ELSEVIER

Antiviral Research 38 (1998) 141–149

Antiviral Research

RD6-2198, a novel betain-type fluoroalkylated oligomer, inhibits the replications of human immunodeficiency virus type 1 and other enveloped viruses

Masatoshi Fujiwara [a,d,*], Noriyuki Ashida [a], Masayuki Okamoto [a], Tadashi Mizuta [a], Teruhiko Ide [b], Yasuaki Hanasaki [b], Kimio Katsuura [b], Hideo Sawada [c], Shiro Shigeta [d], Kenji Konno [a], Tomoyuki Yokota [a], Masanori Baba [e]

[a] *Rational Drug Design Laboratories, 4-1-1 Misato, Matsukawamachi, Fukushima 960-12, Japan*
[b] *Tokyo Research Laboratories, Tosoh Corp., Kanagawa 252, Japan*
[c] *Department of Chemistry, Nara National College of Technology, Yamatokoriyama 639-11, Japan*
[d] *Fukushima Medical College, Fukushima 960-12, Japan*
[e] *Division of Human Retroviruses, Center for Chronic Viral Diseases, Faculty of Medicine, Kagoshima University, Kagoshima 890, Japan*

Received 5 September 1997; accepted 23 March 1998

Abstract

We have examined a novel betain-type fluoroalkylated oligomer, RD6-2198, for its inhibitory effects on the replication of human immunodeficiency virus type 1 (HIV-1) and other enveloped viruses, including herpes simplex virus types 1 and 2 (HSV-1 and HSV-2, respectively) and respiratory syncytial virus (RSV) in cell cultures. We have found that the compound is a potent and selective inhibitor of these viruses. RD6-2198 inhibited the replication of HIV-1_{IIIB} at a concentration of 0.85 μg/ml with a selectivity index greater than 59 in MT-4 cells. Furthermore, its 50% effective concentration (EC_{50}) values for HSV-1, HSV-2 and RSV, were 0.51, 0.94 and 3.0 μg/ml, respectively. We found that the RD6-2198 suppressed the gp120–CD4 interaction (as monitored by an enzyme-linked immunosorbent assay (ELISA) method). RD6-2198 also inhibited the binding of anti-gp120 monoclonal antibody to gp120 expressed on MOLT-4/III_B cells (MOLT-4 cells chronically infected with HIV-1_{IIIB}). However, the compound did not inhibit the interaction of anti-CD4 antibody with CD4. These results suggest that RD6-2198 interacts with the viral envelope glycoprotein and thereby inhibits the viral adsorption process. In addition, RD6-2198 was also found to suppress the proliferation of MOLT-4/III_B cells. When applied topically, RD6-2198 at a concentration of 10 mg/ml completely protected mice from an intravaginal HSV-2 infection.

Keywords: Fluoroalkylated oligomer; HIV-1; HSV-1; HSV-2; RSV; Viral adsorption inhibitor

* Corresponding author. Tel.: +81 24 5673593; fax: +81 24 5675554; e-mail: fuji@rdl.co.jp

0166-3542/98/$19.00
PII S0166-3542(98)00022-9

1. Introduction

Several agents, such as dextran sulfate, heparin, fuchsin acid, aurin tricarboxylic acid, flavane derivatives and ingenol derivatives, have been shown to inhibit the replication of human immunodeficiency virus type 1 (HIV-1) in cell cultures (Balzarini et al., 1986; Baba et al., 1988a,b, 1990; Schols et al., 1989; Mahmood et al., 1993; Witvrouw et al., 1994; Fujiwara et al., 1996; Hashimoto et al., 1996). These compounds act on an early event of the HIV-1 replication cycle. They inhibit the viral adsorption process through interacting with the viral envelope glycoprotein gp120 or blocking the CD4 molecules on the host cell surface. Some of these compounds are inhibitory not only to HIV-1 but also to other enveloped viruses, including herpes simplex virus types 1 and 2 (HSV-1 and HSV-2, respectively), human cytomegalovirus (HCMV), influenza virus type A, and respiratory syncytial virus (RSV) (Schols et al., 1990). However, none of them have been approved for the treatment of AIDS and other viral diseases, mainly due to poor pharmacokinetic profiles. In fact, a clinical trial with dextran sulfate failed to demonstrate its therapeutic efficacy (Abrams et al., 1989).

