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Mechanism of inhibitory effect of dextran sulfate and heparin on replication of human immunodeficiency virus *in vitro*

(acquired immunodeficiency syndrome/antiviral chemotherapy/sulfated polysaccharide)

MASANORI BABA*, RUDI PAUWELS*, JAN BALZARINI*, JEF ARNOUT†, JAN DESMYTER*, AND ERIK DE CLERCQ*

*Rega Institute for Medical Research, Katholieke Universiteit Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium; and †Center for Thrombosis and Vascular Research, Campus Gasthuisberg, O & N, Herestraat 49, B-3000 Leuven, Belgium

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ABSTRACT **The sulfated polysaccharides dextran sulfate and heparin have proved to be potent and selective inhibitors of human immunodeficiency virus type 1 (HIV-1) *in vitro*. Dextran sulfate (M_r 5000) and heparin (M_r 15,000) completely protected MT-4 cells against HIV-1-induced cytopathogenicity at a concentration of 25 μg/ml. Their 50% inhibitory concentrations were 9.1 μg/ml (dextran sulfate) and 7.0 μg/ml (heparin), respectively. No toxicity for the host cells was observed with these compounds at a concentration of 625 μg/ml. The anti-HIV-1 activity of heparins of various molecular weights correlated well with their anticoagulant activity. On the other hand, with dextran sulfates of low molecular weight (5000, 8000) a significant inhibitory effect on HIV-1 was achieved at a concentration that was not markedly inhibitory to the blood coagulation process. Dextran sulfate and heparin were not inhibitory to HIV-1 reverse transcriptase unless they were used at concentrations in excess of those that inhibited HIV-1 replication. They were highly effective against HIV-1 replication even when present only during the 2-hr virus adsorption period. Studies using radiolabeled HIV-1 virions indicated that dextran sulfate and heparin inhibit virus adsorption to the host cells.**

The urgent need for an effective chemotherapy for the acquired immunodeficiency syndrome (AIDS), which is caused by human T-cell lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) (1, 2), now termed human immunodeficiency virus type 1 (HIV-1), has prompted the search for selective anti-HIV agents. Suramin has been shown to inhibit the replication of HIV-1 in cell culture (3) and in AIDS patients (4), and 3′-azido-3′-deoxythymidine (commonly called azidothymidine or AZT) has been proven to improve the clinical and immunological status of patients with AIDS and AIDS-related complex (5, 6). The target of azidothymidine is the viral reverse transcriptase (7), and the phosphorylated products of azidothymidine may be held responsible for the bone marrow suppression that is often associated with the administration of this compound (8).

It has recently been reported that the sulfated polysaccharides dextran sulfate and heparin are highly selective inhibitors of HIV-1 replication *in vitro* (9, 10). Polyanionic substances were, in fact, suggested by De Clercq (11) as potential anti-HIV-1 agents because of their putative effect on the virus adsorption process. Studies have now been undertaken to determine the specificity of the anti-HIV-1 activity of dextran sulfate and heparin, the dependence of this activity on the molecular weight and its relationship with anticoagulant activity, and, finally, the mechanism of anti-HIV-1 action of the compounds.

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MATERIALS AND METHODS

Cells. MT-4, a T4 lymphocyte line carrying human T-cell lymphotropic virus type I (HTLV-I) (12), and HUT-78, a T4 lymphocyte line not carrying HTLV-I (13), were used for the anti-HIV-1 assay. The cells were mycoplasma-negative. The cell lines used for the cytostasis assays—i.e., L1210, FM3A, Raji, Molt/4F, and CEM—have been described elsewhere (14, 15).

Viruses. HIV-1 was obtained from the culture supernatant of a HUT-78 cell line persistently infected with HTLV-III_B (HUT-78/HTLV-III_B). The titer of the virus stock was 2×10^5 50% cell culture infective doses ($CCID_{50}$) per ml.

