ELSEVIER

Antiviral Research 28 (1995) 159–173

Antiviral Research

Potent inhibition of human immunodeficiency virus by MDL 101028, a novel sulphonic acid polymer

D.L. Taylor [a], T.M. Brennan [a], C.G. Bridges [a], M.J. Mullins [b], A.S. Tyms [a], R. Jackson [c], A.D. Cardin [d,*]

[a] *MRC Collaborative Centre, Mill Hill, London, NW7 1AD, UK*
[b] *Dow Chemical Company, Midland, MI, USA*
[c] *Wyeth-Ayerst Research, Princeton, NJ, USA*
[d] *Marion Merrell Dow Research Institute, 2110 East Galbraith Road, P.O. Box 156300, Cincinnati, OH, USA*

Received 16 January 1995; accepted 22 May 1995

Abstract

MDL 101028, a novel biphenyl disulphonic acid urea co-polymer was designed and synthesised as a heparin mimetic. This low molecular weight polymer showed potent inhibition of human immunodeficiency virus type 1 (HIV-1) replication in a number of host-cell/virus systems, including primary clinical isolates of the virus cultured in human peripheral blood mononuclear cells (PBMCs). When compared with the heterogeneous polysulphated molecules, heparin and dextran sulphate, this chemically defined compound showed equivalent antiviral activity with 50% inhibitory concentrations (IC_{50}s) in the range 0.27–3.0 μg/ml in the host-cell/virus systems tested. MDL 101028 also inhibited the replication of HIV type 2 and the simian immunodeficiency virus (SIV), as well as HIV-1 variants resistant to reverse transcriptase inhibitors. Virus growth was blocked when exposure of T-lymphocytes to MDL 101028 was restricted to the virus absorption stage, or even in whole blood conditions. MDL 101028 did not irreversibly inactivate virions, and in contrast to heparin, did not inhibit the attachment of radiolabelled HIV-1 to $CD4^+$ T-cells. MDL 101028 blocked HIV-induced cell-to-cell fusion and this activity appears to explain the mechanism of its antiviral action. The antiviral evaluation of discrete oligomer molecules of MDL 101028 showed that a polymer chain length of six repeating units had optimal potency. The lack of anticoagulant properties and significant antiviral activity in whole blood may allow the development of MDL 101028 as a treatment of HIV infections.

Keywords: Human immunodeficiency virus (HIV); Sulfonic acid; Antiviral activity

* Corresponding author. Fax: +1 (513) 948 7345.

SSDI 0166-3542(95)00046-1

1. Introduction

It is well established that polysulphated compounds which include heparin, dextran sulphate, pentosan polysulphate, chondroitin polysulphate, dextrin sulphate, fucoidan, cyclodextrin sulphates and polyacetal polysulphate are potent inhibitors of human immunodeficiency virus type 1 (HIV-1) replication in cell culture (Ito et al., 1987; Baba et al., 1988a; Jurkiewicz et al., 1989; McClure et al., 1991; Moriya et al., 1991; Witvrouw et al., 1992, 1994). The mechanism of the anti-HIV-1 action of this class of compounds, in general, has been ascribed to inhibition of virus adsorption to permissive cells (Baba et al., 1988b, 1988c; Mitsuya et al., 1988; Nakashima et al., 1989; Schols et al., 1989), although detailed information on structure/activity relationships is limited.

The infection of target host-cells by HIV is initiated by a specific high-affinity reaction between the viral envelope glycoprotein, gp120, and a glycoprotein CD4 that is found predominantly on the surface of a sub-population of T-lymphocytes and cells of the monocyte/macrophage lineage (Capon and Ward, 1991). After attachment to the CD4 receptor, the viral envelope glycoprotein undergoes a conformational change (Sattentau and Moore, 1991), permitting HIV-1 to gain entry to its host-cell via a pH-independent fusion mechanism (Stein et al., 1987; McClure et al., 1988). Although the transmembrane glycoprotein, gp41, is known to be important in the fusion process (Freed et al., 1990), the V3 variable domain of the external glycoprotein, gp120, is considered to initiate and/or trigger the critical events required for fusion of the viral envelope with the host-cell membrane (Freed et al., 1991). The V3 domain of gp120 consists of a loop between two cysteine residues (amino acids 296 and 331), connected by a disulphide bond (Starcich et al., 1986). It is postulated that negatively charged polysulphated compounds exert their anti-HIV effects as a result of interaction with this positively charged region of gp120 (Cardin et al., 1989; Schols et al., 1990; Callahan et al., 1991; Batinic and Robey, 1992; Mbemba et al., 1992).

