Review

Antiviral portrait series: 4. Polysulfates as inhibitors of HIV and other enveloped viruses

M. Witvrouw,* J. Desmyter and E. De Clercq

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

Summary

Polysulfates are highly potent and selective inhibitors of the *in vitro* replication of HIV and other enveloped viruses. They not only inhibit the cytopathic effect of HIV, but also prevent HIV-induced syncytium (giant cell) formation. They also act synergistically with other anti-HIV drugs. The anti-HIV activity of polysulfates is a result of their shielding of the positively charged sites in the V3 loop of the viral envelope glycoprotein gp120. When polysulfates were administered intravenously to rabbits, their half-life was approximately 2 h. Although they are very poorly absorbed following oral administration, they can be made orally bioavailable with the appropriate chemical modifications. Also, polysulfates may lose (much of) their anticoagulant activity upon chemical modification without giving up their anti-HIV activity. Their efficacy in the therapy and/or prophylaxis of retroviral infections remains to be demonstrated both in animal models and in humans.

Introduction

In the early 1960s it was demonstrated that heparin and related polyanionic substances act as herpes simplex virus inhibitors (Nahmias and Kibrick, 1964; Nahmias *et al.*, 1964; Takemoto and Fabisch, 1964; Vaheri, 1964). Shortly after the identification and isolation of the virus responsible for the acquired immune deficiency syndrome (AIDS) (subsequently named the human immunodeficiency virus, HIV), several groups, following a suggestion of De Clercq (1986), demonstrated that heparin and other polysulfates are potent and selective inhibitors of HIV-1 replication in cell culture (Ito *et al.*, 1987, 1991;

Ueno and Kuno, 1987; Baba *et al.*, 1988a,b,c, 1990a; Mitsuya *et al.*, 1988; Mizumoto *et al.*, 1988; Yoshida *et al.*, 1988; Hirabayashi *et al.*, 1989; Jurkiewicz *et al.*, 1989; Sugawara *et al.*, 1989; Tochikura *et al.*, 1989; Anand *et al.*, 1990a,b; Kaneko *et al.*, 1990; Montefiori *et al.*, 1990; Weiler *et al.*, 1990; Schols *et al.*, 1990a, 1991; Von Briesen *et al.*, 1990; Aoki *et al.*, 1991; Bagasra *et al.*, 1988, 1991; Sosa *et al.*, 1991; Handa *et al.*, 1991; Hatanaka *et al.*, 1991; McClure *et al.*, 1991; Moriya *et al.*, 1991, 1993; Otake *et al.*, 1991, 1994; Witvrouw *et al.*, 1991, 1992, 1994; Mizuno *et al.*, 1992; Uryu *et al.*, 1992; Bârză *et al.*, 1993; Beutler *et al.*, 1993; Damonte *et al.*, 1994). Several polysulfates were also found to be active against a wide variety of enveloped viruses (Baba *et al.*, 1988c; Andrei and De Clercq, 1990, 1993; Andrei *et al.*, 1991; Schols *et al.*, 1990a, 1991; Lüscher-Mattli and Glück, 1990; Lüscher-Mattli *et al.*, 1993; Hosoya *et al.*, 1991; Mastromarino *et al.*, 1991a,b; Witvrouw *et al.*, 1991, 1992, 1994; Damonte *et al.*, 1994), such as herpesviruses [herpes simplex virus type 1 (HSV-1), thymidine-kinase-deficient (TK⁻) HSV-1, herpes simplex virus type 2 (HSV-2), cytomegalovirus (CMV)], togaviruses (Sindbis virus, Semliki Forest virus), arenaviruses (Junin virus, Tacaribe virus), rhabdoviruses [vesicular stomatitis virus (VSV)], orthomyxoviruses (influenza A virus) and paramyxoviruses [respiratory syncytial virus (RSV)]. The polysulfates also showed some inhibitory activity against poxviruses [vaccinia virus (VV)] but proved inhibitory to non-enveloped viruses such as picorna-, reo- and adenoviruses and some enveloped myxoviruses (influenza B virus and parainfluenza virus type 3).

All polysulfates reported in this review inhibit the first step in the replicative cycle of HIV-1, that is virus adsorption to CD4⁺ cells. After virus binding, several post-binding events occur in the mechanism of HIV entry, such as fusion and uncoating of the virus core. Heparin fractions specifically interact with the V3 region of gp120 (Cardin *et al.*, 1989; Jackson *et al.*, 1991). Polysulfates inhibit the binding of anti-gp120 mAbs to the V3 loop of gp120 on persistently HIV-1-infected cells (Schols *et al.*, 1990a; Callahan *et al.*, 1991), shielding off the positively charged amino acids at the viral envelope which normally interact with cell-surface heparan sulfate molecules before the more specific interaction with the CD4 receptor. This

general mechanism also explains the broad antiviral activity of polysulfates against enveloped viruses. Variations in the viral envelope glycoprotein region may result in differences in the susceptibility of different enveloped viruses to compounds that interact with their envelope glycoproteins. Polysulfates must fulfil certain structural requirements to be effective against HIV and other enveloped viruses. They prevent syncytium formation between HIV-infected cells and uninfected cells, and act synergistically with other anti-HIV drugs (i.e. AZT). Because of their marked *in vitro* anti-HIV activity, polysulfates have been the subject of pharmacokinetic studies and clinical trials in patients with HIV infection.

In vitro activity

Activity against HIV

Polysulfates are able to block HIV replication in cell culture at concentrations as low as $0.01 \mu\text{g ml}^{-1}$ (Table 3) without toxicity of the host cells at 2.5 mg ml^{-1} (data not shown), achieving *in vitro* selectivity indices of up to 250 000. Sulfated polysaccharides show significant differences in their *in vitro* anti-HIV activities, depending on the HIV virus type. This suggests that marked differences exist in the target molecules of the different virus types with which polysulfates interact. A marked increase in anti-HIV-1 activity was observed (Table 3) with increasing molecular weight (MW), with an optimum at a MW of

10 000, and with increasing degree of sulfation, as demonstrated using various samples of dextran sulfate (DS) prepared from dextran fractions with MW ranging from 1 000 to 500 000 but with identical degrees of sulfation and sulfated cyclodextrins containing one or two sulfate groups per glucose unit, respectively (Figs 1 and 2; Table 1).

A sugar backbone is not strictly needed for the antiviral activity of polysulfates, since sulfated polymers (Fig. 2 and Table 2) based on a polyacetal backbone, such as polyacetal polysulfate (PAPS) (Witvrouw *et al.*, 1992), or simply a carbon-carbon backbone, such as polyvinylalcohol sulfate (PVAS) and its copolymer with acrylic acid (PAVAS) (Baba *et al.*, 1990b; Schols *et al.*, 1990b), have also proved to be highly efficient anti-HIV agents *in vitro* (Table 3), irrespective of their MW or degree of sulfation.

Activity against enveloped viruses other than HIV

A similar structure-activity relationship was found for all other enveloped viruses tested with regard to the MW and degree of sulfation of the sulfated polysaccharides: the antiviral activity increased with increasing MW or degree of sulfation (Tables 3 and 4). These requirements proved less stringent for the sulfated polymers. These compounds were highly efficient against some viruses (i.e. Junin virus, Tacaribe virus, RSV and CMV), irrespective of their MW and degree of sulfation (Tables 3 and 4). While the MW and degree of sulfation certainly play an important role in the antiviral activity of polysulfates, other factors must be involved as well. For instance, PAVAS # 3 is a more potent inhibitor of CMV infection than is DS (MW 5 000), although they have a similar MW and comparable degrees of sulfation.

The importance of the carbohydrate backbone has been previously demonstrated for the plant lectins (Stig Hansen *et al.*, 1991; Balzarini *et al.*, 1992). Only *N*-acetylglucosamine-specific and mannose-specific plant lectins proved active against HIV, HSV and CMV. *N*-acetylgalactosamine-specific plant lectins proved effective only against HSV. These findings are consistent with the nature of the sugars present on HIV, HSV and CMV envelope glycoproteins. The oligosaccharides of HSV and CMV envelope glycoproteins have not been described in the same detail as those of HIV, but HSV is believed to contain additional O-linked oligosaccharides (acetylgalactosamine), known to be present in a localized cluster in gC (the heparin-binding moiety) of HSV. This coincides with the finding that enzymatic digestion of heparan sulfate (containing *N*-acetylglucosamine residues), but not chondroitin sulfate (containing *N*-acetylgalactosamine residues), influences HIV infectivity (Patel *et al.*, 1993) (see section below on 'Mechanism of action'). In addition to MW, degree of sulfation and sugar content, other factors may be involved which are as yet poorly understood. The

Fig. 1. Sulfated polysaccharides: dextran sulfate (DS), pentosan polysulfate (PS) and heparin. Structures are shown as if all hydroxyl groups are substituted by sulfates.

Fig. 2. Circular polysaccharides: α -, β - and γ -cyclodextrin.

