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Short Communication

Inhibitory effect of dextran sulfate and heparin on the replication of human immunodeficiency virus (HIV) in vitro

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Summary

The polyanionic substances dextran sulfate and heparin were investigated for their antiviral effect on the human immunodeficiency virus (HIV) in vitro. Dextran sulfate and heparin effected a 50% reduction in the cytopathogenicity of HIV for MT-4 cells at a concentration of 4.7 and 7.5 μg/ml, respectively. In Molt-4 (clone 8) cells, these values were slightly higher (14.1 and 15.6 μg/ml, respectively). No toxicity for the host cells was noted with these compounds at a concentration up to 400 μg/ml, so that the selectivity indexes, as based on the ratio of the 50% cytotoxic dose to the 50% antiviral effective dose, were well in excess of 100. These findings may have far reaching implications both diagnostically, when attempts are made to isolate HIV from heparinized blood samples, as therapeutically, to the extent that dextran sulfate or heparin may be useful in blocking HIV replication in vivo.

Human immunodeficiency virus (HIV); Dextran sulfate; Heparin; AIDS

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Introduction

The pursuit of an effective antiviral chemotherapy for the acquired immunedeficiency syndrome (AIDS) has yielded various candidates, i.e. suramin (Mitsuya et al., 1984), phosphonoformate (Sandstrom et al., 1985), 3′-azido-2′, 3′-dideoxythymidine (AZT) (Mitsuya et al., 1985), and 2′,3′-dideoxynucleoside analogues (Mitsuya et al., 1986), which show promise as inhibitors of human immunodeficiency virus (HIV), the causative agent of AIDS. As these compounds are assumed to act as inhibitors of the reverse transcriptase, and the latter is of pivotal importance in the replicative cycle of retroviruses, the inhibitory effects of the compounds on HIV replication can be readily accounted for (De Clercq, 1986). Foremost among compounds presently pursued as chemotherapeutic agents against AIDS is AZT which completely protects ATH8 cells against the cytopathogenicity of HIV at a concentration of 1–5, μM (Mitsuya et al., 1985), and has proven efficacious in prolonging the survival of AIDS and ARC (AIDS-related complex) patients (Mitsuya et al., 1987).

However, in view of the severity and complexity of AIDS and the toxicity problems associated with the clinical use of AZT, the search for effective anti-HIV agents should be intensified. Recently, we reported that glycyrrhizin (GL), one of the aqueous extracts of licorice root, is inhibitory to HIV replication in vitro (Ito et al., 1987). GL consists of one molecule of glycyrrhetinic acid (GA) and two molecules of glucuronic acid and, therefore, it shares some structural features with the carboxylate polyanions polyacrylic acid and polymethacrylic acid which are known to interfere with virus infectivity, at least partly through inhibition of virus adsorption (De Somer et al., 1968a,b).

Further extending these observations we have now found that sulfate polyanions such as dextran sulfate and heparin are highly potent and selective inhibitors of HIV replication in vitro.

Materials and Methods

Cells and virus

The HTLV-I-carrying cell line, MT-4 (Harada et al., 1985), and clone 8 of the human leukemic T-cell line Molt-4 (Kikukawa et al., 1986), were used in our experiments. The cells were cultured and maintained in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/ml penicillin G and 100 μg/ml streptomycin (culture medium).

HIV was obtained from the culture supernatant of the Molt-4/HTLV-III cell line, as previously described (Harada et al., 1985).

Drugs

Dextran sulfate (MW = approximately 5000) and sodium heparin (197.1 U/ml) were obtained from Wako Chemical Co., Tokyo, Japan. Another preparation of

dextran sulfate (MW = 5000) was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. This preparation gave the same results as the preparation obtained from Wako Chemical Co. The compounds were dissolved in phosphate-buffered saline (PBS) and stored at 4°C until used.

Anti-HIV assay

Determination of the antiviral activity of the compounds against HIV replication was based on the inhibition of virus-induced cytopathogenicity, measured by trypan-blue exclusion, as previously described (Ito et al., 1987). Briefly, MT-4 cells and Molt-4 (clone 8) cells were infected with HIV at a multiplicity of infection (moi) of 0.002, and incubated for 1 h at 37°C. After virus adsorption, infected cells were washed and resuspended in culture medium. The number of MT-4 cells and Molt-4 (clone 8) cells was adjusted to 2×10^5 and 1×10^5 cells/ml, respectively. Then they brought into each well of a flat bottomed 96-well plastic microtiter tray containing various concentrations of the test compounds. After 4 days of incubation at 37°C, half of the cells and culture medium were removed, and fresh medium containing the same concentration of the compounds was added. After another 2 days of incubation, the number of viable cells was determined microscopically in a hematocytometer by Trypan Blue exclusion.

