

Balzarini, J. and De Clercq, E. (1993) Acyclic purine nucleoside phosphonates as retrovirus inhibitors. In. J. Jeffries and E. De Clercq (Eds.) Antiviral Chemotherapy, John Wiley and Sons, Chichester.

Balzarini, J., Hao, Z., Herdewijn, P., Johns, D.G. and De Clercq, E. (1991a) Intracellular metabolism and mechanism of anti-retrovirus action of 9-(2-phosphonylmethoxyethyl) adenine, a potent anti-human immunodeficiency virus compound. Proc. Natl. Acad. Sci. U.S.A. 88, 1499–1503.

Balzarini, J., Schols, D., Neyts, J., van Damme, E., Peumans, W. and De Clercq, E. (1991b) α-(1–3)- and α-(1–6)-D-mannose-specific plant lectins are markedly inhibitory to human immunodeficiency virus and cytomegalovirus infections in vitro. Antimicrob. Agents Chemother. 35, 410–416.

Balzarini, J., Neyts, J., Schols, D., Hosoya, M., van Damme, E., Peumans, W. and De Clercq, E. (1992) The mannose-specific plant lectins from Cymbidium hybrid and Epipactis helleborine and the (N-acetylglucosamine)$_n$-specific plant lectin from Urtica dioica are potent and selective inhibitors of human immunodeficiency virus and cytomegalovirus replication in vitro. Antiviral Res. 18, 191–207.

Balzarini, J., Holy, A., Jindrich, J., Naesens, L., Snoeck, R., Schols, D. and De Clercq, E. (1993) Differential antiherpesvirus and antiretrovirus effects of the (S) and (R) enantiomers of acyclic nucleoside phosphonates: potent and selective in vitro and in vivo antiretrovirus activities of (R)-9-(2-phosphonomethoxypropyl)-2,6-diaminopurine. Antimicrob. Agents Chemother. 37, 332–338.

Debyser, Z., Pauwels, R., Andries, K. and De Clercq, E. (1992) Specific HIV-1 reverse transcriptase inhibitors. J. Enzyme Inhibition 6, 47–53.

De Clercq, E. (1989) New acquisitions in the development of anti-HIV agents. Antiviral Res. 12, 1–20.

De Clercq, E. (1991) Broad-spectrum anti-DNA virus and anti-retrovirus activity of phosphonylmethoxyalkylpurines and -pyrimidines. Biochem. Pharmacol. 42, 963–972.

De Clercq, E. (1993) Antiviral agents: characteristic activity spectrum depending on the molecular target with which they interact. Adv. Virus Res. 42, 1–55.

De Clercq, E., Yamamoto, N., Pauwels, R., Baba, M., Schols, D., Nakashima, H., Balzarini, J., Debyser, Z., Murrer, B.A., Schwarts, D., Thornton, D., Bridger, G., Fricker, S., Henson, G., Abrams, M. and Picker, D. (1992) Potent and selective inhibition of human immunodeficiency virus (HIV)-1 and HIV-2 replication by a class of bicyclams interacting with a viral uncoating event. Proc. Natl. Acad. Sci. U.S.A. 89, 5286–5290.

De Clercq, E., Yamamoto, N., Pauwels, R., Balzarini, J., Witvrouw, M., De Vreese, K., Debyser, Z., Rosenwirth, B., Peichl, P., Datema, R., Thornton, D., Skerlj, R., Gaul, F., Padmanabhan, S., Bridger, G., Henson, G. and Abrams, M. (1994) Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative JM3100. Antimicrob. Agents Chemother. 38, 668–674.

Gonda, M.A., Wong-Staal, F., Gallo, R.C., Clements, J.E., Narayan, O. and Gilden, R.V. (1985) Sequence homology and morphologic similarity of HTLV-III and visna virus, a pathogenic lentivirus. Science 227, 173–177.

Hsu, M.-C., Schutt, A.D., Holly, M., Slice, L.W., Sherman, M.I., Richman, D.D., Potash, M.J. and Volsky, D.J. (1991) Inhibition of HIV replication in acute and chronic infections in vitro by a Tat antagonist. Science 254, 1799–1802.

Johnson, R.T., McArthur, J.C. and Narayan, O. (1988) The neurobiology of human immunodeficiency virus infections. FASEB J. 2, 2970–2981.

Pauwels, R., Andries, K., Desmyter, J., Schols, D., Kukla, M.J., Breslin, H.J., Raeymaeckers, A., van Gelder, J., Woestenborghs, R., Heykants, J., Schellekens, K., Janssen, M.A.C., De Clercq, E. and Janssen, P.A.J. (1990) Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivatives. Nature 343, 470–474.

Querat, G., Audoly, G., Sonigo, P. and Vigne, R. (1990) Nucleotide sequence analysis of SA-OMVV, a visna-related ovine lentivirus: phylogenetic history of lentiviruses. Virology 175, 434–447.

Reed, L.H. and Muench, H. (1938) A simple method of estimating fifty per cent endpoints. Am. J. Hyg. 27, 493–497.

Sigurdsson, B., Thormar, H. and Pálsson, P.A. (1960) Cultivation of visna virus in tissue cultures. Arch. Ges. Virusforsch. 10, 368–381.

Sonigo, P., Alizon, M., Staskus, K., Klatzmann, D., Cole, S., Danos, O., Retzel, E., Tiollais, P., Haase, A. and Wain-Hobson, S. (1985) Nucleotide sequence of the visna lentivirus: relationship to the AIDS virus. Cell 42, 369–382.

Sundquist, B. and Larner, E. (1977) Phosphonoformate inhibition of visna virus replication. J. Virol. 30, 847–851.

Thormar, H., Balzarini, J., Holy, A., Jindrich, J., Rosenberg, I., Debyser, Z., Desmyter, J. and De Clercq, E. (1993) Inhibition of visna virus replication by 2′,3′-dideoxynucleosides and acyclic nucleoside phosphonate analogs. Antimicrob. Agents Chemother. 37, 2540–2544.

Witvrouw, M., Pauwels, R., Vandamme, A.M., Schols, D., Reymen, D., Yamamoto, N., Desmyter, J. and De Clercq, E. (1992) Cell type-specific anti-human immunodeficiency virus type 1 activity of the transactivation inhibitor RO5-3335. Antimicrob. Agents Chemother. 36, 2628–2633.