Radiolabeled HIV-1 particles were obtained from the supernatant of HUT-78/HTLV-III_B cultures. Briefly, HUT-78/HTLV-III_B cells were cultured with either 1 mCi of [^{32}P]orthophosphate (carrier-free, 10 mCi/ml, Amersham PBS.13; 1 Ci = 37 GBq) or 1 mCi of [5-^{3}H]uridine (30 Ci/mmol, Amersham). After 3 days, the supernatant was collected, centrifuged at low speed, and then ultracentrifuged at 100,000 × *g* for 2 hr. Pellets were resuspended in 1/100th vol of culture medium. The HIV-1 particles labeled with [5-^{3}H]uridine were further purified by isopycnic ultracentrifugation on a 15–60% sucrose gradient. Radioactivity and virus titer of the final preparations were 6.4×10^5 cpm/ml and 2.4×10^6 $CCID_{50}$/ml for [^{32}P]orthophosphate-labeled HIV-1 and 1.1×10^6 cpm/ml and 1×10^7 $CCID_{50}$/ml for [5-^{3}H]uridine-labeled HIV-1.

The Moloney strain of murine leukemia virus (MuLV) was obtained from Electronucleonics (Bethesda, MD).

Compounds. Dextran (M_r approximately 90,000 and 506,000), dextran sulfate (M_r approximately 5000, 8000, and 500,000), glucosamine 2-sulfate, 3-sulfate, 6-sulfate, and 2,3-bissulfate were purchased from Sigma. Sodium heparin (mean M_r 15,000) and fragmented heparins with various molecular weights were kindly provided by W. O. Godtfredsen (Leo Pharmaceutical, Ballerup, Denmark). 2′,3′-Dideoxycytidine was obtained from Pharmacia. Azidothymidine and azidothymidine 5′-triphosphate were synthesized by P. Herdewijn in the Rega Institute.

Antiviral Assay. The procedure for measuring anti-HIV-1 activity in MT-4 cells has been described previously (16). Briefly, MT-4 cells were suspended at 5×10^5 cells per ml and infected with HIV-1 at 1000 $CCID_{50}$/ml. After 2-hr incubation at 37°C, 5×10^4 cells per 100 μl were brought into

Abbreviations: HIV-1, human immunodeficiency virus type 1; AIDS, acquired immunodeficiency syndrome; azidothymidine, 3′-azido-3′-deoxythymidine; $CCID_{50}$, 50% cell culture infective dose; IU, international unit(s); MuLV, (Moloney) murine leukemia virus; APTT, activated partial thromboplastin time.

each well of a flat-bottomed 96-well plastic microtiter tray containing 100 μl of various dilutions of the test compounds in each well. After a 5-day incubation at 37°C, the number of viable cells was determined microscopically in an hemacytometer by trypan blue exclusion. Anti-HIV-1 activity of the compounds was also determined by monitoring viral antigen expression in HUT-78 cells at day 14 after infection. Indirect immunofluorescence was measured by a polyclonal antibody as probe, as previously described (16).

Reverse Transcriptase Assays. The effects of dextran sulfate and heparin on reverse transcriptase activity *in vitro* were evaluated with HIV-1 and MuLV as the sources of the enzyme. The HIV-1 reverse transcriptase assay was performed at 37°C for 60 min with a 50-μl reaction mixture containing 50 mM Tris·HCl at pH 8.4, 2 mM dithiothreitol, 100 mM KCl, 10 mM $MgCl_2$, 1 μCi of [*methyl*-^{3}H]dTTP (30 Ci/mmol)], 0.01 A_{260} unit of poly(rA)·oligo(dT), 0.1% Triton X-100, 10 μl of compound solution (containing various concentrations of the compounds), and 10 μl of the enzyme [which had been partially purified by low centrifugation of the supernatant of HUT-78/HTLV-III_B cell cultures followed by filtration (0.45-μm pores) and ultracentrifugation (100,000 × *g*, 2 hr)]. The reaction was stopped with 200 μl of trichloroacetic acid (5%, vol/vol) and the precipitated material was analyzed for radioactivity. For the MuLV reverse transcriptase assay, endogenous viral RNA served as the template. Otherwise, the assay conditions were as described previously (17).

Virus Adsorption Assay. In the first experiment 2 × 10^6 MT-4 cells were suspended in medium (final volume, 100 μl) containing dextran sulfate (M_r 5000) or heparin at 25 μg/ml, after which 32,000 cpm of ^{32}P-labeled HIV-1 was added. In the second experiment, 2 × 10^6 MT-4 cells were suspended in medium (final volume, 500 μl) containing dextran sulfate or heparin at 25 μg/ml, after which 11,000 cpm of ^{3}H-labeled HIV-1 was added. The samples were incubated at 37°C, and after 0, 30, 60, or 120 min, the cells were collected by centrifugation (at 4°C) and washed three times with phosphate-buffered saline (Oxoid, Conforma, Belgium) (at 4°C) to remove unadsorbed virus particles. Then the cells were precipitated with 5% trichloroacetic acid, and acid-insoluble material was analyzed for radioactivity.