We have previously described fluoroalkylated oligomers as potent inhibitors of HIV-1 replication (Baba et al., 1994a). Like polyanionic compounds, the fluoroalkylated oligomers appeared to inhibit the viral adsorption process. However, further characterization of this class of compounds has not been carried out yet. We have therefore attempted to synthesize more active derivatives, preferably with different characteristic profiles. In this study, we report the novel betain-type fluoroalkylated oligomer RD6-2198, which is a potent and selective inhibitor of HIV-1 and other enveloped viruses in cell culture. Furthermore, it was found to protect mice from intravaginal infection with HSV-2 at a much lower concentration than dextran sulfate.

2. Materials and methods

2.1. Compounds

The chemical synthesis of the betain-type fluoroalkylated oligomer, RD6-2198 (Fig. 1), has been reported (Sawada et al., 1998). Its average molecular weight is 24000 by gel permeation chromatography calibrated with the standard polystyrenes. Dextran sulfate (molecular weight: 8000) was purchased from Sigma (St. Louis, MO). The test compounds were dissolved in distilled water at an appropriate concentration and stored at −20°C until use.

2.2. Cells

MT-4, MOLT-4, and MOLT-4/III_B (MOLT-4 chronically infected with HIV-1_{IIIB}) were grown and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 100 U/ml penicillin G, and 100 μg/ml streptomycin. Peripheral blood mononuclear cells (PBMCs) were isolated from HIV-1-negative healthy donors by Ficoll–Hypaque gradient centrifugation. PBMCs were stimulated with phytohemagglutinin (2 μg/ml) for 3 days and cultured with RPMI 1640 medium containing 20% FCS and interleukin 2 (50 U/ml). Human embryonic lung fibroblast (MRC-5) cells were grown in Eagle's minimal essential medium (MEM) supplemented with 8% FCS and antibiotics. HeLa cells were maintained in MEM supplemented with 2% FCS, 2 mM L-glutamine, and antibiotics.

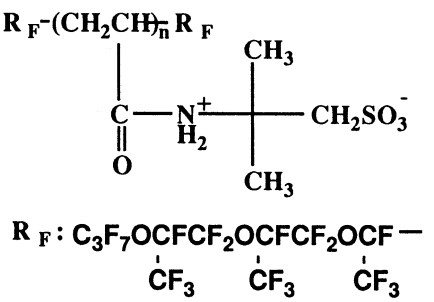

Fig. 1. Chemical structure of the betain-type fluoroalkylated oligomer RD6-2198.

2.3. Viruses

Three strains of HIV-1 (HIV-1_{IIIB}, HIV-$1_{IIIB\text{-}R}$, and HIV-$1_{Ba\text{-}L}$), two strains of HSV-1 (KOS and A4-3), HSV-2 (G), and RSV (Long) were used in the assays. HIV-$1_{IIIB\text{-}R}$, which has a single amino acid change ($Tyr^{181} \rightarrow Cys$) in reverse transcriptase (RT), is a nonnucleoside reverse transcriptase inhibitor (NNRTI)-resistant mutant established by serial passages in cell culture in the presence of escalating concentrations of MKC-442 (Baba et al., 1994b). HIV-1_{Ba-L} is a macrophage tropic HIV-1. HSV-1 (A4-3), provided by Dr. K. Inoue (Osaka University, Osaka, Japan), is an acyclovir-resistant strain derived from the KOS strain in vitro.

2.4. Antiviral assays in vitro

Determination of antiviral activity of the compounds against the replication of HIV-1_{IIIB} and HIV-$1_{IIIB\text{-}R}$ was based on the inhibition of virus-induced cytopathicity in MT-4 cells. Briefly, MT-4 cells were suspended in culture medium at 1×10^5 cells/ml and infected with virus at a multiplicity of infection (MOI) of 0.02. Immediately after virus infection, the cell suspension (100 μl) was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 5-day incubation at 37°C, the number of viable cells was determined by the 3-(4,5-dimethyl-thiazol-2-yl)

-2,5-diphenyltetrazolium bromide (MTT) method (Pauwels et al., 1988). Determination of the activity against the replication of HIV-1 in PBMCs was based on the inhibition of HIV-1 p24 antigen production. In this assay, PBMCs were infected with virus at a MOI of 0.2. After a 2-h viral adsorption, the cells were extensively washed to remove unadsorbed virus particles and incubated at 37°C in the presence of various concentrations of the test compounds. After a 7-day incubation, the amount of p24 antigen in the cell culture supernatants was measured by a p24 antigen-capture enzyme-linked immunosorbent assay (ELISA) Kit (Cellular Products, Buffalo, NY). Antiviral activities were expressed as the 50% effective concentration (EC_{50}).