Although numerous polysulphated compounds with anti-HIV activity have been proposed as drug candidates, little success has been achieved to date with respect to the treatment of HIV-infected patients (Abrams et al., 1989; Flexner et al., 1991). Problems associated with this type of compound include the poor oral bioavailability, a lack of specificity and potential anticoagulant activity. With the objective of producing a molecule with greater selectivity for the CD4/gp120 interaction and with little or no anticoagulant activity, a series of low molecular weight negatively charged polymers were synthesised as heparin mimetics (Mullins et al., 1994). The design of these molecules was based on the predicted interaction of a heparin octasaccharide with a

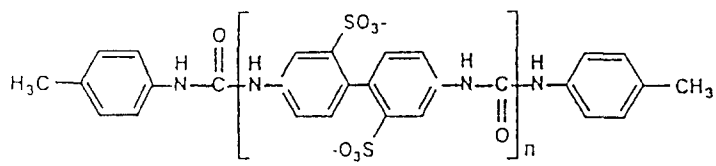

Fig. 1. Structure of MDL 101028, a biphenyl disulphonic acid urea co-polymer ($n = 3–8$).

heparin-binding domain in the V3 region of gp120 (Cardin et al., 1989; Jackson et al., 1991). A number of structures displayed good antiviral activity (data not shown), but MDL 101028, a compound composed of repeating biphenyl disulphonic acid urea moieties (Fig. 1), was selected for more detailed assessment of its anti-HIV activity and mechanism of its action. We show that MDL 101028 is a potent inhibitor of HIV which may have a role in the chemotherapy of the acquired immunodeficiency syndrome (AIDS).

2. Materials and methods

2.1. Compounds

The synthesis of polydisperse MDL 101028 (sodium salt) (average molecular weight 3,500) and the preparation of monodisperse oligomers of specific chain lengths by HPLC fractionation will be described elsewhere (Mullins et al., 1994). Low molecular weight heparin (5000), commercial heparin, 4-methyl-(2*R*)-amino[methoxy(ethoxy-carbamate)]-pentanoyl-glycyl-arginyl-*p*-nitroanilide-hydrochloride (chromogenic Xa-02445), human plasma and Factor Xa were supplied by Celsus Laboratories Inc., (Cincinnati, OH, USA) and dextran sulphate and AZT were from Sigma Chemical Co., (Poole, Dorset, UK).

2.2. Viruses and cells

The T-cell lines MT-4, H9, JM, MT-2 and C8166, the virus strains HIV-1_{RF}, HIV-1_{GB8}, HIV-1_{HXB2}, HIV-1_{MN}, HIV-2_{ROD} and SIV_{mac251} as well as the drug resistant strains of HIV-1_{HXB2} were all obtained from Dr. Harvey Holmes, MRC AIDS Directed Programme Reagent Project, (National Institute for Biological Standards and Control, Potters Bar, UK). Cells were grown in RPMI 1640 supplemented with 10% fetal calf serum, glutamine (290 μg/ml), penicillin (100 U/ml) and streptomycin (50 μg/ml). Human peripheral blood mononuclear cells (PBMCs) separated from buffy coat samples (North London Blood Transfusion Centre, Colindale, London) using Lymphoprep gradient centrifugation were cultured in the same growth medium with the addition of interleukin 2 (10 U/ml) (Boehringer Ingelheim). For the first 3 days of culture, PBMCs were also stimulated with 10 μg/ml phytohaemagglutinin (PHA) (Boehringer Ingelheim). Clinical isolates of HIV-1 were obtained after co-culture of PBMCs separated from blood samples, taken from HIV-infected patients by Dr. Jacqui Parkin, St. Bartholomews Hospital Medical School, London, with uninfected PBMCs.

2.3. Antiviral assays

MDL 101028 was investigated for antiviral activity against a range of laboratory-adapted strains of HIV-1, HIV-2 and SIV in different T-cell lines. The 50% inhibitory

concentrations (IC_{50}s) were determined by measuring either the reduction in p24 viral antigen (Coulter HIV and SIV antigen ELISA assays), syncytium formation (Taylor et al., 1991), or cell viability (Pauwels et al., 1988). For studying the antiviral activity of MDL 101028 against clinical isolates of HIV-1, infected PBMCs were co-cultured with stimulated uninfected PBMCs in a 1 : 10 ratio in the presence or absence of different concentrations of test compound for 7 days and the cell-free culture fluid assayed for levels of p24 viral antigen using a commercial ELISA assay (Coulter Ltd). The phenotype (syncytial inducing (SI) or non-syncytial inducing (NSI)) of the clinical isolates used was determined in parallel with the antiviral assays by co-culturing infected PBMCs with MT-2 cells and observing for the appearance of syncytia, as previously described (Koot et al., 1992).

2.4. Cytotoxicity

JM cells were distributed (5×10^4 cells/well) into wells of 24-well cell culture plates containing 1-ml volumes of test compound at different concentrations, or no compound. Every 2 days, cells obtained from triplicate wells were counted using a Coulter Counter and cell growth curves constructed. In addition, cells were stained with fluorescein diacetate (Cambridge Bioscience) after growth in the absence or presence of MDL 101028, and analysed for fluorescence intensity using a Becton Dickinson FACScan. The effect of MDL 101028 on the ability of PBMCs to proliferate in response to mitogen was studied by distributing cells into 96-well round-bottom plates at a concentration of 2×10^5 per well and stimulating them with concanavalin A (2.5 μg/ml) in the presence or absence of different concentrations of drug for 48 h. The cells were then labelled with [^{3}H]thymidine (1 μCi/well) (Amersham) for 24 h and radiolabel incorporation assessed by cell harvest onto glass fibre paper followed by measurement in a β-spectrometer.