Dextran Sulfate Anticoagulant Activity Test. Anticoagulant activity of dextran sulfate was assessed by the standard methods for measuring the thrombin time and the activated partial thromboplastin time (APTT) of plasma containing the compound at various concentrations, as described previously (18, 19). Anticoagulant activity of heparin and its fragments was expressed in international heparin units (IU).

RESULTS

Anti-HIV-1 Activity of Dextran Sulfate and Heparin. When dextran sulfate (M_r 5000) and heparin were evaluated for their inhibitory effect on the cytopathogenicity of HIV-1 in MT-4 cells, both compounds completely protected the cells against virus-induced destruction at a concentration of 25 μg/ml (Fig. 1). At this concentration dextran sulfate and heparin inhibited virus antigen expression in HUT-78 cells by 85% and 74%, respectively (Fig. 1). The 50% inhibitory concentrations (IC_{50}) of dextran sulfate and heparin for HIV-1 replication measured by virus-induced destruction of MT-4 cells were 9.1 and 7.0 μg/ml, respectively (Table 1).

No cytotoxicity of the compounds for MT-4 cells, as measured by trypan blue exclusion, was noted at concentrations up to 3125 μg/ml (dextran sulfate) or 625 μg/ml (heparin) (Fig. 1), and their IC_{50} values for the viability of MT-4 cells could be estimated at >3125 and 1980 μg/ml, respectively (Table 1). When the more sensitive MTT method, based on the mitochondrial reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

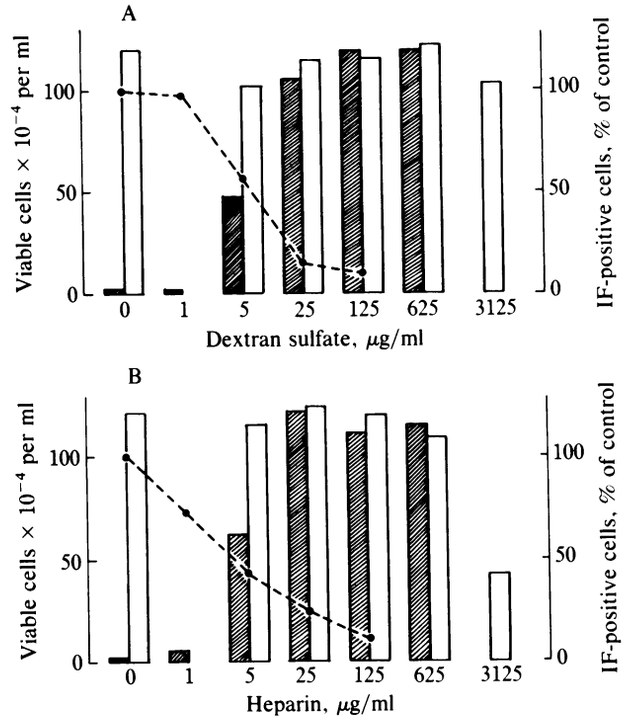

FIG. 1. Inhibitory effects of dextran sulfate (M_r 5000) (*A*) and heparin (*B*) on HIV-1-induced cytopathogenicity in MT-4 cells and antigen expression in HUT-78 cells. MT-4 cells were infected with 100 $CCID_{50}$ of HIV-1 per well, and, after 2-hr adsorption of virus, the cells were incubated with the test compounds at various dilutions for 5 days. Viabilities of HIV-1-infected cells (hatched bars) and mock-infected cells (empty bars) were assessed in parallel. HUT-78 cells were infected with HIV-1 at a multiplicity of infection of 0.1, and, after 2-hr adsorption of virus, the cells were incubated with the test compounds at various dilutions. At day 7, half of the medium in each well was replaced with fresh culture medium containing the same concentrations of the compounds. At day 14, indirect immunofluorescence (IF) was examined with polyclonal antibody as probe. Number of antigen-positive cells was expressed as percent of the control (●). Data represent mean values for two separate experiments.