Table 1. Average MW, percentage of sulfation, sugar content and configuration of the sugar oligomers (i.e. nature and type of interglycosidic linkage) of the polysaccharides

Compound	Average ($\times 10^{-3}$)	% of sulfation	Sugar content	Configuration
Linear				
<i>Chemically synthesized</i>				
DS	from 1 to 500	81	glucose	α 1,6
PS	3	60	xylose	β 1,4
Heparin	11	ND	uronic acid [iduronic (60%) or glucuronic (40%)] -glucosamine	β 1,4
<i>Extracted from seaweed</i>				
Xylomannan sulfate	ND	25	xylose-mannose	α 1,3-linked mannose (98%) having single stubs of β 1,2-xylose
Galactan sulfate	ND	28	galactose	β 1,3
Circular				
α -cyclodextrin hexasulfate	1.7	40	glucose	α 1,6
β -cyclodextrin heptasulfate	2.1	43	glucose	α 1,6
γ -cyclodextrin octasulfate	2.3	40	glucose	α 1,6
α -cyclodextrin dodecasulfate	2.3	70	glucose	α 1,6
β -cyclodextrin tetradecasulfate	2.5	63	glucose	α 1,6
γ -cyclodextrin hexadecasulfate	3.1	73	glucose	α 1,6

Formulae of (the oligomeric units of) sulfated polysaccharides are depicted in Figs 1 and 2.

ND: not determined

Table 2. Average MW, composition and percentage of sulfation of the polymers

Compound	Average ($\times 10^{-3}$)	% of the acrylic acid residues $100 \times (\frac{k}{k+l+m})^a$	% of sulfation ^b
<i>Carbon-carbon backbone</i>			
PVAS # 1	20	—	50
PVAS # 2	40	—	51
PVAS # 3	40	—	84
PAVAS # 1	5	10	31
PAVAS # 3	5	10	67
PAVAS # 6	20	10	31
PAVAS # 8	20	10	82
<i>Polyacetal backbone</i>			
PAPS	32	—	62

^aThe symbols *k*, *l* and *m* are depicted in Fig. 3.^bCalculated with the following formulae for the PVAS and PAVAS congeners: $100 \times (\frac{m}{k+l+m})^a$.**Table 3.** Inhibitory effects of structurally different groups of polysulfates on the replication of enveloped RNA viruses

Compound	IC ₅₀ ^a (µg ml ⁻¹)									
	HIV-1 (III _B)	HIV-2 (ROD)	Sindbis virus	Semliki Forest virus	Junin virus	Tacaribe virus	VSV	Influenza A virus	RSV	
POLYSACCHARIDE										
Linear										
<i>Chemically synthesized</i>										
Dextran sulfate	(MW 1000)	7.1	0.1	300	> 400	0.2	0.2	70	200	12
	(MW 1500)	2.9	0.1	> 400	> 400			70	200	1.4
	(MW 3400)	0.7	0.3	185	100	0.2	0.4	4.5	42	0.4
	(MW 5000)	0.5	0.3	150	120	0.3	0.4	2	5.8	0.2
	(MW 10 000)	0.2	1.2	15	14	0.2	0.3	0.7	1.4	0.1
	(MW 40 000)	0.4	2.3	2	14	0.04	0.2	0.2	1.0	0.1
	(MW 70 000)	0.6	3.9	14	14	0.1	0.2	0.5	1.4	0.1
	(MW 110 000)	0.6	4.1	2	20			0.7		
	(MW 500 000)	0.6	4.1	7	20			0.7		
Pentosan polysulfate		0.8	0.01	20	> 400	1.6	2.8	4	20	0.8
Heparin		0.8	4.5	200	> 400	1.8	2.0	> 50	> 200	—
<i>Extracted from seaweed</i>										
Xylomannan rich		13.7	13.4	—	—	10	7.8	—	0.2	0.9
Galactan rich		0.6	0.5	11	85	> 100	> 100	14	0.4	0.4
Circular										
α-cyclodextrin hexasulfate		26.2	1.9	> 400	> 400	> 400	> 400	> 400	> 400	> 400
β-cyclodextrin heptasulfate		3.0	1.8	> 400	> 400	> 400	> 400	> 400	> 400	> 400
γ-cyclodextrin octasulfate		0.8	1.6	> 400	> 400	> 400	> 400	> 400	> 400	> 400
α-cyclodextrin dodecasulfate		6.5	0.4	> 400	> 400	> 400	> 400	> 400	> 400	> 400
β-cyclodextrin tetradecasulfate		0.8	1.0	> 400	> 400	> 400	> 400	> 400	> 400	> 400
γ-cyclodextrin hexadecasulfate		0.2	0.4	> 400	> 400	> 400	> 400	> 400	> 400	> 400
POLYMER										
Carbon-carbon backbone										
PVAS # 1		0.3	0.3	2	> 400	0.4	1	0.7	—	—
PVAS # 2		0.3	0.4	1.4	40	10	10	0.6	—	3.6
PVAS # 3		0.4	0.4	2	20	3.2	3.2	0.2	—	1.1
PAVAS # 1		0.3	0.2	70	> 400	> 50	> 50	20	—	27
PAVAS # 3		0.3	0.4	10	> 400	3.7	3.7	0.7	—	3
PAVAS # 6		0.3	0.3	2	> 400	5	3.2	2	—	12
PAVAS # 8		0.3	0.5	20	3.2	2	0.7	> 200	3.6	—
Polyacetal backbone										
PAPS		0.4	0.04	20	> 400	4	4	2	0.8	0.8

^a50% inhibitory concentration, or concentration required to inhibit by 50% the cytopathic effect of HIV-1 or HIV-2 in MT-4 cells, Sindbis virus, Semliki forest virus, Junin virus or Tacaribe virus in Vero cells, VSV or RSV in HeLa cells and influenza A virus in MDCK cells.Data are taken from Baba *et al.* (1988c), Andrei and De Clercq (1990), Schols *et al.* (1990b, 1992), Hosoya *et al.* (1991), Witvrouw *et al.* (1991, 1992, 1994) and Damonte *et al.* (1994).

configuration of the sugar oligomers [i.e. the nature and type of the interglycosidic linkage (α or β , 1-3 or 1-6 binding)] may contribute to the antiviral specificity demonstrated by these compounds.

Inhibition of syncytium formation

After a 24-h period of cocultivation of MOLT-4 (clone 8) cells with an equal number of HUT-78/HIV cells, multinucleated giant cells were induced by the interaction between the gp120 glycoprotein expressed on the surface of cells infected with HIV and the CD4 receptor of uninfected CD4⁺ cells. Syncytium formation may play an important

role in the depletion of T4 lymphocytes in AIDS patients (Haseltine, 1988). All polysulfates that are inhibitory to HIV-1 replication are able to block HIV-1-induced multinucleated giant cell formation; yet, higher concentrations are required for inhibition of syncytium formation than for inhibition of HIV-1 replication (Tables 3 and 5). A similar correlation was found between the MWs of the DS samples and their inhibitory effects on HIV-1-induced syncytium formation to that mentioned above between their MWs and their inhibitory effects on HIV-1-induced cytopathic effect. We noted a marked increase in anti-HIV activity when the MW increased from 1000 to 5000 or 10 000. At higher MW (10 000–500 000), the activity of DS against

Table 4. Inhibitory effects of different structural groups of polysulfates on the replication of enveloped DNA viruses

Compound	IC ₅₀ ^a (µg ml ⁻¹)					
	HSV-1 (KOS)	TK ⁻ HSV-1 (B2006)	HSV-2 (G)	CMV (AD-169)	CMV (Davis)	VV
POLYSACCHARIDE						
Linear						
<i>Chemically synthesized</i>						
Dextran sulfate (MW 1000)	110	100	95	2.5	3	275
(MW 1500)	10	20	7	0.3	0.8	400
(MW 3400)	4.5	2	2	0.7	0.8	35
(MW 5000)	2	2	1.4	0.7	0.7	80
(MW 10 000)	2	1.4	1.4	0.6	0.5	10
(MW 40 000)	2	0.7	0.3	0.3	0.3	4.5
(MW 70 000)	2	0.9	0.6	0.6	0.6	20
(MW 110 000)	1	1.4	0.7	0.1	0.3	20
(MW 500 000)	2	2	0.7	0.1	0.3	40
Pentosan polysulfate	0.7	1.1	0.5	0.4	0.1	20
Heparin	3.6	5	2	11	3	> 50
<i>Extracted from seaweed</i>						
Xylomannan rich	1.6	–	2.5	10	2.8	–
Galactan rich	0.7	0.7	2.5	–	4.0	7
Circular						
α -cyclodextrin hexasulfate	300	150	200	50	15	> 400
β -cyclodextrin heptasulfate	150	20	150	40	7	> 400
γ -cyclodextrin octasulfate	150	70	70	25	20	> 400
α -cyclodextrin dodecasulfate	150	70	70	1.5	7	> 400
β -cyclodextrin tetradecasulfate	70	70	40	2	9	> 400
γ -cyclodextrin hexadecasulfate	70	20	20	1.5	10	> 400
POLYMER						
Carbon-carbon backbone						
PVAS # 1	1.4	1.5	1.4	–	0.1	14
PVAS # 2	0.7	0.6	0.6	–	0.3	12
PVAS # 3	0.9	0.5	0.5	0.08	0.2	9
PAVAS # 1	7	7	7	–	0.03	> 400
PAVAS # 3	0.7	2	1	0.04	0.03	7
PAVAS # 6	4.5	2	11	–	0.08	> 400
PAVAS # 8	0.7	1.3	1.3	0.08	0.07	20
Polyacetal backbone						
PAPS	2	2	7	0.08	0.1	10

^a50% inhibitory concentration, or concentration required to inhibit by 50% the cytopathic effect of HSV-1, TK⁻ HSV-1, HSV-2 or VV in E₆ SM cells and CMV in HEL cells.