2.5. Antiviral activity of MDL 101028 in whole blood

Citrated whole blood obtained from the North London Blood Transfusion Centre, (Colindale, London), was spiked with C8166 T-cells at a concentration of 2×10^5/ml. A portion of this preparation was immediately separated on a discontinuous density gradient (Lymphoprep, Nycomed, Norway) and 800-μl volumes of the resulting washed mononuclear cell fraction in RPMI containing 10% fetal calf serum were dispensed into 24-well culture plates at cell concentrations equivalent to the original blood volume. This represented the tissue culture (TC) preparation. The whole blood (WB) preparation was dispensed in the same way. Varying concentrations of the test compounds were added in duplicate to both preparations and the resulting mixtures were immediately infected with 50 μl of HIV-1_{RF} (10^7 $TCID_{50}$/ml). After 24 h incubation at 37°C, the WB preparations were separated on Lymphoprep, washed and resuspended in RPMI growth medium at a concentration equivalent to 5×10^5 C8166 cells/ml. This preparation was dispensed (200 μl/well) into 'U'-bottom 96-well plates and incubated at 37°C

for 4 days. The cells were then observed for syncytium formation and the supernatant fluid assayed for levels of p24. The TC mixtures of PBMC and C8166 cells which were placed in cell culture conditions before infection were washed and subsequently treated in the same way as the preparations derived from whole blood.

2.6. HIV-induced cell fusion assay

H9 cells chronically infected with HIV-1_{RF}, HIV-1_{HXB2}, HIV-1_{MN}, the clinical isolates HIV-1_{CC1} and HIV-1_{CC5} or HIV-2_{ROD} were mixed with uninfected C8166 cells in a 1 : 2 ratio, in duplicate 24-well cell culture plates, in the presence or absence of different concentrations of test compound and incubated at 37°C. After approximately 4 h, cells in the untreated control cultures began to fuse and the degree of syncytium formation in both treated and untreated cultures was scored subjectively.

2.7. Anticoagulant activity

Prothrombin (PT) and activated partial thromboplastin (APTT) times on human plasma were measured with an Electra 800 automatic coagulation timer (Medical Laboratory Automation, Inc., Pleovatville, New York, USA) using the standard programs and reagents recommended by the manufacturer. The anticoagulant activity of MDL 101028 was measured with sheep plasma as described in the US Pharmacopeia (US Pharmacopeial Convention, Inc., Rockville, MD) and expressed in USP units (U/mg). The activity was calculated from a standard curve using the USP K2 heparin standard. An ACL 300 Coagulation Systems (Instrumentation Laboratories, Lexington, MA) was used to determine anti-factor Xa activities. The anti-factor Xa activities were determined by amidolytic assay with normal human plasma as a source of antithrombin III, bovine factor Xa and chromagenic Xa substrate. Activities were expressed as IU/mg calculated from a standard curve prepared with the USP K2 heparin.

2.8. Virus infectivity assay

The supernatant fluid obtained from cultures of H9 cells chronically infected with HIV-1_{RF} was incubated for 3 h at 37°C in the presence of MDL 101028 (100 μg/ml), heparin (100 μg/ml) or left untreated. The culture fluid was then subjected to ultracentrifugation to pellet the virions which were washed once in RPMI, prior to titration in C8166 cells. The infectivity titre was determined by cytopathic endpoint.

2.9. Virus attachment

MT-4 cells (2×10^6) were simultaneously exposed to ^{14}C-labelled HIV-1_{RF} and different concentrations of test compounds, in a 200-μl volume of HBSS, for 1 h at room temperature. The cells were then washed 3 times in HBSS and levels of cell associated radioactivity determined. Each sample was done in triplicate. Radiolabelled

virus was obtained by culturing H9 cells chronically infected with HIV-1_{RF} in the presence of 5 μCi/ml [^{14}C]amino acids (Amersham) for 3 days and the cell-free culture fluid subjected to ultracentrifugation to precipitate the virions. The virus pellets were washed once in RPMI to remove unincorporated radiolabel. The anti-CD4 monoclonal antibodies OKT-4 and OKT-4A (Ortho Diagnostics) were used as controls.

3. Results

3.1. Antiviral activity of MDL 101028

MDL 101028 showed potent inhibitory activity against the growth of laboratory-adapted strains of HIV-1, HIV-2 and SIV in the T-cell lines C8166, JM and MT-4, with IC_{50} values in the range 0.27–3.0 μg/ml (Table 1). In these assays, the low molecular weight biphenyl disulphonic acid polymer had greater antiviral activity than heparin but equivalent potency to high molecular weight (500,000) dextran sulphate. MDL 101028 also prevented the growth of both syncytial-inducing (SI) and non-syncytial-inducing (NSI) clinical isolates of HIV-1 in cultures of PBMCs (Table 2), as well as variants of the genomic clone HIV-1_{HXB2}, which had been manipulated by site directed mutagenesis to have resistance to the reverse transcriptase inhibitors, AZT, ddI, 3TC and nevirapine (Table 3).

Potent antiviral activity was observed with MDL 101028 when treatment was restricted to the period of virus adsorption. For these experiments, JM cells were infected with HIV-1_{GB8} in the presence of different concentrations of MDL 101028, but then washed and cultured either in the absence or continued presence of compound. The IC_{50} value for MDL 101028 was 0.38 μg/ml when present only during virus adsorption, and 0.17 μg/ml when present both at adsorption and postinfection. Infection was measured by p24 antigen production. This finding indicates, consistent with other polyanionic molecules, that MDL 101028 antagonised an early stage in the virus replication cycle.