(MTT) (20), was used, comparable results for both cytotoxicity and anti-HIV-1 activity were obtained (data not shown). Furthermore, dextran sulfate and heparin did not inhibit the proliferation of various cell lines—i.e., murine leukemia L1210, murine mammary FM3A, human B-lymphoblast Raji, human T-lymphoblast Molt/4F, and human T4-lymphocyte CEM cells—at concentrations up to 5000 and 2500 μg/ml, respectively (data not shown). These results indicate that dextran sulfate and heparin are selective inhibitors of HIV-1 replication *in vitro*.

Dextran sulfates of different molecular weight (5000, 8000, and 500,000) showed a similar inhibitory effect on HIV-1 replication in MT-4 cells. In contrast, dextran itself and glucosamine 2-, 3-, or 6-sulfate or 2,3-bissulfate did not show any anti-HIV-1 activity even at 125 μg/ml (Table 1 and data not shown). Neither did these compounds show any cytotoxicity at concentrations up to 3125 μg/ml. When assayed under the same conditions, azidothymidine proved active against HIV-1 at 0.02 μg/ml and cytotoxic at 3.5 μg/ml.

Inhibitory Effects of Dextran Sulfate and Heparin on the Activity of HIV-1 and MuLV-Associated Reverse Transcriptase. When dextran sulfate (M_r 5000, 8000, or 500,000) and heparin were evaluated for their inhibitory effect on HIV-1-associated reverse transcriptase, the IC_{50} values of the compounds were 41.9, 66.2, 17.9, and 99.0 μg/ml, respectively (Table 1). These values, except for dextran sulfate (M_r 500,000), were well above the respective IC_{50} values for anti-HIV-1 activity. Neither dextran sulfate (M_r 5000 or

Table 1. Inhibitory effects of dextran sulfate, heparin, and related compounds on replication of HIV-1 in MT-4 cells, on cell viability, and on HIV-1 reverse transcriptase activity

Compound	IC_{50}, μg/ml		
	Viral activity*	Cell viability†	HIV-1 RT‡
Dextran sulfate			
5,000	9.1	>3125	41.9
8,000	10.7	1650	66.2
500,000	10.9	>3125	17.9
Dextran			
90,000	>125	>3125	>1000
506,000	>125	>3125	ND
Heparin	7.0	1980	99.0
Azidothymidine	0.02	3.5	0.02§

Approximate molecular weights are given for dextran sulfate and dextran. Data represent mean value for two separate experiments. ND, not determined.

*Inhibition of viral activity is expressed as the 50% inhibitory concentration (IC_{50}), which reduces by 50% the number of cells destroyed in a virus-infected culture.

†The IC_{50} for cell viability is the concentration that reduces by 50% the number of cells in a mock-infected culture.

‡HIV-1 reverse transcriptase was assayed with poly(rA)·$(dT)_{12-18}$ as template-primer.

§IC_{50} of azidothymidine 5′-triphosphate.

500,000) nor heparin proved inhibitory to MuLV-associated reverse transcriptase unless used at very high concentrations (237, >1000, and >400 μg/ml, respectively). Under the same conditions azidothymidine triphosphate showed an IC_{50} of 0.30 μg/ml for MuLV reverse transcriptase. These results indicate that dextran sulfate and heparin are not markedly inhibitory to reverse transcriptase whatever the origin of the enzyme (human or murine retroviruses).

Influence of Various Treatment Periods with Dextran Sulfate and Heparin on Their Anti-HIV-1 Activity. In attempts to determine the mechanism of action of dextran sulfate and heparin on HIV-1 replication, various treatment periods with the compounds for MT-4 cells were examined. In treatment A, the cells were exposed to HIV-1 in the presence of the test compounds. After a 2-hr incubation at 37°C, both compound and unadsorbed virus were removed, and the cells were washed with culture medium and further incubated in the absence of the compounds. In treatment B, the cells were exposed to HIV-1, and after a 2-hr incubation at 37°C, unadsorbed virus was removed, and the cells were washed and further incubated in the presence of the compounds. Fig. 2*A* shows that treatment A with dextran sulfate (M_r 5000) was equally active as treatment B, and that treatment A combined with treatment B further enhanced the activity, so that complete protection was achieved at a compound concentration of 5 μg/ml. In marked contrast, treatment A with azidothymidine was approximately 1/100th as effective as treatment B, and the activity of azidothymidine was not further enhanced when the two treatment regimens were combined (Fig. 2*B*). When heparin and dideoxycytidine were examined under the same conditions, heparin exhibited a behavior similar to that of dextran sulfate, whereas dideoxycytidine behaved similarly to azidothymidine. Treatment of the MT-4 cells with dextran sulfate or heparin for 24 hr, before the cells were infected with HIV-1 did not offer any protection (Table 2).