For HSV-1 and HSV-2, confluent MRC-5 cells in a microtiter tray were inoculated with 100 $CCID_{50}$ (50% cell culture infective dose) of the virus per well in the presence of various concentrations of the test compounds. After a 7-day incubation at 37°C, the number of viable cells was determined by the MTT method, as previously described (Sudo et al., 1994). The antiviral activity of the compounds against RSV replication was determined by the inhibition of virus-induced cytopathicity in HeLa cells. Briefly, HeLa cells were infected with RSV at a multiplicity of infection of 0.01, and incubated in the presence of various concentrations of the test compounds in a round-bottomed microtiter tray. After a 5-day infection at 35°C, the number of viable cells was determined by the MTT method (Watanabe et al., 1994). The antiviral activity is expressed as the EC_{50}.

Cytotoxicity of the compounds was always evaluated in parallel with their antiviral activity. It was based on the viability of mock-infected cells and expressed as the 50% cytotoxic concentration (CC_{50}).

2.5. Binding inhibition assays

The inhibitory effect of the compounds on gp120–CD4 interaction was examined by a gp120 antigen-capture ELISA Kit (Immuno Diagnostic, Buffalo, NY). The test compounds and gp120 (1 ng/well) were added to a microtiter tray coated with CD4, and incubated at room temperature. After a 60-min incubation period, the amount of gp120 bound to CD4 was determined according to the Manufacturer's instructions.

The inhibitory effect of the compounds on the binding of anti-gp120 antibody to gp120 was determined by FACScan (Becton-Dickinson, Mountain View, CA) analysis. Briefly, MOLT-4/III_B cells (1×10^6) were washed twice with phosphate-buffered saline (PBS) and incubated in the presence of various concentrations of the test compounds. After a 60-min incubation at 4°C, the cells were washed with PBS and incubated with anti-gp120 monoclonal antibody. After two washing steps with PBS, the cells were incubated with FITC-conjugated anti-mouse immunoglobulin an-

Table 1
Inhibitory effects of RD6-2198 and dextran sulfate on HIV-1 replication

Compound	Strain	Cell	EC_{50} (μg/ml)	CC_{50} (μg/ml)
RD6-2198	III_B	MT-4	0.85 ± 0.20	>50
	$III_{B\text{-}R}$	MT-4	0.71 ± 0.20	
	III_B	PBMC	7.7 ± 2.0	54.6 ± 0.1
	Ba-L	PBMC	18 ± 4	
Dextran sulfate	III_B	MT-4	3.2 ± 0.3	>100
	$III_{B\text{-}R}$	MT-4	2.5 ± 0.7	
	III_B	PBMC	5.2 ± 0.5	12.3 ± 2.0
	Ba-L	PBMC	>12	

Values are mean $\pm$ S.D. for at least three separate experiments. EC_{50}, 50% effective concentration for HIV-1 replication; CC_{50}, 50% cytotoxic concentration for mock-infected cells.

tibody (rabbit) for 30 min at 4°C. The cells were then washed twice again with PBS, fixed with 1% formamide in PBS, and subjected to FACScan.

The inhibitory effect of the compounds on the binding of anti-CD4 antibody to CD4 was also determined by FACScan analysis. Briefly, MT-4 cells (1×10^6) were incubated with FITC-labeled anti-CD4 monoclonal antibody (Leu-3a) in the absence or presence of the compounds in PBS containing 1% bovine serum albumin and 0.1% sodium azide. After a 60-min incubation at 4°C, the cells were washed twice with PBS, fixed with 1% formamide in PBS, and subjected to FACScan analysis.