Table 1
Anti-HIV activity of MDL 101028 with various T-cell lines

Compound	50% Inhibitory concentration [a]					
	JM/HIV-1_{GB8}		C8166/ HIV-1_{RF} p24	MT-4/ HIV-2_{ROD} Cell viability	MT-4/ HIV-1_{RF} Cell viability	C8166/ SIV_{mac251} p24
	p24	Syncytium				
MDL 101028	0.27	0.3	1.8	3.0	1.9	0.72
Heparin	0.42	0.44	16	58	10.0	19.0
DS (500,000) [b]	0.92	0.85	3.2	3.4	1.5	3.2
AZT	> 100	> 100	0.06	0.022	0.055	0.18

[a] IC_{50} is in μg/ml for polysulphated compounds and μM for AZT.
[b] DS (500,000) is dextran sulphate of molecular weight 500,000.

Table 2
Activity of MDL 101028 against clinical isolates of HIV-1 in peripheral blood mononuclear cells

HIV-1 clinical isolate	IC50		Cell culture adaptation (weeks)	Phenotype [a]
	MDL 101028 [b] (μg/ml)	AZT (nM)		
HIV-1_{CC1}	1.6	130	0	SI
HIV-1_{CC5}	1.5	8	0	SI
HIV-1_{CC6}	1.5	30	2	n.d.
HIV-1_{CC7}	1.1	10	5	n.d.
HIV-1_{CC8}	0.55	3	3	n.d.
HIV-1_{CC9}	0.3	10	3	n.d.
HIV-1_{CC10}	2.1	10	3	SI
HIV-1_{CC11}	2.2	1	14	NSI

[a] SI, syncytial inducing; NSI, non-syncytial inducing; n.d., not determined.
[b] 1 μg/ml MDL 101028 is approximately equal to 285 nM.

To examine the potential biostability of MDL 101028, the inhibitory effect on virus infection was investigated in whole blood. Aliquots of citrated human blood were spiked with C8166 cells and these cells were targeted for infection with HIV-1_{RF} in the presence or absence of the polymer. PBMCs were subsequently recovered using a density gradient and cultured for p24 production (Fig. 2). In whole blood conditions MDL 101028 was effective, although the IC_{50} value (20 μg/ml) was approximately 10-fold greater than that required in normal culture conditions (3.0 μg/ml). This finding most probably reflects non-specific binding of the polymer to blood proteins. In the absence of compound, the efficiency of virus infection was also reduced, as indicated by reduced levels of p24.

Table 3
Activity of MDL 101028 against drug resistant variants of HIV-1

Compound	Wild-type HIV-1_{HXB2}	50% Inhibitory concentration [a]			
		AZT res [b]	ddI res [c]	3TC res [d]	AZT [e], ddI, nevirapine res
AZT	0.018	0.53 (29) [f]	–	–	0.062 (3)
ddI	2.9	–	16 (5)	–	14 (5)
3TC	0.4	–	–	> 100 (> 250)	–
Nevirapine	0.06	–	–	–	12 (200)
MDL 101028	2.8	3.2 (1.14)	2.7 (0.96)	2.8 (0)	2.8 (0)

Antiviral activity was determined using the MTT cell viability assay.
[a] IC_{50} is in μg/ml for MDL 101028; and in μM for all others.
[b] AZT resistant virus (67N, 70R, 215F, 219Q).
[c] ddI resistant virus (74V).
[d] 3TC resistant virus (184V).
[e] AZT, ddI, nevirapine resistant virus (41L, 74V, 106A, 215Y).
[f] Numbers in parentheses indicate the fold increase in IC_{50} compared with the wild-type strain.

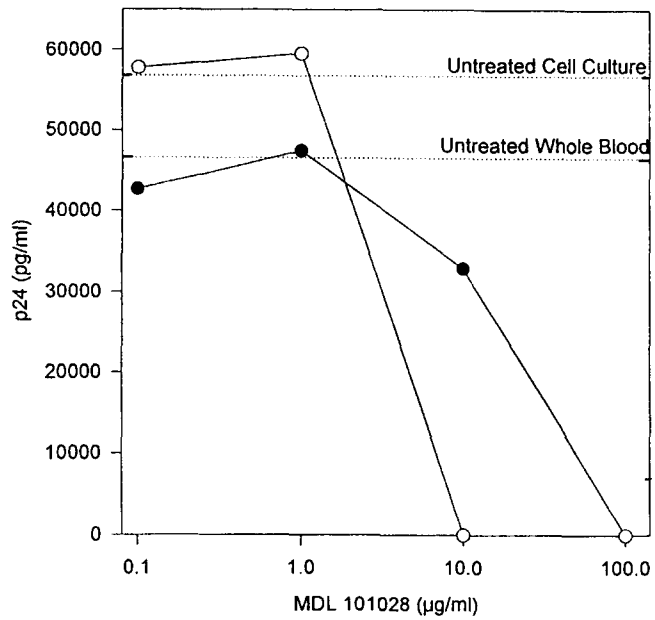

Fig. 2. Effect of MDL 101028 on the ability of HIV-1 to infect cells in whole blood conditions, compared with cell culture. Blood samples were spiked with C8166 cells and infected with HIV-1 in the presence of different concentrations of MDL 101028, in either whole blood conditions (●) or after separation by Lymphoprep gradient centrifugation and resuspension in culture medium (○). After 24 h the T-cells in the whole blood cultures were also separated using Lymphoprep. The cells in both cultures were then washed and incubated in fresh medium for 3 days, prior to determination of levels of p24 antigen in the cell-free supernatant.