Direct Virus-Neutralizing Effects of Dextran Sulfate and Heparin. Neither dextran sulfate nor heparin brought about a reduction in virus titer (data not shown), after they had been incubated with the virus (input: 10^5 $CCID_{50}$/ml) for 30, 60, or 120 min at 37°C at a concentration (25 μg/ml) that was found to be fully protective if present on the cells only during the 2-hr virus adsorption period [treatment A (Table 2)]. Thus, dextran sulfate and heparin do not directly neutralize HIV-1.

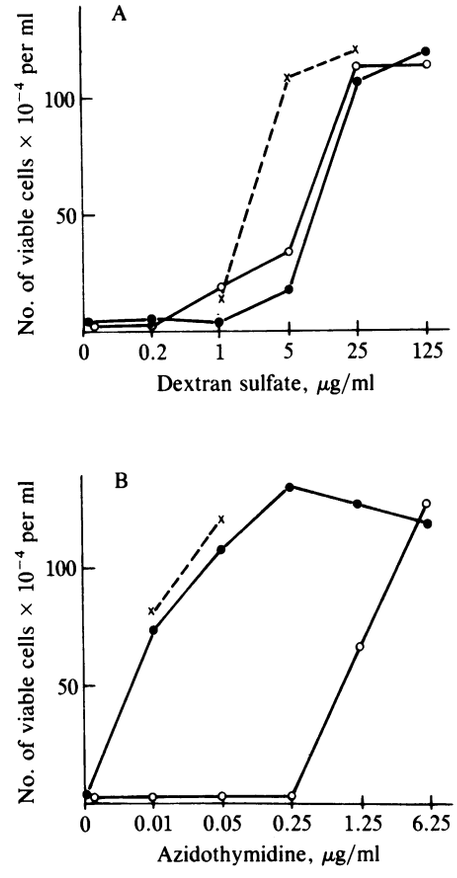

FIG. 2. Influence of various treatment periods with dextran sulfate (M_r 5000) (*A*) or azidothymidine (*B*) on their anti-HIV-1 activity in MT-4 cells. MT-4 cells were infected with 100 $CCID_{50}$ of HIV-1 per well and exposed simultaneously to the test compounds at various dilutions. After a 2-hr incubation at 37°C, unadsorbed virus together with the test compound were removed, and the cells were washed and further incubated in the absence of the compounds [treatment A (○)]. MT-4 cells were infected with HIV-1, and after a 2-hr incubation at 37°C unadsorbed virus was removed and the cells were washed. The cells were then exposed to the compounds at various dilutions and further incubated until determination of cell viability [treatment B (●)]. Combined treatment A and B was also examined (×). After 5 days, the number of viable cells was determined by trypan blue exclusion. Data represent mean values for two separate experiments.

Inhibitory Effect of Dextran Sulfate and Heparin on Virus Adsorption. When [^{32}P]orthophosphate or [5-^{3}H]uridine-labeled virus was incubated with the MT-4 cells (in the absence of the test compounds), cell-associated radioactivity

Table 2. Influence of various treatment periods on anti-HIV-1 activity of heparin and dextran sulfate in MT-4 cells

Compound	IC_{50}, μg/ml			
	Pre-treatment	Treat. A	Treat. B	Treat. A + B
Dextran sulfate	>125	8.8	12.1	2.3
Heparin	>125	5.0	13.0	1.0
Azidothymidine	ND	1.1	0.009	0.007
Dideoxycytidine	ND	12.0	1.0	0.9

MT-4 cells were incubated with the test compounds at various dilutions for 24 hr prior to virus infection (pretreatment), or during periods A, B, or A + B, as described in the legend to Fig. 2. Dextran sulfate $M_r \approx 5000$. Data represent mean value for two separate experiments. ND, not determined.

increased with the incubation time (Fig. 3). When, however, dextran sulfate or heparin was added to the suspensions, little, if any, increase in cell-associated radioactivity was observed (Fig. 3). These results indicate that dextran sulfate and heparin prevented the adsorption of HIV-1 to the MT-4 cells.