Data are taken from Schols *et al.* (1990b, 1992), Witvrouw *et al.* (1991, 1992, 1994) and Damonte *et al.* (1994).

HIV-1 tended to level off (Table 5). For HIV-2-induced syncytium formation, the 1000 MW sample proved more inhibitory than the higher MW samples. Low-MW DS samples differ markedly from higher MW DS samples in their inhibitory effect on HIV-induced syncytium formation: while not inhibiting HIV-1-induced giant cell formation, they inhibit HIV-2-induced giant cell formation at a concentration of approximately $1.8 \mu\text{g ml}^{-1}$, which is at least 15-fold lower than the concentration at which DS (MW 5000) inhibits HIV-2-induced syncytium formation. Yet, low-MW DS are active in inhibiting the cytopathic effect of HIV-1. This means that the MW requirements are more stringent for inhibition of HIV-1-induced syncytium formation than for inhibition of HIV-1-induced cytopathic effect.

The sulfated polymers PVAS, PAVAS and PAPS (Fig. 3) achieve 50% reduction in HIV-1- and HIV-2-induced syncytium formation at a concentration of $1\text{--}2 \mu\text{g ml}^{-1}$ (Table 5), irrespective of their MW or degree of sulfation.

Polysulfates are known to interact with the V3 region of the viral envelope glycoprotein gp120 (Schols *et al.*, 1990a) and probably with gp41 (see section below on 'Mechanism of action'), and this would explain why they inhibit fusion (syncytium formation) of HIV-infected cells with uninfected CD4⁺ cells. The fact that the low-MW DS samples inhibit HIV-2-induced, but not HIV-1-induced, syncytium formation suggests that there exist marked differences in those parts of the HIV-1 and HIV-2 gp120 glycoproteins that are involved in virus-cell fusion.

Table 5. Inhibitory effects of polysulfates on retroviral-induced syncytium formation

Compound	IC ₅₀ ^a ($\mu\text{g ml}^{-1}$)	
	HIV-1 (IIIB)	HIV-2 (ROD)
POLYSACCHARIDE		
Linear		
<i>Chemically synthesized</i>		
Dextran sulfate		
(MW 1000)	500	1.8
(MW 1500)	200	12
(MW 3400)	3.4	11
(MW 5000)	2.2	30
(MW 10 000)	1.2	40
(MW 40 000)	1.2	30
(MW 70 000)	2.0	40
(MW 11 0000)	3.0	40
(MW 50 0000)	3.0	28
Pentosan polysulfate	14	6
Heparin	12.3	—
<i>Extracted from seaweed</i>		
Xylomannan sulfate	200	200
Galactan sulfate	> 5	> 5
Circular		
α -cyclodextrin hexasulfate	> 500	—
β -cyclodextrin heptasulfate	> 500	—
γ -cyclodextrin octasulfate	> 500	1
α -cyclodextrin dodecasulfate	> 500	1
β -cyclodextrin tetradecasulfate	> 500	1
γ -cyclodextrin hexadecasulfate	> 500	1
POLYMER		
Carbon-carbon backbone		
PVAS # 1	2	2
PVAS # 2	2	1
PVAS # 3	2	2
PAVAS # 1	1	1
PAVAS # 3	1	1
PAVAS # 6	2	1
PAVAS # 8	2	2
Polyacetal backbone		
PAPS	1	1

^a50% inhibitory concentration, or concentration required to inhibit by 50% the syncytium formation between HIV-infected HUT-78 cells and uninfected Molt-4 (clone 8) cells.

Data are taken from Schols *et al.* (1990b, 1992), Witvrouw *et al.* (1991, 1992, 1994) and Damonte *et al.* (1994).

Fig. 3. Sulfated polymers: polyvinylalcohol sulfate (PVAS), its copolymer with acrylic acid (PAVAS) and polyacetal polysulfate (PAPS). Percent sulfation is mentioned in Table 2.

In conclusion, most of the polysulfates that have been found to inhibit HIV replication also inhibit syncytium formation, although the structural requirements with respect to molecular mass and number of sulfate groups seem to be more stringent for inhibition of syncytium formation than for inhibition of HIV replication (cytopathic effect) (Tables 3 and 5). For all polysulfates tested, the concentration required to block HIV replication (Table 3) was consistently lower than the concentration required to block syncytium formation (Table 5).

***In vitro* anti-HIV activity of polysulfates in combination with nucleoside analogues**

AZT, ddI and ddC are presently the only drugs approved by the US Food and Drug Administration (FDA) for the treatment of AIDS patients. The significant toxicity associated with administration of these inhibitors (myelosuppression, neuropathy and pancreatitis) and the appearance of drug-resistant strains of HIV (Larder *et al.*, 1989; St. Clair *et al.*, 1991) emphasize the critical need to identify new classes of anti-HIV agents to be used alone or in combination with other antiviral agents. The combination of different antiviral drugs has three possible benefits: (1) it may prevent or delay the emergence of resistant strains; (2) it may lead to increased activity (synergism) and extend the antiviral spectrum to include viruses that would otherwise not be affected by the individual compounds, and (3) it may allow a reduction of the individual dosages and thus decrease the likelihood of toxicity.

In 1987, Ueno and Kuno reported synergism between DS and AZT. This observation was confirmed by Hayashi *et al.* (1990) and Busso and Resnick (1990). Schols *et al.* (1991) showed a sub-synergistic interaction between the pyrimidine nucleoside analogues AZT, ddC, D4T and β -cyclodextrin tetradecasulfate (β -CDS), whereas the purine nucleoside analogues ddA and ddI acted mildly synergistically with β -CDS. The synergistic action may result from the capturing of the nucleoside analogues within the central cavity of the cyclodextrins. The higher synergistic activity achieved by the purine nucleoside analogues may suggest that they are captured more easily than the pyrimidine nucleoside analogues. Anand *et al.* (1990a,b) also reported a strong antiviral synergism with three polyanionic compounds [α -cyclodextrin dodecasulfate (α -CDS), β -CDS, PS] and AZT. Various other drugs, i.e. soluble CD4 (Johnson *et al.*, 1989a), castanospermine (Johnson *et al.*, 1989b), PMEA (Smith *et al.*, 1989), phosphonoformate (foscarnet) (Eriksson and Shinazi, 1989), TIBO derivatives (Buckheit *et al.*, 1993) and α A interferon (Hartshorn *et al.*, 1987), have been previously reported to act synergistically with AZT.

Optimal AIDS therapy may require the use of a combination of agents that exhibit synergistic antiviral effects (e.g. polysulfates and nucleoside analogues). Ideally, such combinations should target different sites in the HIV replicative cycle (e.g. virus attachment and reverse transcriptase), affect viral replication in a broad range of cell types, and yet not display accrued toxicity. Combination therapies may help to prevent the emergence of drug-resistant HIV mutants and may also allow the use of the individual drugs below their toxic concentrations.

Mechanism of action

The mechanism by which the polysulfates inhibit HIV replication can be attributed to the inhibition of the binding of the virions to CD4⁺ cells (Kowalski *et al.*, 1987; Baba *et al.*, 1988b; Mitsuya *et al.*, 1988; Nakashima *et al.*, 1989; Schols *et al.*, 1989) and subsequent syncytium formation (Bagasra *et al.*, 1988; Mitsuya *et al.*, 1988; Baba *et al.*, 1990b). However, conflicting results have been published as to the target molecule for this action. Heparin fractions specifically react with the V3 loop region of gp120, which is characterized as a heparin-binding region. These fractions showed increased antiviral activity (Cardin *et al.*, 1989; Jackson *et al.*, 1991). Polysulfates interact with positively charged amino acids concentrated on the principal neutralizing domain of gp120 (V3) (Callahan *et al.*, 1991; Batinic and Robey, 1992; Mbemba *et al.*, 1992). They interfere with the binding of a specific mAb to the V3 loop of the gp120 of HIV-1 (9284) (Schols *et al.*, 1990a; Witvrouw *et al.*, 1994). This interaction is probably mediated by the binding of negatively charged sulfate groups to positively charged amino acid side-chains in the V3 loop. We envisage that polysulfate binding to gp120 disrupts two (related) processes critical to HIV infection: inhibition of HIV binding to the cell surface, followed by interference with the virus-cell fusion step. The initial virus-binding event depends on the interaction with the CD4 membrane receptor, but in addition has to overcome the electrostatic repulsive forces between the cell membrane and the virion envelope. The early inhibitory effect of polysulfates can be the result of a disruption of ionic interactions between charged regions of viral surface glycoproteins, including gp120, and membrane phospholipids. Enveloped viruses that bud from cells carry part of the cell membrane and therefore may interact more effectively with the negatively charged cell surface if the outer part of the envelope protein contains regions of high positive charge density (Stegmann *et al.*, 1989). The V3 loop in HIV may provide this function, and the binding of polysulfates to the loop not only abolishes positive charge but also adds an additional negative potential, thereby electrostatically preventing the interaction between the virion and the cell. This general mechanism also explains the broad antiviral activity of polysulfates against enveloped viruses.