3.2. Cytotoxicity and anticoagulant properties of MDL 101028

No loss of cell viability was observed at 100 μg/ml of MDL 101028 in the antiviral assays described above. At this concentration, JM cells grew at the same rate as untreated cells over a 7-day period. At a concentration of 1000 μg/ml, cell growth was significantly restricted (results not shown). Similarly, flow cytometry indicated that JM cells grown in 100 μg/ml MDL 101028 showed no reduction in fluorescence after staining with fluorescein diacetate, but a significant reduction was seen after growth in 1000 μg/ml, indicating a loss of viability. When cytotoxicity was measured by [^{3}H]thymidine uptake by PBMCs in response to the mitogen concanavalin A, the 50% cytotoxic concentration was 104 ± 45 μg/ml.

MDL 101028 showed negligible anticoagulant activity, in contrast to heparin (Table 4). Both the APTT and PT were marginally affected at doses exceeding the IC_{50} value for inhibition of virus growth in whole blood. Under USP conditions, MDL 101028 showed less than 7 U/mg of anti-clotting activity vs 160 U/mg for commercial heparin, and < 10 IU/mg of anti-Factor Xa activity vs 120 IU/mg for low molecular weight heparin and 70 IU/mg for commercial heparin.

Table 4
Anticoagulant properties of MDL 101028

Compound concentration	Clot time (s) [a]			
	PT		APTT	
	Heparin	MDL 101028	Heparin	MDL 101028
None	20	19	94	87
3.3 μg/ml	24	20	161	90
10.0 μg/ml	58	20	NCD	83
16.7 μg/ml	91	21	NCD	91
23.3 μg/ml	NCD	23	NCD	113
30.0 μg/ml	NCD	25	NCD	132

[a] PT, prothrombin; APTT, activated partial thromboplastin; NCD, no clot detected.

3.3. Effect of MDL 101028 on virion infectivity

Virions contained in clarified supernatant medium from H9 cells chronically infected with HIV-1_{RF}, were exposed to MDL 101028 (100 μg/ml), heparin (100 μg/ml) or

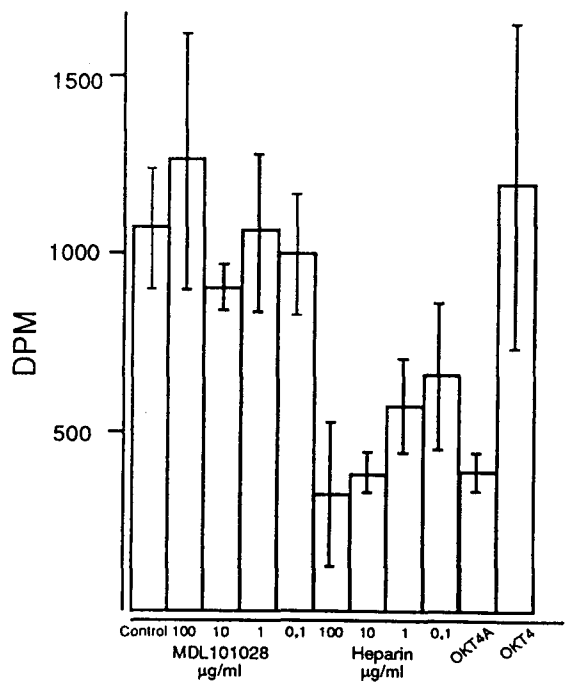

Fig. 3. Attachment of radiolabelled HIV to MT-4 cells in the presence of MDL 101028. MT-4 cells were exposed to ^{14}C-labelled HIV in the presence of different concentrations of MDL 101028 and heparin, OKT-4A (10 μg/ml) OKT-4 (10 μg/ml), or no compound. The cells were then washed and cell-associated radioactivity determined. Each sample was done in triplicate, and the mean DPM has been plotted with the standard deviation shown.

left untreated. Virus was recovered after centrifugation and assayed for infectivity by cytopathic endpoints. Infectivity titres after treatment with either compound were similar to the untreated control (results not shown) and indicated that neither compound bound irreversibly to HIV.

3.4. Virus attachment to $CD4^+$ cells in the presence of MDL 101028

MDL 101028 failed to inhibit the binding of radiolabelled HIV-1_{RF} to MT-4 cells (Fig. 3) while heparin showed a dose-dependent inhibition of virion binding. The anti-CD4 monoclonal antibodies OKT-4A and OKT-4 which react with epitopes in the gp120 binding region and the membrane proximal domain of the CD4 ligand, respectively, were used as controls. As expected OKT-4A, but not OKT-4, inhibited virus attachment. Similar results were obtained when flow cytometry was used to quantify virus binding in the presence of MDL 101028 or heparin (results not shown).