Anticoagulant Activity of Dextran Sulfate. When the anticoagulant activity of the dextran sulfates was examined, the thrombin time was found to be doubled with dextran sulfate (M_r 5000 or 8000) at a concentration of 50 μg/ml. Dextran sulfate of M_r 5000 caused a doubling of the APTT at 20 μg/ml, whereas dextran sulfate of M_r 8000 did so at a concentration of 50 μg/ml. At 10 μg/ml, which corresponds to the IC_{50} for HIV-1 replication in MT-4 cells (Table 1), dextran sulfate (M_r 5000 or 8000) caused only a marginal prolongation of the APTT. Dextran sulfate of M_r 500,000 was the most potent anticoagulant, doubling the thrombin time and the APTT at 2 and 5 μg/ml, respectively.

Inhibitory Effect of Fragmented Heparins on HIV-1 Replication. Since it has been demonstrated that the biological (i.e., anticoagulant) activity of heparin depends on its molecular weight (21), the anti-HIV-1 and anticoagulant effects of various fragmented heparins with molecular weights ranging from 1400 to 11,000 were examined. Table 3 shows that the fragmented heparins were clearly less active against HIV-1 than the original (nonfragmented) heparin, and the antiviral activity closely correlated with the anticoagulant activity. Both the antiviral and anticoagulant activity decreased with decreasing molecular weights of the heparin fragments. Dermatan sulfate did not exhibit either antiviral or anticoagulant activity (Table 3).

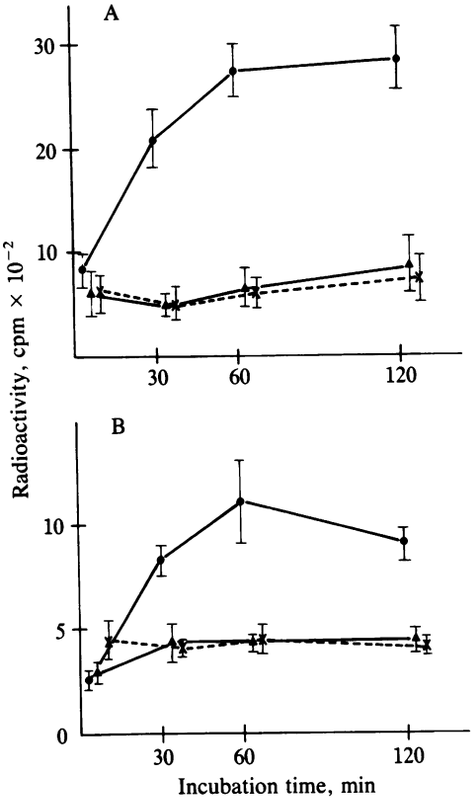

FIG. 3. Effect of dextran sulfate and heparin on adsorption of radiolabeled HIV-1 to MT-4 cells. Suspensions of MT-4 cells with the test compounds and either [^{32}P]orthophosphate-labeled HIV-1 particles (*A*) or [5-^{3}H]uridine-labeled purified HIV-1 particles (*B*) were prepared. The samples were incubated at 37°C for 0, 30, 60, or 120 min with heparin at 25 μg/ml (▲), dextran sulfate at 25 μg/ml (×), or no compound (control) (●). At the end of incubation the cells were collected and washed with phosphate-buffered saline to remove unadsorbed virus particles. Cell-associated acid-insoluble material was analyzed for radioactivity after precipitation with 5% trichloroacetic acid. Radioactivity background (in the absence of radiolabeled virus) was less than 150 cpm (*A*) or 30 cpm (*B*). Data are represented as mean values ± SD.

Table 3. Anticoagulant activities and inhibitory effects of various fragmented heparins on replication of HIV-1 in MT-4 cells

Heparin fragment	Mean M_r*	IC_{50}, μg/ml	Anticoagulant activity,* IU/mg: Factor Xa	Anticoagulant activity,* IU/mg: Factor IIa
A	11,000	41.7	97	117
B	9,400	61.3	77	81
C	6,800	125	70	59
D	6,070	95.1	82	65
E	5,700	>125	55	44
F	3,770	>125	59	34
G	3,400	>125	42	9
H	1,400	>125	31	4
Nonfragmented	15,000	7.0	175	220
Dermatan sulfate	31,000	>125	<0.01	<0.01

*Data provided by Leo Pharmaceutical Products.