Enveloped viruses, for example HSV, CMV and pseudorabies virus (PRV), have been shown to interact with cell-surface heparan sulfate as an initial binding site (WuDunn and Spear, 1989; Sawitzky *et al.*, 1990; Lycke *et al.*, 1991; Neyts *et al.*, 1992). They are also inhibited by the polysulfates. By analogy, heparan sulfate has recently been reported to participate in HIV-cell attachment and virus entry (Patel *et al.*, 1993). The importance of the V3 loop in the attachment to heparan sulfate molecules at the

cell surface is consistent with features previously reported for the heparin-binding moieties of, for example, CMV gC-II and PRV gIII (Robbins *et al.*, 1986; Sawitzky *et al.*, 1990; Kari and Gehrz, 1993). The binding of gC-II to immobilized heparin depends on structures maintained by disulfide bonds. Moreover, the PRV gIII glycoprotein also contains an arginine-rich region, as previously documented for the HIV V3 loop. The function of heparan sulfate depends on the sulfation of these molecules, again suggesting that ionic interactions contribute to virus binding to heparan sulfate. A hypothetical model for the mechanism of HIV binding to CD4⁺ cells is shown in Fig. 4. The specific binding of gp120 to CD4 is preceded by an electrostatic interaction between positively charged amino acids in the V3 loop and the heparan sulfate molecules on the surface of CD4⁺ cells. Based on previous data showing a potent inhibitory effect of sulfated polymers on binding of mAb 9284 to the gp120 V3 loop, positively charged amino acids in the V3 domain may bind to cell-surface heparan sulfate. The antiviral activity of heparin [which is chemically very similar to heparan sulfate (Kjellén and Lindahl, 1991)] may be achieved by occupation of the sites on the virion envelope that are necessary for attachment of the virus particles to cell-surface heparan sulfate.

Fig. 4. Mechanism of HIV binding to CD4⁺ cells. (a) The V3 loop of gp120 of HIV-1 contains a high concentration of positively charged amino acids. Cells contain heparan sulfate molecules at their surface. (b) The positively charged amino acids interact with the negative charges of the heparan sulfate molecules before (c) a more specific binding between gp120 and CD4 occurs.

It is likely that molecules that are related to heparin, such as DS, PS, CDS, PVAS and their congeners and PAPS, achieve their antiviral activities in a similar fashion to heparin. This mechanism of anti-HIV action of polysulfates is presented in Fig. 5. The interaction of polysulfates with an accessory binding mechanism (second receptor) for HIV was suggested by Thiele *et al.* in 1989.

The most likely role of the V3 loop is to assist in membrane fusion after gp120 binds to CD4. Once again, the high positive charge density may be essential to this function, presumably bringing two negatively charged membranes together (Stegmann *et al.*, 1989). Specifically, this could be accomplished by binding to a negatively charged region on the cell membrane, thereby orienting the fusogenic portion of gp41 and the viral membrane so that fusion occurs. The V3 region may be physically close to gp41, since mutations in the loop have been reported to lead to gp41-gp120 dissociation (Kowalski *et al.*, 1987). Proteolysis has also been shown to occur within the V3 loop and could be involved in inducing fusion (Hattori *et al.*, 1989). Binding of the polysulfate to the V3 region may neutralize the local positive charge density and thus disturb binding of the V3 region to the CD4 receptor, proteolysis and fusion. On the other hand, antibodies directed against the V3 domain may not inhibit virion binding (Skinner *et al.*, 1988a), because they do not eliminate the positive charge density but still physically block an essential interaction between the V3 loop and either a cell-surface protease or the cell-surface membrane.

Moreover, the fact that the polysulfates are inhibitory to some enveloped viruses and not to others seems to be a result of the composition of the amino acid sequences of the viral envelope glycoproteins that are involved in virus-cell fusion (Hosoya *et al.*, 1991). The viruses sensitive to the polysulfates share a tripeptide segment (Phe-Leu-Gly). This tripeptide segment may be involved either directly

(as a target sequence) or indirectly (*via* its influence on the overall conformation) in the inhibitory effects of these sulfated compounds.

In conclusion, polysulfates are assumed to exert their anti-HIV activity by occupying the positively charged sites of the V3 loop of gp120 that are necessary for attachment of HIV to cell-surface heparan sulfate molecules. It is most likely that sulfated polymers interact with more than one viral epitope. Polysulfates may interact with epitopes of gp41. Since the gp120 V3 loop and gp41 are believed to assist in HIV attachment and fusion to CD4⁺ cells, it is clear that inhibition of these functions by polysulfates should result in the inhibition of virus binding to CD4⁺ cells and syncytium formation between HIV-infected cells and CD4⁺-uninfected cells.

Pharmacokinetic profile

Because of its marked *in vitro* activity against HIV and other enveloped viruses, DS and PS have been subjected to pharmacokinetic studies (Abrams *et al.*, 1989; Lorentsen *et al.*, 1989; Peters *et al.*, 1989, 1991; Hartman *et al.*, 1990; Witvrouw *et al.*, 1990; Flexner *et al.*, 1991; Maffrand *et al.*, 1991). Initial studies were conducted with oral DS (MW 8000) in patients with AIDS and AIDS-related complex (Abrams *et al.*, 1989). The compound appeared to be well tolerated, but did not show much benefit. It was later ascertained that, after oral administration, DS (MW 8000) is very poorly absorbed in humans (Lorentsen *et al.*, 1989), as has also been demonstrated in rats (Flexner *et al.*, 1991).

There are several assay methods to monitor the blood drug levels achieved by sulfated polymers. MacGregor *et al.* (1985) examined the plasma concentration of PS using a competitive binding assay, in which radiolabelled PS competed with plasma samples for binding to polybrene-sepharose (MacGregor *et al.*, 1985). The plasma half-life and peak levels of PS in humans following intravenous (iv) injection of 100 mg PS were 50–58 min and 16–24.5 $\mu\text{g ml}^{-1}$, respectively (MacGregor *et al.*, 1985). Moreover, after an iv dose of 225 mg DS in humans, peak plasma concentrations by competitive binding assay were 26 to 35 $\mu\text{g ml}^{-1}$. DS disappeared from the plasma with a half-life of 84–120 min. Only 25% of the dose was recovered in the urine of healthy volunteers with normal renal function, indicating that most of the drug was cleared by a non-renal mechanism (Lorentsen *et al.*, 1989). This chemical method suffers from the drawback that it may also detect degradation products that are no longer active as anti-HIV agents.

Since polysulfates are primarily known for their anticoagulant activity and are used clinically for this purpose, a bioassay could, in principle, be based on the measure-

Fig. 5. Mechanism of anti-HIV action of polysulfates. The antiviral activity of polysulfates is achieved by shielding the positively charged V3 loop and preventing an initial binding with heparan sulfate molecules.

ment of this anticoagulant activity (Dawes *et al.*, 1986). Using this procedure, the plasma half-life of heparin in humans has been found to be approximately 35 min (after iv injection of 23 mg heparin) (Dawes *et al.*, 1986). However, the anti-HIV activity and anticoagulant effects of sulfated polymers are not necessarily correlated (Baba *et al.*, 1990a; Bâzu *et al.*, 1993).

The ideal bioassay for monitoring the concentration of these compounds in biological specimens should be based on the measurement of anti-HIV activity. We have established a simple and sensitive bioassay system to measure serum concentrations of sulfated polymers (Witvrouw *et al.*, 1990). This assay is based on the inhibitory effect of the serum samples (containing polysulfates) on HIV-2 replication in cell culture. As mentioned above, DS and PS inhibit HIV-2 replication at a markedly lower concentration than HIV-1 replication. In addition, HIV-2 replication should not be influenced by the presence of anti-HIV-1 neutralizing antibodies in the plasma samples from AIDS patients. This method permits the detection in (rabbit) serum samples of DS and PS concentrations as low as 3.0 and 0.5 $\mu\text{g mL}^{-1}$, respectively. The serum concentrations of iv administered DS and PS declined biphasically with an initial half-life of approximately 84 min (Witvrouw *et al.*, 1990).