2.6. Cell proliferation assays

MOLT-4 and MOLT-4/III_B cells (1×10^4) were cultured in the presence of various concentrations of the test compounds at 37°C. After a 5-day incubation period, the number of viable cells was determined by the MTT method.

2.7. Anti-HSV-2 assays in vivo

Vaginal secretions of 6-week-old female BALB/c mice (CrSlc) were carefully removed with a small cotton swab at 30 min before viral inoculation. Then 10 μl of HSV-2 (strain G) suspension (1×10^5 plaque-forming units) were instilled intravaginally using a pipette with a small plastic tip. Test compounds were dissolved in PBS, and 10 μl of the compound solution was administered intravaginally to mice. The treatment and viral infections were carried out simultaneously. The infected mice were observed for vaginal lesions and death for 21 days. Differences in mortality and mean survival time between the control and the treated groups were evaluated by the chi-square (χ^2) test with Yates' correction, and the Mann–Whitney U-test, respectively. The mice that died on or before day 21 were included in the calculations of the mean survival time.

3. Results

When RD6-2198 was examined for its inhibitory effects on HIV-1 replication in MT-4 cells and PBMCs, it proved inhibitory to the replication of HIV-1_{IIIB} (Table 1). Its EC_{50} values were 0.85 and 7.7 μg/ml in MT-4 cells and PBMCs, respectively. These values are approximately 57- and 7-fold lower than the CC_{50} values. RD6-2198 also inhibited the replication of the NNRTI-resistant mutant HIV-$1_{IIIB\text{-}R}$. When dextran sulfate was examined under the same assay conditions, the compound was found to be less active than RD6-2198. Furthermore, RD6-2198 was also inhibitory to the replication of the macrophage-tropic strain HIV-$1_{Ba\text{-}L}$ in PBMCs, whereas dextran sulfate did not show any selective inhibition of HIV-$1_{B\text{-}L}$ (Table 1).

To determine whether RD6-2198 was also inhibitory to other enveloped viruses, the compounds was examined for its antiviral activity

Table 2
Inhibitory effects of RD6-2198 and dextran sulfate on the replication of enveloped viruses

Compound	Virus (strain)	Cell	EC_{50} (μg/ml)	CC_{50} (μg/ml)
RD6-2198	HSV-1 (KOS)	MRC-5	0.51 ± 0.15	>50
	HSV-1 (A4-3)	MRC-5	0.41 ± 0.10	
	HSV-2 (G)	MRC-5	0.94 ± 0.10	
	RSV (Long)	Hela	3.0 ± 0.3	>50
Dextran sulfate	HSV-1 (KOS)	MRC-5	2.6 ± 0.2	>100
	HSV-1 (A4-3)	MRC-5	3.3 ± 2.0	
	HSV-2 (G)	MRC-5	2.0 ± 0.6	
	RSV (Long)	Hela	20 ± 15	>100

Values are mean $\pm$ S.D. for at least three separate experiments. EC_{50}, 50% effective concentration for viral replication; CC_{50}, 50% cytotoxic concentration for mock-infected cells.

against HSV-1, HSV-2 and RSV. RD6-2198 was found to be highly active against the replication of these viruses (Table 2). Its EC_{50} values were 0.51, 0.94 and 3.0 μg/ml for HSV-1, HSV-2 and RSV, respectively. Furthermore, the compound was equally inhibitory to the acyclovir-resistant HSV-1 mutant A4-3 (Table 2). The antiviral activity of RD6-2198 was stronger than that of dextran sulfate. However, RD6-2198 did not show any inhibition of HCMV or influenza virus type A (data not shown).