Assay for HIV-antigen expression

Virus-specific antigen expression in HIV-infected MT-4 cells or Molt-4 (clone 8) cells was determined by indirect immunofluorescence (IF), using a polyclonal antibody from a seropositive anti-HIV human serum and fluorescein isothiocyanate (FITC)-conjugated rabbit anti-human IgG (Dakopatts A/S, Copenhagen, Denmark). More than 500 cells were counted under a fluorescent microscope and the percentage of fluiorescent-positive cells was calculated.

Reverse transcriptase (RT) assay

The inhibitory effect of dextran sulfate on cell-free RT activity of HIV was examined with the HIV suspension concentrated by ultracentrifugation and poly(rA)·oligo(dT) as template, as previously described (Ito et al., 1987).

Results

When dextran sulfate and heparin were evaluated for their inhibitory effect on the cytopathogenicity of HIV in MT-4 cells, dextran sulfate and heparin completely protected the cells against destruction by the virus at a concentration of 8 and 40 μg/ml, respectively (Fig. 1A,C). When evaluated in Molt-4 (clone 8) cells, dextran sulfate and heparin achieved complete protection against HIV at a concentration of 25 and 50 μg/ml, respectively (Fig. 1B,D).

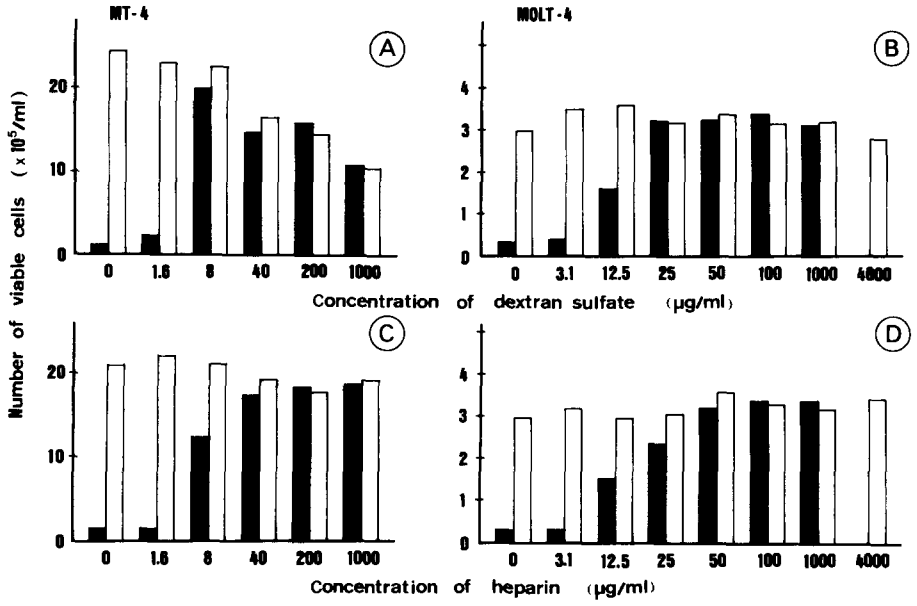

Fig. 1. Inhibition of the cytopathogenicity of HIV for MT-4 and Molt-4 (clone 8) cells by extran sulfate (A, B) or heparin (C, D). The cell viability was measured by trypan blue exclusion on day 6 after virus infection. The infected cells are indicated by solid columns (■) and the mock-infected cells are indicated by open columns (□).

When assayed for cytotoxicity in mock-infected MT-4 cells and Molt-4 (clone 8) cells, dextran sulfate reduced MT-4 cell viability to 42% of control at a concentration of 1000 μg/ml, whereas it did not reduce the viability of Molt-4 (clone 8) cells at a concentration up to 4000 μg/ml (Fig. 1A,B). Heparin did not affect the viability of either MT-4 cells or Molt-4 (clone 8) cells at a concentration up to 1000 and 4000 μg/ml, respectively (Fig. 1C,D). The 50% antiviral effective dose (ED_{50}) of dextran sulfate and heparin for HIV-infected cells and their 50% cytotoxicity dose (CD_{50}) for mock-infected cells are presented in Table 1. The selectivity indexes (SI), as based on the ratio of CD_{50} to ED_{50}, were 100 for dextran sulfate in MT-4 cells, >284 for dextran sulfate in Molt-4 cells, >133 for heparin in MT-4 cells and >256 for heparin in Molt-4 cells.