This anti-HIV bioassay system was used to monitor serum concentrations of PS following iv administration at doses of 600, 300 and 100 mg to six patients with stage III or IV HIV infection. One hour after administration, serum concentrations reached peak values of 113, 54 and 28 $\mu\text{g mL}^{-1}$, respectively. The serum levels of PS decreased rapidly, but the compound remained detectable at 48, 24 and 4 h after injection, respectively. The estimated half-life of PS in humans was approximately 2 h (Peters *et al.*, 1991). This result confirmed a previously reported half-life of PS in humans of 1 h 14 min (Gerard *et al.*, 1990).

When several original polysulfates were administered iv to rabbits at a dose of 0.1 g kg^{-1} , serum peak concentrations ranging from 0.3 mg mL^{-1} for PS to 1.5 mg mL^{-1} for PVAS and PAVAS were reached (Schols *et al.*, 1990b, 1991; Witvrouw *et al.*, 1990, 1991, 1992). DS and PS were injected iv to rabbits at 0.5 g kg^{-1} . Serum peak levels of 2.2–3.0 mg mL^{-1} were reached (Witvrouw *et al.*, 1990). Sulfated polymers disappeared from the serum with half-lives of 20 min for γ -cyclodextrin hexadecasulfate (γ -CDS) and up to 2 h for PVAS and PAVAS (Schols *et al.*, 1990, 1991; Witvrouw *et al.*, 1990, 1991, 1992).

After oral administration of polysulfates via gastric intubation, only the modified β -CDS that had three benzylthio groups as a lipophilic moiety (mCDS11) gave concentrations above the minimum detectable concentration (Otake *et al.*, 1994). The serum drug concentration at 1 h after oral administration of mCDS11 at a dose of 0.25 g kg^{-1} was 0.5 mg mL^{-1} , thus indicating high oral bioavailability.

Its half-life was 4 h (Otake *et al.*, 1994). Similar findings were also obtained following oral administration of mCDS11 to rats (Moriya *et al.*, 1991). Toxicity was not observed after oral administration of mCDS11 to mice at a dose of 3 g kg^{-1} (Moriya *et al.*, 1991) and at doses of 0.5 and 2 g $\text{kg}^{-1} \text{ day}^{-1}$ for 5 days (Otake *et al.*, 1994). Recently, Moriya *et al.* (1993) reported an advanced investigation of modified CDS. A series of C-2 modified CDS were synthesized instead of C-6 modified analogues for the development of a more satisfactory oral anti-HIV agent with high activity. The plasma peak level of the modified β -CDS mCDS71, determined by the anti-HIV bioassay, after oral administration to rats at 1 g kg^{-1} , was 0.32 mg mL^{-1} (Moriya *et al.*, 1993). These compounds should be pursued for their potential as oral drug candidates in the prophylaxis and/or therapy of HIV infections in humans.

In conclusion, sulfated polymers are very poorly absorbed following oral administration. Only a modified β -CDS gave concentrations above the minimum detectable concentration with a half-life of 4 h.

Clinical efficacy

In marked contrast to the wealth of *in vitro* antiviral data is the paucity of antiviral data for polysulfates in animal models. Beneficial effects have only been reported for PS (Biesert *et al.*, 1988), sulfatoverman (Weiler *et al.*, 1990) and Viva Natural, a dietary seaweed extract (Furusawa *et al.*, 1991), in the murine leukemia virus (MLV) system. It is essential to examine whether other polysulfates are as effective as those previously reported in the MLV model and/or whether the remarkable efficacy of these compounds, especially of sulfatoverman, extends to other *in vivo* models. Attempts to demonstrate activity with DS in cats infected with feline leukemia virus (Mathes *et al.*, 1991) and ducklings infected with duck hepatitis B virus (Offensperger *et al.*, 1991) failed. In the cat model, DS proved to be prohibitively toxic, and in the ducklings it was ineffective against the hepatitis B virus infection.

Because polysulfates are potent and selective inhibitors of HIV-1 and HIV-2 *in vitro* (Table 1), DS (MW 8000) and PS have been introduced in the clinic for the treatment of AIDS patients. Serum drug concentrations achieved by these sulfated polymers were highly dependent on the route of administration. Clinical trials of oral DS (Abrams *et al.*, 1989) and PS (Peters *et al.*, 1989) were terminated soon after the drug was shown to be poorly absorbed (bioavailability < 1%) (Lorentsen *et al.*, 1989). No significant changes in surrogate markers (p24) or clinical endpoints (CD4), suggesting anti-HIV activity, were recorded from those trials. Not only are sulfated polyanions poorly

absorbed when given perorally, but their oral administration may also cause intestinal overgrowth of facultative bacilli in the colonic and faecal flora, accompanied by histological alterations in the intestinal mucosa (Wells and Rhame, 1990).

Since DS and PS appear to be poorly absorbed through the gastro-intestinal tract, parenteral administration of these drugs seems essential. DS has been administered as a continuous iv infusion to symptomatic HIV patients, at the maximally tolerated dose (i.e. the highest dose to produce the greatest acceptable increase in activated partial thromboplastin time), thus achieving the greatest possible exposure to drug over time and maximizing any potential effect on the virus (Flexner *et al.*, 1991). Although plasma peak concentrations far exceeded (200-fold) the IC_{50} ($0.5 \mu g ml^{-1}$) at which DS inhibits HIV-1 replication *in vitro*, circulating HIV p24 levels increased in all subjects who received the drug for more than 3 days. Continuous iv infusion of DS proved toxic, producing reversible thrombocytopenia (Flexner *et al.*, 1991).

Recently, Pluda and colleagues from the U.S. National Institutes of Health (NIH) administered PS to 16 HIV-positive patients with Kaposi's sarcoma (KS) in a Phase I clinical trial. Since PS inhibits fibroblast growth factor (FGF), it was the goal to prevent or block the neovascularization thought to play a role in the pathogenesis of KS. Administration of PS as a continuous iv infusion yielded no significant tumour response or anti-HIV activity. However, plasma PS levels of at least $7 \mu g ml^{-1}$ were reached. The IC_{50} at which PS inhibits HIV-1 replication *in vitro* is $0.8 \mu g ml^{-1}$.

A possible explanation for the failure of DS and PS to achieve a response in these studies is that effective levels of these sulfated compounds were lower in tissue than predicted from serum or plasma concentrations, either because of poor penetration or because of extensive binding to plasma proteins. Also, given the MW and strong anionic nature of sulfated compounds, they might be expected to remain confined to the intravascular space, and not attain therapeutic tissue levels.

In conclusion, in several models for retroviral infections there was no evidence for the efficacy of sulfated polyanions. Also, when DS and PS were introduced in clinical trials by continuous iv infusion to symptomatic HIV patients, no beneficial effect on surrogate markers was noted. Continuous iv infusion of DS or PS was toxic. The main dose-limiting side-effects were anticoagulation and thrombocytopenia. Because of their toxicity and lack of beneficial effect on surrogate markers, DS and PS are unlikely to have a practical role in parenteral treatment of symptomatic HIV infection. Nor may other polysulfates have any practical utility unless they have been modified to such an extent that they lose (some of) their undesirable features.

Conclusion

As potential anti-HIV drug candidates, polysulfates offer a number of promising features. The activity spectrum of these polysulfates extends to various enveloped viruses, including viruses (i.e. HSV and CMV) that emerge as opportunistic pathogens in immunosuppressed (e.g. AIDS) patients. They are highly selective inhibitors of HIV replication *in vitro*, achieving selectivity indexes of up to 25000. Their antiviral activity increases with increasing MW and degree of sulfation. Other, yet to be defined, structural features may also play an important role. Polysulfates prevent syncytium formation between HIV-infected cells and uninfected cells. This occurs at a significantly higher concentration than the concentration required to block HIV-induced cytopathicity. Polysulfates act synergistically with other anti-HIV drugs (i.e. AZT). They are not known to lead to virus-drug resistance development and they show activity against HIV mutants that have become resistant to reverse transcriptase inhibitors (i.e. AZT, TIBO and others). The sulfated polymers probably exert their anti-HIV activity by shielding off the positively charged sites in the V3 loop of the viral envelope glycoprotein (gp120). The V3 loop is necessary for attachment to cell-surface heparan sulfate.

Polysulfates suffer from a number of disadvantages. They are very poorly absorbed following oral administration. Only the modified β -cyclodextrin hexadecasulfate with three benzylthiogroups showed oral bioavailability (with a half-life of 4 h). When polysulfates were administered intravenously, half-lives of a maximum of 2 h were noted. When DS and PS were introduced in clinical trials by continuous iv infusion to symptomatic HIV patients, no beneficial effect on surrogate markers (i.e. p24) or clinical endpoints (i.e. CD4), suggesting anti-HIV activity, were noted. Moreover, continuous iv infusion of DS and PS proved toxic, causing thrombocytopenia. Because of their toxicity and lack of beneficial effect on surrogate markers, polysulfates such as unmodified DS or PS are unlikely to have practical utility in parenteral treatment of symptomatic HIV infection.