In the next experiments, we examined whether RD6-2198 directly inhibited the binding of gp120 to CD4. As shown in Table 3, the compound could block the binding of gp120 to CD4 in a concentration-dependent fashion. At a concentration of 0.8 μg/ml, which is close to the EC_{50} value for HIV-1_{IIIB}, the binding was inhibited by 60%. Similar binding inhibition was achieved with dextran sulfate (Table 3). When RD6-2198 and dextran sulfate were examined for their inhibitory effects on the binding of anti-gp120 monoclonal antibody to gp120 expressed in MOLT-4/III_B cells, both compounds appeared to interfere concentration-dependently with the binding process (Table 4). In contrast, neither RD6-2198 nor dextran sulfate interfered with the binding of anti-CD4

Table 3
Inhibitory effects of RD6-2198 and dextran sulfate on the binding of gp120 with immobilized CD4

Compounds	Concentration (μg/ml)	Inhibition (%)
RD6-2198	100	88 ± 10
	20	76 ± 19
	4	72 ± 27
	0.8	60 ± 18
	0.16	38 ± 3
Dextran sulfate	100	66 ± 8
	20	59 ± 7
	4	67 ± 10
	0.8	52 ± 35
	0.16	29 ± 13

Values are mean $\pm$ S.D. for at least three separate experiments.

Table 4
Inhibitory effects of RD6-2198 and dextran sulfate on the binding of anti-gp120 antibody with gp120 expressed in MOLT-4/III_B cells

Compounds	Concentration (μg/ml)	Inhibition (%)
RD6-2198	100	65 ± 9
	20	66 ± 15
	4	33 ± 23
	0.8	31 ± 1
	0.16	26 ± 22
Dextran sulfate	100	89 ± 15
	20	81 ± 7
	4	54 ± 26
	0.8	38 ± 21
	0.16	28 ± 20

Values are mean $\pm$ S.D. for at least three separate experiments.

Table 5
Inhibitory effects of RD6-2198 and dextran sulfate on the binding of anti-CD4 antibody (Leu-3a) with CD4 expressed in MT-4 cells

Compounds	Concentration (μg/ml)	Inhibition (%)
RD6-2198	100	12 ± 10
	20	3.6 ± 5
	4	8.0 ± 11
	0.8	6.5 ± 13
	0.16	22 ± 17
Dextran sulfate	100	14 ± 22
	20	12 ± 20
	4	14 ± 20
	0.8	15 ± 9
	0.16	2.2 ± 3.0

Values are mean $\pm$ S.D. for at least three separate experiments.

mon oclonal antibody to the CD4 molecule expressed in MT-4 cells' surface (Table 5). These results suggest that RD6-2198 inhibits HIV-1 replication through a mechanism similar to that of dextran sulfate.

When RD6-2198 and dextran sulfate were examined for their effects on the growth and viability of MOLT-4 and MOLT-4/III_B cells, RD6-2198 was found to be more cytotoxic for MOLT-4/III_B cells than for MOLT-4 cells (Fig. 2). The CC_{50} values for MOLT-4 and MOLT-4/III_B cells were >100 and 2.3 μg/ml, respectively. Such an effect could not be seen with dextran sulfate at these concentrations. At a concentration of 100 μg/ml, the viability of MOLT-4 and MOLT-4/III_B cells was 92 and 61%, respectively, indicating that the CC_{50} of dextran sulfate for both cell lines was over 100 μg/ml.

When mice were infected with HSV-2 in the presence of RD6-2198 (10 mg/ml, locally), the development of vaginal lesions and virus-induced death was completely prevented (Table 6). Treatment with 3 mg/ml RD6-2198 increased the mean survival time (8.8 ± 1.3 days) compared with that of the control mice (6.9 ± 0.4 days). In contrast, although dextran sulfate significantly prolonged the survival time of infected mice at a concentration of 100 mg/ml (8.8 ± 0.9 days), it did not achieve a significant increase in survivors at concentrations of 100 or 300 mg/ml (Table 6).