DISCUSSION

The sulfated polysaccharides dextran sulfate and heparin are potent and selective inhibitors of HIV-1 replication *in vitro* (Fig. 1, Table 1). These compounds were also evaluated for their activity against other viruses and found to be effective against human immunodeficiency virus type 2, herpes simplex virus type 1 and type 2, and some other enveloped viruses (data not shown). The data presented in Table 2 indicate that, in all likelihood, dextran sulfate and heparin interfere with an early event of the virus replicative cycle, presumably virus adsorption. Full protective activity was achieved when the compounds were present only during the 2-hr virus adsorption period (Fig. 2). No effect was seen when the compounds were incubated on the cells for 24 hr prior to virus infection (Table 2), which suggests that they do not exert their anti-HIV activity indirectly, for example *via* induction of interferon or other lymphokines. Nor do they neutralize the virus directly, as established by experiments in which HIV-1 was incubated with the compounds before being inoculated on the cells.

As dextran sulfate and heparin were most effective when added to the cells during the virus adsorption period and thereafter [combined treatment A and B (Table 2)], one may postulate that they interfere not only with the initial virus adsorption process but also with the subsequent virus adsorption stages as the virus goes through its successive cycles of replication. The virus is assumed to follow such multiple growth cycles because of the low multiplicity of infection used in our experiments.

The effect of dextran sulfate and heparin on the virus adsorption process was assessed directly, using radiolabeled HIV-1 particles. From Fig. 3 it is clear that dextran sulfate and heparin prevented the adsorption of either [^{32}P]orthophosphate- or [5-^{3}H]uridine-labeled HIV-1 to MT-4 cells. Thus, the mechanism of anti-HIV action of these sulfated polysaccharides may be attributed to an inhibition of the virus adsorption process. This inhibition cannot be attributed to a specific interaction of dextran sulfate and heparin with the CD4 antigen receptor, since the compounds did not interfere with the binding of monoclonal antibodies (anti-OKT-4a, anti-Leu-3a) to the CD4 receptor on MT-4 cells (data not shown).

An important issue is whether dextran sulfate, heparin, or both may be adapted to the treatment of patients with AIDS

and AIDS-related complex. For the fragmented heparins the anti-HIV-1 activity closely correlated with their anticoagulant activity (Table 3), which means that solely by fragmentation the anti-HIV-1 activity of heparin cannot be dissociated from its anticoagulant activity. Also, heparin was found to inhibit HIV-1 replication at an IC_{50} of 7.0 μg/ml, which corresponds to 1.4 IU/ml, a dose that should still be able to interfere with the coagulation process *in vivo*. However, with dextran sulfate anti-HIV-1 activity was obtained at a concentration (IC_{50} 9.1 μg/ml) that had no anticoagulant activity. Under optimized conditions (compound present from the time the virus is inoculated onto the cells) the IC_{50} of heparin and dextran sulfate can be further decreased to about 1–2 μg/ml, which clearly falls below the threshold concentration that interferes with blood coagulation. Furthermore, dextran sulfate, heparin, and other sulfated polysaccharides as well, may be subjected to various chemical modifications—i.e. "supersulfation" (22)—that may allow dissociation of antiretroviral activity from anticoagulant activity.

Clinical trials carried out with intermittent intravenous injections of dextran sulfate have demonstrated that a plasma drug concentration of approximately 50 μg/ml, which doubles the clotting time, is easily attainable in humans without unacceptable side effects (23). This concentration is considerably higher than the antivirally effective dose *in vitro*. In future clinical studies, it will be necessary to carefully monitor plasma levels of the compounds. This could be readily done on the basis of their anticoagulant activity (24). Also, competitive binding assays could be designed to measure plasma concentrations of heparin and heparin derivatives (24). Oral administration of the compounds may be feasible as shown before with heparin fragments in mice (25).

In view of the high selectivity of heparin and dextran sulfate as inhibitors of HIV-1 replication *in vitro*, the selectivity of dextran sulfate (M_r 5000) exceeding that of azidothymidine (Table 1), these polyanions should be further pursued for their potential in the prophylaxis and therapy of retrovirus infections in humans.

Note. During the time that this paper was under review, a report addressing, in part, the same subject was submitted by Mitsuya *et al.* Their published report (26) confirms the mechanism of action described above.

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