Acknowledgements

The original investigations of the authors were supported in part by the Biomedical Research Programme of the European Community and by grants from the Belgian Nationaal Fonds voor Wetenschappelijk Onderzoek (NFWO), the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek (FGWO) and the Belgian Geconcerteerde Onderzoeksacties (GOA). We thank Christiane Callebaut for her dedicated editorial help, and Hilde Azijn, Anita Camps, Frieda De Meyer, Patrick Seldeslachts, Anita Van Lierde, Anja Van Cauwenberge and Barbara Van Remoortel for excellent technical assistance.

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.
- Anand, R., Nayyar, S., Galvin, T.A., Merrill, C.R., and Bigelow, L.B. (1990a) Sodium pentosan polysulfate (SPP), as anti-HIV agent also exhibits synergism with AZT, lymphoproliferative activity, and virus enhancement. *AIDS Res Hum Retroviruses* **6**: 679–689.
- Anand, R., Nayyar, S., Pitha, J., and Merrill, C.R. (1990b) Sulfated sugar alfa-cyclodextrin sulfate, a uniquely potent anti-HIV agent, also exhibits marked synergism with AZT, and lymphoproliferative activity. *Antiviral Chem Chemother* **1**: 41–46.
- Andrei, G., and De Clercq, E. (1990) Inhibitory effect of selected antiviral compounds on arenavirus replication *in vitro*. *Antiviral Res* **14**: 287–300.
- Andrei, G., and De Clercq, E. (1993) Molecular approaches for the treatment of hemorrhagic fever virus infections. *Antiviral Res* **22**: 45–75.
- Andrei, G., Snoeck, R., Goubau, P., Desmyter, J., and De Clercq, E. (1991) Comparative activity of selected antiviral compounds against clinical isolates of human cytomegalovirus. *Eur J Microbiol Infect Dis* **10**: 1026–1033.
- Aoki, T., Kaneko, Y., Stefanski, M.S., Nguyen, T., and Ting, R.C. (1991) Curdlan sulfate and HIV-1. *In vitro* effects of curdlan sulfate on HIV-1 infection. *AIDS Res Hum Retroviruses* **7**: 409–415.
- 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., Pauwels, R., Balzarini, J., Arnout, J., Desmyter, J., and De Clercq, E. (1988b) Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus *in vitro*. *Proc Natl Acad Sci USA* **85**: 6132–6136.
- Baba, M., Snoeck, R., Pauwels, R., and De Clercq, E. (1988c) Sulfated 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., 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. (1990a) Novel sulfated polysaccharides: dissociation of anti-human immunodeficiency virus activity from antithrombin activity. *J Infect Dis* **161**: 208–213.
- Baba, M., Schols, D., De Clercq, E., Pauwels, R., Nagy, M., Györgyi-Edelényi, J., Löw, M., and Görög, S. (1990b) Novel sulfated polymers as highly potent and selective inhibitors of human immunodeficiency virus replication and giant cell formation. *Antimicrob Agents Chemother* **34**: 134–138.
- Bagasra, O., and Lischner, H.W. (1988) Activity of dextran sulfate and other polyanionic polysaccharides against human immunodeficiency virus. *J Infect Dis* **158**: 1084–1087.
- Bagasra, O., Whittle, P., Heins, B., and Pomerantz, R.J. (1991) Anti-human immunodeficiency virus type 1 activity of sulfated monosaccharides: comparison with sulfated polysaccharides and other polyions. *J Infect Dis* **164**: 1082–1090.
- 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.
- Bărză, T., Level, M., Petitou, M., Lormeau, J.-C., Choay, J., Schols, D., Baba, M., Pauwels, R., Witvrouw, M., and De Clercq, E. (1993) Preparation and anti-HIV activity of O-acylated heparin and dermatan sulfate derivatives with low anticoagulant effect. *J Med Chem* **36**: 3546–3555.
- 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.
- Beutler, J.A., McKee, T.C., Fuller, R.W., Tischler, M., Cardellino, J.H., Snader, K.M., McCloud, T.G., and Boyd, M.R. (1993) Frequent occurrence of HIV-inhibitory sulfated polysaccharides in marine invertebrates. *Antiviral Chem Chemother* **4**: 167–172.
- Biesert, L., Suhartono, H., Winkler, I., Meichsner, C., Helsenberg, M., Hewlett, G., Klimetzek, V., Molling, K., Schlumberger, H.D., Schrinner, E., Brade, H.-D., and Rübsamen-Waigmann, H. (1988) Inhibition of HIV and virus replication by polysulfated polyxylan: HOE/BAY 946, a new antiviral compound. *AIDS* **2**: 449–457.
- Buckheit, R.W., Jr, Germany-Decker, J., Hollingshead, M.G., Allen, L.B., Shannon, W.M., Janssen, P.A.J., and Chirigos, M.A. (1993) Differential antiviral activity of two TIBO derivatives against the human immunodeficiency and murine leukemia viruses alone and in combination with other anti-HIV agents. *AIDS Res Hum Retroviruses* **9**: 1097–1106.
- Busso, M.E., and Resnick, L. (1990) Anti-human immunodeficiency virus effects of dextran sulfate are strain dependent and synergistic or antagonistic when dextran sulfate is given in combination with dideoxynucleosides. *Antimicrob Agents Chemother* **34**: 1991–1995.
- Callahan, L.N., Phelan, M., Mallinson, M., and Norcross, M.A. (1991) Dextran sulfate blocks antibody binding to the principal neutralizing domain of human immunodeficiency virus type 1 without interfering with gp120-CD4 interactions. *J Virol* **65**: 1543–1550.
- 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 Physicians* **102**: 101–109.
- Damonte, E., Neyts, J., Pujol, C.A., Snoeck, R., Andrei, G., Ikeda, S., Witvrouw, M., Haines, H., Matulewicz, M.C., Cerezo, A., Coto, C.E., and De Clercq, E. (1994) Antiviral activity of a sulfated polysaccharide from the red seaweed *Nothogenia fastigiata*. *Biochem Pharmacol*, in press.
- Dawes, J., Bara, L., Billaud, E., and Samama, M. (1986) Relationship between biological activity and concentration of a low-molecular-weight heparin (PK 10169) and unfractionated heparin after intravenous and subcutaneous administration. *Haemostasis* **16**: 116–122.
- De Clercq, E. (1986) Chemotherapeutic approaches to the treatment of the acquired immune deficiency syndrome (AIDS). *J Med Chem* **29**: 1561–1569.
- Eriksson, B.F.H., and Schinazi, R.F. (1989) Combinations of 3'-azido-3'-deoxythymidine (zidovudine) and phosphonoformate (foscarnet) against human immunodeficiency virus type 1 and cytomegalovirus *in vitro*. *Antimicrob Agents Chemother* **33**: 663–669.