4. Discussion

We have recently described fluoroalkylated oligomers as novel HIV-1 inhibitors (Baba et al., 1994a). Although the anti-HIV-1 activity of the fluoroalkylated oligomers was potent and selective, it was not superior to that of dextran sulfate. Hence, we synthesized novel derivatives of the fluoroalkylated oligomers in an attempt to improve their antiviral activity and spectrum. In this study, we have found one of the betain-type fluoroalkylated oligomers, RD6-2198, to be a highly potent and selective inhibitor of the replication of HIV-1 and other enveloped viruses in cell cultures. RD6-2198 strongly inhibited the replication of not only HIV-1_{IIIB} and its NNRTI-resistant strain HIV-$1_{IIIB\text{-}R}$, but also of macrophage-tropic strain HIV-$1_{Ba\text{-}L}$ (Table 1). This may be a great advantage of RD6-2198 over dextran sulfate, since dextran sulfate has been shown to enhance the replication of macrophage-tropic strains of HIV-1 (Meylan et al., 1994). In fact, we could not detect any antiviral activity of dextran sulfate against HIV-$1_{Ba\text{-}L}$ in PBMCs (Table 1). This may be attributed to the difference of amino sequences in the third variable region (V3) of gp120 between macrophage- and T-cell-tropic strains (De Jong et al., 1992; Jiang, 1997). The V3 domain of T-cell-tropic viruses is positively charged, thereby allowing the negatively charged molecule of dextran sulfate to interact with gp120. In contrast, the V3 domain of macrophage-tropic strains is variable, with less positively charged amino acid residues. We assume that RD6-2198 can interact with the V3 domain of macrophage-tropic HIV-1, because RD6-2198 contains positively and negatively charged groups.

RD6-2198 proved more inhibitory to the proliferation of the chronically HIV-1-infected cell line MOLT-4/III_B, as compared with its uninfected parental line MOLT-4 (Fig. 2). Although the precise mechanism of this selective inhibition remains to be elucidated, we assume that the high viscosity of RD6-2198 solution contributes to a more potent effect on membrane function of MOLT-4/III_B cells (for instance, through a change of osmotic pressure of the cell culture medium). It

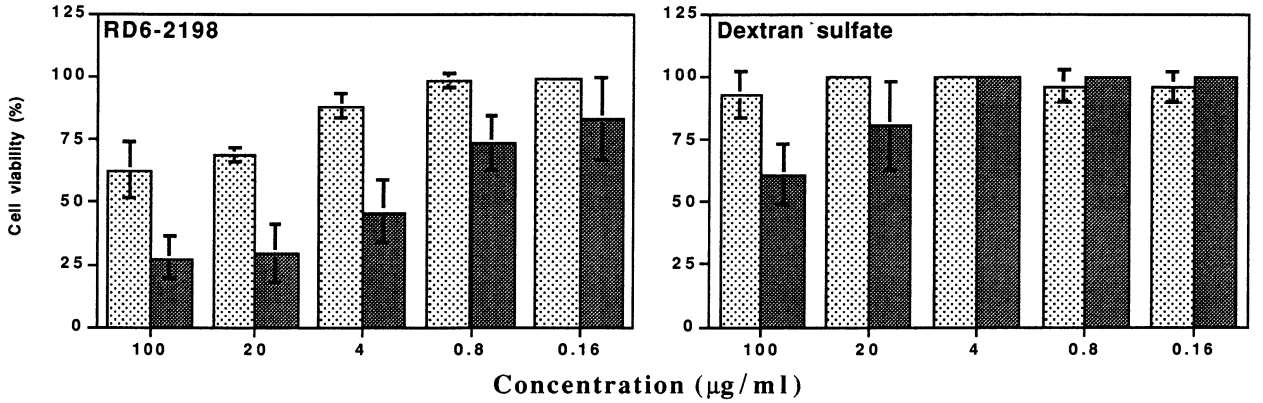

Fig. 2. Inhibitory effects of RD6-2198 and dextran sulfate on the proliferation of MOLT-4 (▩) and MOLT-4/III_B (▩). The cells were grown in the presence of various concentrations of RD6-2198 or dextran sulfate. After a 5-day incubation, the viable cell number was determined by the MTT method. Datarepresent mean values for three separate experiments.

Table 6
Inhibitory effects of RD6-2198 and dextran sulfate on intravaginal HSV-2 infection in mice

Compounds	Concentration (mg/ml)	Survivor/total	Mean survival time (days ± S.E.)
RD6-2198	10	10/10 ($P<0.01$)[a]	
	3	8/10 ($P<0.05$)[a]	8.8 ± 1.3
	1	4/10	7.3 ± 0.5
Dextran sulfate	300	6/10	8.1 ± 1.3
	100	4/10	8.8 ± 0.9 ($P<0.05$)[b]
	30	1/10	6.7 ± 0.2
Control	0	2/10	6.9 ± 0.4

[a] Significantly different from PBS-treated control (χ^2 analysis with Yates' correction).
[b] Significantly different from PBS-treated control (Mann–Whitney *U*-test).

has been postulated that HIV-1-infected cells may play an important role in primary HIV-1 infection (Phillips, 1994; Bomsel, 1997). These authors have demonstrated cell-to-cell transmission of HIV-1 from an infected cell to an uninfected cell. RD6-2198 might be effective against HIV-1 primary infection by preventing this cell-to-cell transmission of HIV-1.