Next, the compounds were evaluated for their inhibitory efect on viral antigen expression. Dextran sulfate and heparin achieved a complete inhibition of viral antigen expression in MT-4 cells at a concentration of 8 and 40 μg/ml, respectively, and in Molt-4 (clone 8) cells at a concentration of 25 and 50 μg/ml, respectively (Table 2). These concentrations were the same as those required for complete protection of the MT-4 and Molt-4 cells against HIV cytopathogenicity.

When the effect of dextran sulfate on HIV RT activity was determined, it did not prove inhibitory, even at a concentration of 125 μg/ml, that is 15-fold higher than the concentration required to achieve complete protection against HIV replication in cell culture.

TABLE 1

Inhibitory effects of dextran sulfate and heparin on the replication of HIV in MT-4 and Molt-4 (clone 8) cell cultures.

Compound	Cell line	ED_{50}[a] (μg/ml)	CD_{50}[b] (μg/ml)	SI[c]
Dextran sulfate	MT-4	4.7	470	100
	Molt-4	14.1	>4000	>284
Heparin	MT-4	7.5	>1000	>133
	Molt-4	15.6	>4000	>256

[a] 50% Antiviral effective dose, based on the protection against the cytopathic effect of HIV.
[b] 50% Cytotoxic dose, based on a reduction in the viability of the mock-infected cells.
[c] Selectivity index (ratio of CD_{50} to ED_{50}).

Discussion

The antiviral effects of the sulfated polysaccharides, dextran sulfate and heparin, have been reported in the early years of antiviral research (Takemoto et al., 1965; Vaheri et al., 1964). The results presented here demonstrate that these compounds are potent and selective inhibitors of HIV replication in vitro. The polyanionic character of these compounds is reminiscent of other anionic compounds, such as suramin and Evans Blue, which contain six (suramin) or four (Evans Blue) sulfonic acid groups per molecule, and which have been shown previously to completely protect ATH8 cells against the cytopathogenicity of HIV at a concentration of 50 and 25 μg/ml, respectively (Balzarini et al., 1986). These values are

TABLE 2

Inhibitory effects of dextran sulfate and heparin on the expression of HIV-specific antigens in MT-4 and Molt-4 (clone 8) cells.

Concentration of compound (μg/ml)	Percentage of fluorescent MT-4 cells	
	Dextran sulfate	heparin
0	87.5	66.3
1.6	81.2	67.8
8	1.2	13.0
40	1.2	2.9
200	0.9	4.0
1000	2.7	2.1
	Percentage of fluorescent Molt-4 cells	
0	81.9	81.9
3.125	48.5	50.3
6.25	20.0	31.3
12.5	4.5	8.4
25	0.4	3.7
50	2.1	0.7
100	1.9	0.3
1000	0.2	0.6

identical to those of dextran sulfate and heparin in Molt-4 (clone 8) cells [25 and 50 μg/ml, respectively (Fig. 1B,D)]. However, the selectivity indexes of dextran sulfate and heparin (>284 and >256, respectively in Molt-4 cells) are much higher than those of suramin and Evans Blue (5 and >4, respectively) (De Clercq, 1986).

The exact mechanism(s) of action of dextran sulfate and heparin against HIV replication remain to be elucidated. Dextran was found to be non-inhibitory to HIV replication (data not shown), which suggests that the anionic (sulfate) groups of dextran sulfate are necessary for its anti-HIV activity. It would now seem mandatory to examine whether dextran sulfate and heparin, akin to other anionic polymers (De Somer et al., 1968a,b) might suppress an early event (adsorption/penetration) in the virus replicative cycle.

The present findings are clinically relevant from both a diagnostic and therapeutic viewpoint. The inhibitory activity of heparin on the infectivity of HIV may explain why on the one hand, it has often proven impossible to isolate and cultivate the virus from heparinized blood samples, and, on the other hand, HIV, in contrast with hepatitis B virus (HBV), infections rarely occur in dialysis patients who are heavily heparinized during the dialysis sessions. Should heparinization prove responsible for the reduced incidence of HIV infections in dialysis patients, it may be worth considering the heparinization procedure in aborting incipient HIV infections (J. Desmyter, personal communication).