3.5. Inhibition of virus-induced cell fusion by MDL 101028

The effect of MDL 101028 on HIV-induced cell fusion was investigated by co-culturing uninfected C8166 cells with H9 cells chronically infected with 5 different strains of HIV-1 as well as HIV-2_{ROD}. At a concentration of 10 μg/ml or greater, MDL 101028 inhibited both HIV-1 and HIV-2-induced cell fusion, although little inhibitory effect was seen at 1 μg/ml. In contrast, heparin showed differential effects depending on the virus strain (Table 5). This glycosaminoglycan had no inhibitory effect at 100 μg/ml on the fusion of uninfected $CD4^+$ cells to H9 cells infected with either HIV-1_{RF}, HIV-1_{CC5} or HIV-2_{ROD}. In contrast, treatment with heparin inhibited HIV-1_{HXB2}-, HIV-1_{MN}- and HIV-1_{CC1}-induced cell fusion at a concentration of 10 μg/ml, indicating that MDL 101028 and heparin have different mechanisms of action.

Table 5
Inhibition of virus-induced cell-fusion by MDL 101028

Compound	Concentration (μg/ml)	Syncytium formation (C8166 : HIV-infected H9 cells) [a]					
		HIV-1_{HXB2}	HIV-1_{MN}	HIV-1_{RF}	HIV-1_{CC1}	HIV-1_{CC5}	HIV-2_{ROD}
Control	–	+++	+++	+++	+++	+++	+++
MDL 101028	100	0	0	0	0	0	0
	10	0	0	0	0	0	0
	1	+	+++	+++	++	+++	++
	0.1	+++	+++	+++	+++	+++	+++
Heparin	100	0	0	+++	0	++	+++
	10	0	0	+++	0	+++	+++
	1	++	+++	+++	+++	+++	+++
	0.1	+++	+++	+++	+++	+++	+++

[a] 0, no syncytium formation; +, <10%; ++, 10–50%; +++, >50% of cells showing syncytium formation.

Table 6
Anti-HIV activity of polymers of specific chain lengths in JM and MT-4 cells

Compound/chain length	IC_{50} μg/ml (μM)	
	MT-4/HIV-1_{RF}	JM/HIV-1_{GB8}
MDL 100642 ($n = 8$)	1.2 (0.37)	0.22 (0.06)
MDL 101509 ($n = 7$)	1.2 (0.42)	0.15 (0.05)
MDL 100719 ($n = 6$)	1.0 (0.41)	0.25 (0.10)
MDL 100211 ($n = 5$)	3.3 (1.58)	0.35 (0.16)
MDL 102749 ($n = 4$)	> 20 (> 11)	1.9 (1.10)
MDL 101848 ($n = 3$)	> 20 (> 15)	7.8 (5.8)
MDL 101028 (mixture)	2.2	0.18

3.6. Investigation of polymers of specified lengths

MDL 101028 when synthesised is polydisperse and consists of polymers of variable chain lengths. Pure, monodisperse polymers of specified chain lengths (range 3–8 monomeric units) were obtained by HPLC and assayed for anti-HIV-1 activity. In both JM cells infected with HIV-1_{GB8}, scored by syncytium reduction assay, and MT-4 cells infected with HIV-1_{RF}, scored by MTT cell viability assay (Table 6), a polymer of 6 repeat monomeric units was the minimum chain length with optimum anti-HIV-1 activity.

4. Discussion

Inhibition of virus adsorption to $CD4^+$ cells is a possible strategy for controlling the spread of HIV. Numerous agents including rsCD4 (Smith et al., 1987), synthetic peptides (Lifson et al., 1988), and polyanionic molecules, both naturally occurring and synthetically produced (Witvrouw et al., 1994), have been described which exert potent anti-HIV activity in vitro by inhibiting this process. Of these molecules, only dextran sulphate, pentosan polysulphate and rsCD4 have been studied in the clinic and all have shown disappointing results (Abrams et al., 1989; Schooley et al., 1990; Flexner et al., 1991; Peters et al., 1991; Witvrouw et al., 1994).

In this study, we have now shown that an anionic heparin-like molecule MDL 101028, is a potent inhibitor of all strains of HIV-1 investigated with activity also against HIV-2 and SIV. The potent inhibitory effect of MDL 101028 on HIV-induced cell fusion was shown both with SI strains of HIV-1, and with the cell fusion assay system which employs co-culture of chronically infected and non-infected cells. This novel polymer was well tolerated by T-cells with a selectivity index in excess of 100. When investigated for anticoagulant activity, in comparison with heparin, it had negligible effect. Further studies relating to the bioavailability, toxicity and pharmacokinetics of this compound in animals are currently being undertaken to determine the potential role of this molecule in the treatment of patients with HIV infections.

As with other polyanionic compounds, MDL 101028 exerted its antiviral effects by inhibition at some stage during the virus/host-cell interaction, thus preventing the subsequent infection of cells. However, it appears likely that the precise mechanism of antiviral action of this novel polymer may be different from that previously described for heparin and other polysulphated compounds (Baba et al., 1988c). MDL 101028 behaved like heparin in that neither compound negated virion infectivity, but clear differences were evident with the attachment and cell-to-cell fusion experiments. The variability of response seen with heparin (dependent upon HIV strain) to antagonise cell-to-cell fusion, suggests an amino acid sequence dependence of this particular interaction. Previous reports have claimed that polysulphated compounds may exert their antiviral effects as a result of ionic interaction with the positively charged V3 fusion domain on gp120 (Cardin et al., 1989; Callahan et al., 1991; Batinic and Robey, 1992). Preliminary results (not shown) indicate that MDL 101028 can interfere with the binding of a monoclonal antibody to this fusion domain, and interaction with this region would effectively prevent fusion of the viral and cellular membranes, critical for virus entry, whilst not inhibiting virus attachment. The effect of MDL 101028 and heparin on the binding of monoclonal antibodies to alternative epitopes on gp120, as well as to cell surface molecules including CD4, is currently under investigation and will be reported elsewhere.