Another interesting observation was that RD6-2198 is a more potent inhibitor of HSV-1, HSV-2 and RSV than dextran sulfate. Neyts and De Clercq (1995) have reported that topical treatment with PAVAS (a copolymer of acrylic acid with vinylalcohol sulfate), but not dextran sulfate, completely prevented intracutaneous and intravaginal infections with HSV-2 in mice. There might be an important indication for the intravaginal use of RD6-2198 as a prophylactic agent for sexually transmitted HIV-1 and/or HSV-2 infections. In our study in vivo, intravaginal RD6-2198 completely prevented vaginitis and death of mice intravaginally infected with HSV-2 at a concentration of 10 mg/ml, whereas dextran sulfate only showed a partial inhibition of HSV-2-induced vaginitis and death, even at a concentration of 300 mg/ml (Table 6). These results are in agreement with the previous report by Neyts and De Clercq (1995), and suggest that RD6-2198 is a more potent inhibitor of intravaginal HIV-1 and HSV-2 infections than dextran sulfate, and equipotent as PAVAS which required 500 mg/ml for complete inhibition. Thus, the betain-type

fluoroalkylated oligomers are worth pursuing as candidate drugs for the prophylaxis and chemotherapy of various viral infections, including HIV-1, HSV-1, HSV-2 and RSV.

Acknowledgements

We thank Ms. E. Sato and Ms. N. Yamaguchi for their excellent technical assistance.

References

Abrams, D.I., Kuno, S., Wong, R., Jeffords, K., Nash, M., Molaghan, J.B., Gorther, R., Ueno, R., 1989. Oral dextran sulfate (UA001) in the treatment of the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Ann. Intern. Med. 110, 183–188.

Baba, M., Schols, D., Pauwels, R., Balzarini, J., De Clercq, E., 1988a. Fuchsin acid selectively inhibits human immunodeficiency virus (HIV) replication in vitro. Biochem. Biophys. Res. Commun. 155, 1404–1411.

Baba, M., Pauwels, R., Balzarini, J., Arnout, J., Desmyter, J., De Clercq, E., 1988b. Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immnodeficiency virus in vitro. Proc. Natl. Acad. Sci. USA 85, 6132–6136.

Baba, M., Schols, D., Pauwels, R., Nakashima, H., De Clercq, E., 1990. Sulfated polysaccharides as potent inhibitors of HIV-induced syncytium formation: a new strategy towards AIDS chemotherapy. J. Acquir. Immun. Defic. Syndr 3, 493–499.

Baba, M., Kira, T., Shigeta, S., Matsumoto, T., Sawada, H., 1994a. Selective inhibition of human immunodeficiency virus type 1 replication by novel fluoroalkylated oligomers in vitro. J. Acquir. Immun. Defic. Syndr. 7, 24–30.

Baba, M., Shigeta, S., Yuasa, S., Takashima, H., Sekiya, K., Ubasawa, M., Tanaka, H., Miyasaka, T., Walker, R.T., De Clercq, E., 1994b. Preclinical evaluation of MKC-442, a highly potent and specific inhibitor of human immunodeficiency virus type 1 in vitro. Antimicrob. Agents Chemother. 38, 688–692.

Balzarini, J., Mitsuya, H., De Clercq, E., Broder, S., 1986. Aurintricarboxylic acid and evans blue represent two different classes of anionic compounds which selectively inhibit the cytopathogenicity of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus. Biochem. Biophys. Res. Commun. 136, 64–71.

Bomsel, M., 1997. Transcytosis of infectious human immunodeficiency virus across a tight human epithelial cell line barrier. Nature Med. 3, 42–47.

De Jong, J.-J., Goudsmit, J., Keulen, W., Klaver, B., Krone, W., Tersmette, M., De Ronde, A., 1992. Human immunodeficiency virus type 1 clones chimeric for the envelope V3 domain differ in syncytium formation and replication capacity. J. Virol. 66, 757–765.