Obviously, additional experiments remain to be done before heparin or dextran sulfate could be advocated for clinical use in the prophylaxis or therapy of retrovirus infections in humans. Aspects that need particular attention are the anticoagulant activity of the compounds at concentrations required to achieve inhibition of HIV infectivity and replication, and the dependence of both the anti-HIV activity and anticoagulant activity of dextran sulfate and heparin on the molecular weights of the compounds. These aspects, as well as the mechanism of anti-HIV action of heparin and dextran sulfate, represent interesting leads for further research that should help to establish the true potential of these compounds in the prophylaxis and therapy of retrovirus infections.

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References

Balzarini, J., Mitsuya, H., De Clercq, E. and Broder, S. (1986) Aurintricarboxylic acid and Evans Blue represent two different classes of anionic compounds which selectively inhibit the cytopathogenicity

of human T-cell lymphotropic virus type III/lymphadenopathy-associated virus. Biochem. Biophys. Res. Commun. 136, 64–71.

De Clercq, E., (1986) Chemotherapeutic approaches to the treatment of the acquired immune deficiency syndrome (AIDS). J. Med. Chem. 29, 1561–1569.

De Somer, P., De Clercq, E., Billiau, A., Schonne, E. and Claesen, M. (1968a) Antiviral activity of polyacrylic acid and polymethacrylic acids. I. Mode of action in vitro. J. Virol. 2, 878–885.

De Somer, P., De Clercq, E., Billiau, A., Schonne, E. and Claesen, M. (1968b) Antiviral activity of polyacrylic acid and polymethacrylic acids. II. Mode of action in vivo. J. Virol. 2, 886–893.

Harada, S., Koyanagi, Y. and Yamamoto, N. (1985) Infection of human T-lymphotropic virus type-I (HTLV-I) being MT-4 cells with HTLV-III (AIDS-virus): chronological studies of early events. Virology 146, 272–281.

Ito, M., Nakashima, H., Baba, M., Pauwels, R., De Clercq, E., Shigeta, S. and Yamamoto, N. (1987). Inhibitory effect of glycyrrhizin on the in vitro infectivity and cytopathic activity of the human immunodefiency virus [HIV (HTLV-III/LAV)]. Antiviral Res. 7, 127–137.

Kikukawa, R., Koyanagi, Y., Harada, S., Kobayashi, N., Hatanaka, M. and Yamamoto, N. (1986) Differential susceptibility to the acquired immunodeficiency syndrome retrovirus in clone cells of human leukemic T-cell line Molt-4. J. Virol. 57, 1159–1162.

Mitsuya, H., Popovic, M., Yarchoan, R., Matsushita, S., Gallo, R.C. and Broder, S. (1984) Suramin protection of T-cells in vitro against infectivity and cytopathic effect of HTLV-III. Science 226, 172–174.

Mitsuya, H., Weinhold, K.J., Furman, P.A., St. Clair, M.H., Nusinoff Lehrman, S., Gallo, R.C., Bolognesi, D., Barry, D.W. and Broder, S. (1985) 3′-Azido-3′-deoxythymidine (BW A509U): an antiviral agent that inhibits the infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy-associated virus in vitro. Proc. Natl. Acad. Sci. U.S.A. 82, 7096–7100.

Mitsuya, H. and Broder, S. (1986) Inhibition of the in vitro infectivity and cytopathic effect of human T-lymphotropic virus type III/lymphadenopathy associated virus (HTLV-III/LAV) by 2′, 3′-dideoxynucleosides. Proc. Natl. Acad. Sci. U.S.A. 83, 1911–1915.

Mitsuya, H. and Broder, S. (1987) Strategies for antiviral therapy in AIDS. Nature 325, 773–778.

Sandstrom, E.G., Kaplan, J.C., Byington, R.E. and Hirsch, M.S. (1985) Inhibition of human T-cell lymphotropic virus type III in vitro by phosphonoformate. Lancet i, 1480–1482.

Takemoto, K.K. and Spicer, S.S. (1965) Effects of natural and synthetic sulfated polysaccharides on viruses and cells. Ann. N.Y. Acad. Sci. 130, 365–373.

Vaheri, A. (1964) Heparin and related polyanionic substances as virus inhibitors. Acta Pathol. Microbiol. Scand., Suppl. 171, 1–98.