- 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.
- Furusawa, E., Furusawa, S., and Chou, S.C. (1991) Antileukemic activity of *Viva-Natural*, a dietary seaweed extract, on Rauscher murine leukemia in comparison with anti-HIV agents, azidothymidine, dextran sulfate and pentosan polysulfate. *Cancer Lett* **56**: 197-205.
- Gerard, M., Weerts, D., De Wit, S., Sommereyns, B., Witvrouw, M., Schols, D., Desmyter, J., and De Clercq, E. (1990) Pentosan polysulfate (PPS) in HIV patients (P): a phase 1 study. 30th ICAAC, Atlanta, GA, October 1990.
- Handa, A., Hoshino, H., Nakajima, K., Adachi, M., Ikeda, K., Achiwa, K., Itoh, T., and Suzuki, Y. (1991) Inhibition of infection with human immunodeficiency virus type 1 by sulfated gangliosides. *Biochem Biophys Res Commun* **175**: 1-9.
- Hartman, N.R., Johns, D.G., and Mitsuya, H. (1990) Pharmacokinetic analysis of dextran sulfate in rats as pertains to its clinical usefulness for therapy of HIV infection. *AIDS Res Hum Retroviruses* **6**: 805-812.
- Hartshorn, K.L., Vogt, M.W., Chou, T.C., Blumberg, R.S., Byington, R., Schooley, R.T., and Hirsch, M.S. (1987) Synergistic inhibition of human immunodeficiency virus *in vitro* by azidothymidine and recombinant alpha A interferon. *Antimicrob Agents Chemother* **31**: 168-172.
- Haseltine, W.A. (1988) Replication and pathogenesis of the AIDS virus. *J AIDS* **1**: 217-240.
- Hatanaka, K., Kurihara, Y., and Yryu, T. (1991) A strong inhibition of HIV-induced cytopathic effects by synthetic (1,6)- α -D-mannopyranan sulfate. *Carbohydrate Res* **214**: 147-154.
- Hattori, T., Koito, A., Takatsuki, K., Kido, H., and Katunuma, N. (1989) Involvement of tryptase-related cellular protease(s) in human immunodeficiency virus type 1 infection. *FEBS Lett* **8**: 48-52.
- Hayashi, S., Fine, R.L., Chou, T.-C., Currens, M.J., Broder, S., and Mitsuya, H. (1990) *In vitro* inhibition of the infectivity and replication of human immunodeficiency virus type 1 by combination of anti-retroviral 2',3'-dideoxynucleosides and virus-binding inhibitors. *Antimicrob Agents Chemother* **34**: 82-88.
- Hirabayashi, K., Iwata, S., Ito, M., Shigeta, S., Narui, T., Mori, T., and Shibata, S. (1989) Inhibitory effect of a lichen polysaccharide sulfate, GE-3-S, on the replication of human immunodeficiency virus (HIV) *in vitro*. *Chem Pharm Bull* **37**: 2410-2412.
- Hosoya, M., Balzarini, J., Shigeta, S., and De Clercq, E. (1991) Differential inhibitory effects of sulfated polysaccharides and polymers on the replication of various myxoviruses and retroviruses, depending on the composition of the target amino acid sequences of the viral envelope glycoproteins. *Antimicrob Agents Chemother* **35**: 2515-2520.
- Ito, M., Baba, M., Sato, A., Pauwels, R., De Clercq, E., and Shigeta, S. (1987) Inhibitory effect of dextran sulfate and heparin on the replication of human immunodeficiency virus (HIV) *in vitro*. *Antiviral Res* **7**: 361-367.
- Ito, M., Baba, M., Shigeta, S., Wada, S., Takagi, M., Kimura, T., and Okuyama, T. (1991) Potent and selective activity of dextran sulfate against human immunodeficiency virus type 1 *in vitro*. *Antiviral Chem Chemother* **2**: 41-44.
- Jackson, R.L., Busch, S.J., and Cardin, A.D. (1991) Glycosaminoglycans: molecular properties, protein interactions, and role in physiological processes. *Physiol Rev* **71**: 481-539.
- Johnson, V.A., Barlow, M.A., Chou, T.C., Fisher, R.A., Walker, B.D., Hirsch, M.S., and Schooley, R.T. (1989a) Synergistic inhibition of human immunodeficiency virus type 1 (HIV-1) replication *in vitro* by recombinant soluble CD4 and 3'-azido-3'-deoxythymidine. *J Infect Dis* **159**: 837-844.
- Johnson, V.A., Walker, B.D., Barlow, M.A., Paradis, T.J., Chou, T.C., and Hirsch, M.S. (1989b) Synergistic inhibition of human immunodeficiency virus type 1 and type 2 replication *in vitro* by castanospermine and 3'-azido-3'-deoxythymidine. *Antimicrob Agents Chemother* **33**: 53-57.
- Jurkiewicz, E., Panse, P., Jentsch, K.D., Hartmann, H., and Hunsmann, G. (1989) *In vitro* anti-HIV-1 activity of chondroitin polysulphate. *AIDS* **3**: 423-427.
- Kaneko, Y., Yoshida, O., Nakagawa, R., Yoshida, T., Date, M., Ogihara, S., Shioya, S., Matsuzawa, Y., Nagashima, N., Irie, Y., Mimura, T., Shinkai, H., Yasuda, N., Matsuzaki, K., Uryu, T., and Yamamoto, N. (1990) Inhibition of HIV-1 infectivity with curdlan sulfate *in vitro*. *Biochem Pharmacol* **39**: 793-797.
- Kari, B., and Gehrz, R. (1993) Structure, composition and heparin binding properties of a human cytomegalovirus glycoprotein complex designated gC-II. *J Gen Virol* **74**: 255-264.
- Kjellén, L., and Lindahl, U. (1991) Proteoglycans: structures and interactions. *Ann Rev Biochem* **60**: 443-475.
- Kowalski, M., Potz, J., Basiripour, L., Dorfman, T., Goh, W.C., Terwilliger, E., Dayton, A., Rosen, C., Halseltine, W., and Sodroski, J. (1987) Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. *Science* **237**: 1351-1355.
- Larder, B.A., Darby, G., and Richman, D.D. (1989) HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **243**: 1731-1734.
- Lorentsen, K.J., Hendrix, C.W., Collins, J.M., Kornhauser, D.M., Petty, B.G., Klecker, R.W., Flexner, C., Eckel, R.H., and Lietman, P.S. (1989) Dextran sulfate is poorly absorbed after oral administration. *Ann Intern Med* **111**: 561-566.
- Lüscher-Mattli, M., and Glück, R. (1990) Dextran sulfate inhibits the fusion of influenza virus with model membranes, and suppresses influenza virus replication *in vitro*. *Antiviral Res* **14**: 39-50.
- Lüscher-Mattli, M., Glück, R., Kempf, C., and Zanoni-Grassi, M. (1993) A comparative study of the effect of dextran sulfate on the fusion and the *in vitro* replication of influenza A and B, Semliki forest, vesicular stomatitis, rabies, Sendai, and mumps virus. *Arch Virol* **130**: 317-326.
- Lycke, E., Johansson, M., Svennerholm, B., and Lindahl, U. (1991) Binding of herpes simplex virus to cellular heparan sulfate, an initial step in the adsorption process. *J Gen Virol* **72**: 1131-1137.
- 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.
- MacGregor, I.R., Dawes, J., Pepper, D.S., Prowse, C.V., and Stocks, J. (1985) Metabolism of sodium pentosan polysulfate in man measured by a new competitive binding assay for sulfated polysaccharides - comparison with effects upon anticoagulant activity, lipolysis and platelet α -granule proteins. *Thromb Haemost* **53**: 411-414.
- Maffrand, J.-P., Herbert, J.-M., Bernat, A., Defreyn, G., Delebassee, D., Savi, P., Pinot, J.-J., and Sampol, J. (1991) Experimental and clinical pharmacology of pentosan polysulfate. *Seminars Thromb Hemost* **17**: 186-198.