MDL 101028, one of a series of sulphonic acid polymers, was specifically designed and synthesised as a result of molecular modelling studies. The identification of a heparin-binding motif (Cardin and Weintraub, 1989) on the V3 loop immunodominant region of gp120 (Cardin et al., 1989), led to molecular modelling of a representative gp120 peptide, with a hypothetical octasaccharide of heparin (Jackson et al., 1991). The results indicated that a 30-amino acid peptide of V3 was the minimum binding unit for this heparin molecule. Evidence of a heparin-produced conformational change in a soluble V3 synthetic peptide was seen by circular dichroism studies (Cardin et al., 1989). The chain length dependence for antiviral activity supports the notion that a polymer of $n = 6$ provides the minimal structure with optimal potency, which is consistent with a limited structure of heparin proposed from the modelling studies.

In common with a number of other polyanionic molecules, MDL 101028 also had antiviral activity against the enveloped viruses herpes simplex types 1 and 2 (Ahmed et al., 1994). This broad-spectrum antiviral activity may be important in the chemotherapy of AIDS, where opportunistic herpes infections are a common complication.

Acknowledgements

We would like to thank Jessie Ngyou for help with the preparation of this manuscript.

References

Abrams, D.I., Kuno, S., Wong, R., Jeffords, K., Nash, M., Molaghan, J.B., Gorter, R. and 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.

Ahmed, S.P., Cardin, A.D., Bridges, C.G., Jackson, R.L., Mullins, M.J. and Tyms, A.S. (1994) Potent inhibition of herpes simplex virus by MDL 101028 a novel biphenyl disulphonic acid urea co-polymer. Antiviral Chem. Chemother. 6, 34–42.

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

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

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

Batinic, D. and Robey, F.A. (1992) The V3 region of the envelope glycoprotein of human immunodeficiency virus type 1 binds sulfated polysaccharides and CD4-derived synthetic peptides. J. Biol. Chem. 267, 6664–6671.

Callahan, L.N., Phelan, M., Mallinson, M. and Norcross, M.A. (1991) Dextran sulfate blocks antibody binding to the principal neutralising domain of human immunodeficiency virus type 1 without interfering with gp120–CD4 interactions. J. Virol. 65, 1543–1550.

Capon, D.J. and Ward, R.H. (1991) The CD4–gp120 interaction and AIDS pathogenesis. Annu. Rev. Immunol. 9, 649–678.

Cardin, A.D. and Weintraub, H.J.R. (1989) Molecular modelling of protein–glycosaminoglycan interactions. Arteriosclerosis 9, 21–32.

Cardin, A.D., Taylor, D.L., Krstenansky, J.L., Tyms, A.S. and Jackson, R.L. (1989) A heparin which binds to the envelope glycoprotein gp120 inhibits human immunodeficiency virus replication. Trans. Assoc. Am. Phys. CII, 101–109.

Flexner, C., Barditch-Crovo, P.A., Kornhauser, D.M., Farzadegan, H., Nerhood, L.J., Chaisson, R.E., Bell, K.M., Lorentsen, K.J., Hendrix, C.W., Petty, B.G. and Lietman, P.S. (1991) Pharmacokinetics, toxicity, and activity of intravenous dextran sulfate in human immunodeficiency virus infection. Antimicrob. Agents Chemother. 35, 2544–2550.

Freed, E.O., Myers, D.J. and Risser, R. (1990) Characterisation of the fusion domain of the human immunodeficiency virus type 1 envelope glycoprotein gp41. Proc. Natl. Acad. Sci. USA 87, 4650–4654.

Freed, E.O., Myers, D.J. and Risser, R. (1991) Identification of the principal neutralising determinant of human immunodeficiency virus type 1 as a fusion domain. J. Virol. 65, 190–194.

Ito, M., Baba, M., Sato, A., Pauwels, R., De Clercq, E. and Shigeta, S. (1987) Inhibitory effect of dextran sulphate and heparin on the replication of human immunodeficiency virus type 1 (HIV) in vitro. Antiviral Res. 7, 361–367.

Jackson, R.L., Busch, S.J. and Cardin, A.D. (1991) Glycosaminoglycans: protein interactions and role in physiological processes. Physiol. Rev. 71, 481–539.

Jurkiewicz, E., Panse, P., Jentsch, K.D., Hartman, H. and Hunsmann, G. (1989) In vitro anti-HIV-1 activity of chondroitin polysulphate. AIDS 3, 423–427.

Koot, M., Vos, A.H.V., Keet, R.P.M., de Goede, R.E.Y., Dercksen, M.W., Terpstra, F.G., Coutinho, R.A., Miedema, F. and Tersmette, M. (1992) HIV-1 biological phenotype in long-term infected individuals evaluated with an MT-2 cocultivation assay. AIDS 6, 49–54.

Lifson, J.D., Hwang, K.M., Nara, P.L., Fraser, B., Padgett, M., Dunlop, N.M. and Eiden, L.E. (1988) Synthetic CD4 peptide derivatives that inhibit HIV infection and cytopathicity. Science 241, 712–716.