Fujiwara, M., Ijichi, K., Tokuhisa, K., Katsuura, K., Wang, G.-Y.-S., Uemura, D., Shigeta, S., Konno, K., Yokota, T., Baba, M., 1996. Ingenol derivatives are highly potent and selective inhibitors of HIV replication in vitro. Antiviral Chem. Chemother. 7, 230–236.

Hashimoto, K., Kodama, E., Mori, S., Watanabe, J., Baba, M., Okutani, K., Matsuda, M., Shigeta, S., 1996. Antiviral activity of a sulphated polysaccharide extracted from the marine *Pseudomonas* and marine plant *Dinoflagellata* against human immunodeficiency viruses and other enveloped viruses. Antiviral Chem. Chemother. 7, 189–196.

Jiang, S., 1997. HIV-1–co-receptors binding. Nature Med. 3, 367–368.

Mahmood, N., Pizza, C., Aquino, R., De Tommasi, N., Piacente, S., Colman, S., Burke, A., Hay, A.J., 1993. Inhibition of HIV infection by flavanoids. Antiviral Res. 22, 189–199.

Meylan, P.R.A., Kornbluth, R.S., Zbinden, I., Richman, D.D., 1994. Influence of host cell type and V3 loop of the surface glycoprotein on susceptibility of human immunodeficiency virus type 1 to polyanion compounds. Antimicrob. Agents Chemother. 38, 2910–2916.

Neyts, J., De Clercq, E., 1995. Effect of polyanionic compounds on intracutaneous and intravaginal herpesvirus infection in mice: impact on the search for vaginal microbicides with anti-HIV activity. J. Acquir. Immune Defic. Syndr. Hum. Retrovirol 10, 8–12.

Pauwels, R., Balzarini, J., Baba, M., Snoeck, R., Schols, D., Herdewijn, P., Desmyter, J., De Clercq, E., 1988. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. J. Virol. Methods 20, 309–321.

Phillips, D.M., 1994. The role of cell-to-cell transmission in HIV infection. AIDS 8, 719–731.

Sawada, H., Katayama, S., Nakamura, Y., Kawase, T., Hayakawa, Y., Baba, M., 1998. Gelation of fluoroalkylated 2-acrylamido-2-methylpropanesulfonic acid oligomers as potential for prevention of HIV-1 transmission. Polymer, 39, 743–746.

Schols, D., Baba, M., Pauwels, R., Desmyter, J., De Clercq, E., 1989. Specific interaction of aurintricarboxylic acid with the human immunodeficiency virus/CD4 cell receptor. Proc. Natl. Acad. Sci. USA 86, 3322–3326.

Schols, D., De Clercq, E., Balzarini, J., Baba, M., Witvrouw, M., Hosoya, M., Andrei, G., Snoeck, R., Pauwels, R., Nagy, M., Gyorgyi-Edelenyi, J., Machovich, R., Horvath, I., Low, M., Gorog, S., 1990. Sulfated polymers are potent and selective inhibitors of various enveloped viruses, including herpes simplex

virus, cytomegalovirus, vesicular stomatitis virus, respiratory syncytial virus, and toga-, arena-, and retro-viruses. Antiviral Chem. Chemother. 4, 233–240.

Sudo, K., Konno, K., Yokota, T., Shigeta, S., 1994. A sensitive assay system screening antiviral compounds against herpes simplex virus type 1 and type 2. J. Virol. Methods 49, 169–178.

Watanabe, W., Konno, K., Ijichi, K., Inoue, H., Yakota, T., Shigeta, S., 1994. MTT colorimetric assay system for the screening of anti-orthomyxo- and anti-paramyxoviral agents. J. Virol. Methods 48, 257–265.

Witvrouw, M., Este, J.A., Mateu, M.Q., Reyman, D., Andrei, G., Snoeck, R., Ikeda, S., Pauwels, R., Bianchini, N.V., Desmyter, J., De Clercq, E., 1994. Activity of a sulfated polysaccharide extracted from the red seaweed *Aghardhiella tenera* against human immunodeficiency virus and other enveloped viruses. Antiviral Chem. Chemother. 5, 297–303.