- Mastromarino, P., Conti, C., Petruzzello, R., Lapadula, R., and Orsi, N. (1991a) Effect of polyions on the early events of Sindbis virus infection of Vero cells. *Arch Virol* **121**: 19–27.
- Mastromarino, P., Seganti, L., Petruzzello, R., Gabrieli, R., Divizia, M., Panà, A., and Orni, N. (1991b) Influence of polyanions on the early steps of enterovirus infection. *J Chemother* **3**: 203–208.
- Mathes, L.E., Hayes, K.A., Swenson, C.L., Polas, P.J., Weisbrode, S.E., and Kociba, G.J. (1991) Evaluation of antiviral activity and toxicity of dextran sulfate in feline leukemia virus-infected cats. *Antimicrob Agents Chemother* **35**: 2147–2150.
- 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.
- Mitsuya, H., Looney, D.J., Kuno, S., Ueno, R., Wong-Staal, F., and Broder, S. (1988) Dextran sulfate suppression of viruses in the HIV family: inhibition of virion binding to CD4⁺ cells. *Science* **240**: 646–649.
- Mizumoto, K., Sugawara, I., Ito, W., Kodama, T., Hayami, M., and Mori, S. (1988) Sulfated homopolysaccharides with immunomodulating activities are more potent anti-HTLV-III agents than sulfated heteropolysaccharides. *Jpn J Exp Med* **58**: 145–151.
- Mizuno, T., Uchino, K., Toukairin, T., Tanabe, A., Nakashima, H., Yamamoto, N., and Ogowara, H. (1992) Inhibitory effect of tannic acid sulfate and related sulfates on infectivity, cytopathic effect, and giant cell formation of human immunodeficiency virus. *Planta Med* **58**: 535–539.
- Montefiori, D.C., Robison, W.E., Modliszewski, A., Rowland, J.M., Schuffman, S.S., and Mitchell, W.M. (1990) Differential inhibition of HIV-1 cell binding and HIV-1-induced syncytium formation by low molecular weight sulphated polysaccharides. *J Antimicrob Chemother* **25**: 313–318.
- 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.
- Moriya, T., Saito, K., Kurita, H., Matsumoto, K., Otake, T., Mori, H., Morimoto, M., Ueba, N., and Kunita, N. (1993) A new candidate for an anti-HIV-1 agent: modified cyclodextrin sulfate (mCDS71). *J Med Chem* **36**: 1674–1677.
- Nahmias, A.J., and Kibrick, S. (1964) Inhibitory effect of heparin on herpes simplex virus. *J Bacteriol* **87**: 1060–1066.
- Nahmias, A.J., Kibrick, S., and Bernfeld, P. (1964) Effect of synthetic and biological polyanions on herpes simplex virus. *Proc Soc Exp Biol Med* **115**: 993–996.
- 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.
- Neyts, J., Snoeck, R., Schols, D., Balzarini, J., Esko, J.D., Van Schepdael, A., and De Clercq, E. (1992) Sulfated polymers inhibit the interaction of human cytomegalovirus with cell surface heparan sulfate. *Virology* **189**: 48–58.
- Offensperger, W.B., Offensperger, S., Walter, E., Blum, H.E., and Gerok, W. (1991) Inhibition of duck hepatitis B virus infection by lysosomotropic agents. *Virology* **183**: 415–418.
- Otake, T., Miyano, K., Mori, H., Morimoto, M., Ueba, N., Kunita, N., Nakashima, H., and Kurimura, T. (1991) Anti-HIV activity of sulfated amphotericin B *in vitro*. *Antiviral Res* **16**: 243–266.
- Otake, T., Schols, D., Witvrouw, M., Naesens, L., Nakashima, H., Moriya, T., Kurita, H., Matsumoto, K., Ueba, N., and De Clercq, E. (1994) Modified cyclodextrin sulfates (mCDS) have potent inhibitory activity against HIV and high oral bioavailability. *Antiviral Chem Chemother* **5**: 155–161.
- Patel, M., Yanagishita, M., Roderiquez, G., Bou-Habib, D.C., Oravec, T., Hascall, V.C., and Norcross, M.A. (1993) Cell-surface heparan sulfate proteoglycan mediates HIV-1 infection of T-cell lines. *AIDS Res Hum Retroviruses* **9**: 167–174.
- Peters, M., Ruf, B., and Pohle, H.D. (1989) Intravenous versus oral application of pentosan polysulfate (HOE/BAY 946). Vth International Conference on AIDS. Montreal, June 1989 (abstract WBP299).
- Peters, M., Witvrouw, M., De Clercq, E., and Ruf, B. (1991) Pharmacokinetics of intravenous pentosan polysulfate (HOE/BAY 946) in HIV-positive patients. *AIDS* **5**: 1534–1535.
- Robbins, A.K., Watson, R.J., Whealy, M.E., Hays, W.W., and Enquist, L.W. (1986) Characterization of a pseudorabies virus glycoprotein gene with homology to herpes simplex virus type 1 and type 2 glycoprotein C. *J Virol* **58**: 339–347.
- St. Clair, M.H., Martin, J.L., Tudor-Williams, G., Bach, M.C., Vavro, C.L., King, D.M., Kellam, P., Kemp, S.D., and Larder, B.A. (1991) Resistance to ddI and sensitivity to AZT induced by a mutation in HIV-1 reverse transcriptase. *Science* **245**: 1557–1559.
- Sawitzky, D., Hampl, H., and Habermehl, K.-O. (1990) Comparison of heparin-sensitive attachment of pseudorabies virus (PRV) and herpes simplex virus type 1 and identification of heparin-binding PRV glycoproteins. *J Gen Virol* **71**: 1221–1225.
- 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 AIDS* **2**: 10–15.
- Schols, D., Pauwels, R., Desmyter, J., and De Clercq, E. (1990a) 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.
- Schols, D., De Clercq, E., Balzarini, J., Baba, M., Witvrouw, M., Hosoya, M., Andrei, G., Snoeck, R., Neyts, J., Pauwels, R., Nagy, M., Györgyi-Edelényi, J., Machovich, R., Horváth, I., Löw, M., and Görög, S. (1990b) Sulphated 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 retroviruses. *Antiviral Chem Chemother* **1**: 233–240.
- Schols, D., De Clercq, E., Witvrouw, M., Nakashima, H., Snoeck, R., Pauwels, R., Van Schepdael, A., and Claes, P. (1991) Sulphated cyclodextrins are potent anti-HIV agents acting synergistically with 2',3'-dideoxynucleoside analogues. *Antiviral Chem Chemother* **2**: 45–53.
- Skinner, M.A., Ting, R., Langlois, A.J., Weinhold, K.J., Lyerly, H.K., Javaherian, K., and Matthews, T.J. (1988a) Characteristics of a neutralizing monoclonal antibody to the HIV envelope glycoprotein. *AIDS Res Hum Retroviruses* **4**: 187–197.
- Smith, M.S., Brian, E.L., De Clercq, E., and Pagano, J.S. (1989) Susceptibility of human immunodeficiency virus type 1 replication *in vitro* to cyclic adenosine analogs and synergy of the analogs with 3'-azido-3'-deoxythymidine. *Antimicrob Agents Chemother* **33**: 1482–1486.
- Sosa, M.A., Fazely, F., Koch, J.A., Vercelotti, S.V., and Ruprecht, R.M. (1991) N-carboxymethylchitosan-N, O-sulfate as an anti-HIV-1 agent. *Biochem Biophys Res Commun* **174**: 489–496.

- Stegmann, T., Doms, R.W., and Helenius, A. (1989) Protein-mediated membrane fusion. *Ann Rev Biophys Chem* **18**: 187-211.
- Stig Hansen, J.-E., Nielsen, C., and Vestergaard, B.F. (1991) Inhibition of human immunodeficiency virus 1 (HIV-1) and herpes simplex virus 1 (HSV-1) infectivity with a broad range of lectins. *Scand J Infect Dis* **23**: 425-430.
- Sugawara, I., Ito, W., Kimura, S., Moro, S., and Shimada, K. (1989) Further characterization of sulfated homopolysaccharides as anti-HIV agents. *Experientia* **45**: 996-998.
- Takemoto, K.K., and Fabisch, P. (1964) Inhibition of herpes virus by natural and synthetic acid polysaccharides. *Proc Soc Exp Biol Med* **116**: 140-144.
- Thiele, B., Braig, H.R., Ehm, I., Kunze, R., and Ruf, B. (1989) Influence of sulfated carbohydrates on the accessibility of CD4 and other CD molecules on the cell surface and implications for human immunodeficiency virus infection. *Eur J Immunol* **19**: 1161-1164.
- Tochikura, T.S., Nakashima, H., and Yamamoto, N. (1989) Antiviral agents with activity against human retroviruses. *J AIDS* **2**: 441-447.
- Ueno, R., and Kuno, S. (1987) Dextran sulfate, a potent anti-HIV agent *in vitro* having synergism with zidovudine. *Lancet* **i**: 1379.
- Uryu, T., Ikushima, N., Katsuraya, K., Shoji, T., Takashashi, N., Yoshida, T., Kanno, K., Murakami, T., Nakashima, H., and Yamamoto, N. (1992) Sulfated alkyl oligosaccharides with potent inhibitory effects on human immunodeficiency virus infection. *Biochem Pharmacol* **43**: 2385-2392.
- Vaheri, A. (1964) Heparin and related polyanionic substances as virus inhibitors. *Acta Pathol Microbiol Scand* (Suppl) **171**: 7.
- Von Briesen, H., Meichsner, C., Andreesen, R., Esser, R., Schrinner, E., and Rubsamen-Waigmann, H. (1990) The polysulphated polyxylan Hoe/Bay-946 inhibits HIV replication on human monocytes/macrophages. *Res Virol* **141**: 251-257.
- Weiler, B.E., Schroder, H.C., Stefanovich, V., Stewart, D., Forrest, J.M., Allen, L.B., Bowden, B.J., Kreuter, M.H., Voth, R., and Muller, W.E. (1990) Sulphoeverynan, a polyanionic polysaccharide, and the *narcissus* lectin potentially inhibit human immunodeficiency virus infection by binding to viral envelope protein. *J Gen Virol* **71**: 1957-1963.
- Wells, C.L. and Rhame, F.S. (1990) Effect of oral dextran sulfate on the mouse intestinal tract. *J AIDS* **3**: 361-365.
- Witvrouw, M., Baba, M., Balzarini, J., Pauwels, R., and De Clercq, E. (1990) Establishment of a bioassay to determine serum levels of dextran sulfate and pentosan polysulfate, two potent inhibitors of human immunodeficiency virus. *J AIDS* **3**: 343-347.
- Witvrouw, M., Schols, D., Andrei, G., Snoeck, R., Hosoya, M., Pauwels, R., Balzarini, J., and De Clercq, E. (1991) Antiviral activity of low-MW dextran sulphate (derived from dextran MW 1,000) compared to dextran sulphate samples of higher MW. *Antiviral Chem Chemother* **2**: 171-179.
- 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* **3**: 351-360.
- Witvrouw, M., Este, J.A., Quinones Mateu, M.E., Reyman, D., Andrei, G., Snoeck, R., Ikeda, S., Pauwels, R., Vittori Bianchini, N., Desmyter, J., and 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.
- WuDunn, D., and Spear, P.G. (1989) Initial interaction of herpes simplex virus with cells is binding to heparan sulfate. *J Virol* **63**: 52-58.
- Yoshida, O., Nakashima, H., Yoshida, T., Kaneko, Y., Yamamoto, I., Matsuzaki, K., Uryu, T., and Yamamoto, N. (1988) Sulfation of the immunomodulating polysaccharide lentinan: a novel strategy for antivirals to human immunodeficiency virus (HIV). *Biochem Pharmacol* **37**: 2887-2891.