Mbemba, E., Chams, V., Gluckman, J.-C., Klatzmann, D. and Gattegno, L. (1992) Molecular interactions between HIV-1 major envelope glycoprotein and dextran sulfate. Biochim. Biophys. Acta 1138, 62–67.

McClure, M., Marsh, M. and Weiss, R.A. (1988) Human immunodeficiency virus infection of CD4 bearing cells occurs by a pH-independent mechanism. EMBO J. 7, 513–518.

McClure, M.O., Whitby, D., Patience, C., Gooderham, N.J., Bradshaw, A., Cheingsong Popov, R., Weber, J.N., Davies, D.S., Cook, G.M.W., Keynes, R.J. and Weiss, R.A. (1991) Dextrin sulfate and fucoidan are potent inhibitors of HIV infection in vitro. Antiviral Chem. Chemother. 2, 149–156.

Mitsuya, H., Looney, D.J., Kuno, S., Ueno, R., Wong-Staal, F. and Broder, S. (1988) Dextran sulphate suppression of viruses in the HIV family: inhibition of virion binding to CD4+cells. Science 240, 646–649.

Moriya, T., Kurita, H., Matsumoto, K., Otake, T., Mori, H., Morimoto, M., Ueba, N. and Kunita, N. (1991) Potent inhibitory effect of a series of modified cyclodextrin sulfates (mCDS) on the replication of HIV-1 in vitro. J. Med. Chem. 34, 2301–2304.

Mullins, M.J., Cardin, A.D. and Jackson, R. (1994) Synthesis of novel sulphonic acid polymers which inhibit the replication of HIV-1, in preparation.

Nakashima, H., Yoshida, O., Baba, M., De Clercq, E. and Yamamoto, N. (1989) Anti-HIV activity of dextran sulfate as determined under different experimental conditions. Antiviral Res. 11, 233–246.

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

Peters, M., Witvrouw, M., De Clercq, E. and Ruf, B. (1991) Pharmocokinetics of intravenous pentosan polysulphate (HOE/BAY 946) in HIV-positive patients. AIDS 5, 1534–1535.

Sattentau, Q.J. and Moore, J.P. (1991) Conformational changes induced in the human immunodeficiency virus envelope glycoprotein by soluble CD4 binding. J. Exp. Med. 174, 407–415.

Schols, D., Baba, M., Pauwels, R. and De Clercq, E. (1989) Flow cytometric method to demonstrate whether anti-HIV-1 agents inhibit virion binding to T4+cells. J. Acquired Immune Defic. Syndr. 2, 10–15.

Schols, D., Pauwels, R., Desmyter, J. and De Clercq, E. (1990) Dextran sulfate and other polyanionic anti-HIV compounds specifically interact with the viral gp120 glycoprotein expressed by T cells persistently infected with HIV-1. Virology 175, 556–561.

Schooley, R.T., Merigan, T.C., Gaut, P., Hirsch, M.S., Holodniy, M., Flynn, T., Liu, S., Byington, R.E., Henochowicz, S., Guubish, E., Spriggs, D., Kufe, D., Schindler, J., Dawson, A., Thomas, D., Hanson, D.G., Letwin, B., Liu, T., Gulinello, J., Kennedy, S., Fisher, R. and Ho, D.D. (1990) Recombinant soluble CD4 therapy in patients with the acquired immunodeficiency syndrome (AIDS) and AIDS-related complex. Ann. Intern. Med. 112, 247–253.

Smith, D.H., Byrn, R.A., Marsters, S.A., Gregory, T., Groopman, J.E. and Capon, D.J. (1987) Blocking of HIV-1 infectivity by a soluble, secreted form of the CD4 antigen. Science 238, 1704–1707.

Starcich, B.R., Hahn, B.H., Shaw, G.M., McNeely, R.D., Morrow, S., Wolf, H., Parks, E.S., Parks, W.P., Josephs, S.F. and Gallo, R.C. (1986) Identification and characterisation of conserved and variable regions in the envelope gene of HTLVIII/LAV, the retrovirus of AIDS. Cell 45, 637–648.

Stein, B.S., Gaoda, S.D., Lifson, J.D., Penhallow, R.C., Bensch, K.G. and Engleman, E.G. (1987) pH-Independent HIV entry into CD4-positive T cells via virus envelope fusion to the plasma membrane. Cell 49, 659–668.

Taylor, D.L., Sunkara, P.S., Liu, P.S., Kang, M.S., Bowlin, T.L. and Tyms, A.S. (1991) 6-O-Butanoyl castanospermine (MDL 28,574) inhibits glycoprotein processing and the growth of HIVs. AIDS 5, 693–698.

The United States Pharmacopeia (1985) Heparin/Monographs 21, 482.

Witvrouw, M., Schols, D., Andrei, G., Snoeck, R., Ikeda, S., Pauwels, R., Van Schepdael, A., Arnout, J., Claes, P., Desmyter, J. and De Clercq, E. (1992) New polyacetal polysulphate active against human immunodeficiency virus and other enveloped viruses. Antiviral Chem. Chemother. 2, 171–179.

Witvrouw, M., Desmyter, J. and De Clercq, E. (1994) Polysulphates as inhibitors of HIV and other enveloped viruses. Antiviral Chem. Chemother. 5, 345–359.