Antiviral Research, 11 (1989) 233–246
Elsevier

AVR 00355

Anti-HIV activity of dextran sulphate as determined under different experimental conditions

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(Received 30 January 1989; accepted 20 March 1989)

Summary

Dextran sulphate is a potent and selective inhibitor of human immunodeficiency virus type 1 (HIV-1). Its anti-HIV-1 activity has been investigated under varying experimental conditions. MT-4 cells were infected with HIV-1 at different multiplicities of infection (MOI), and treated with either dextran sulphate, 3′-azido-2′,3′-dideoxythymidine (AZT), or anti-HIV-1 serum obtained from an ARC patient. Dextran sulphate suppressed HIV-1 replication (as monitored by viral antigen expression) when the MOI was 0.01 or 0.1. It was ineffective at an MOI of 1.0. The anti-HIV-1 serum was only partially effective at an MOI of 0.01 and ineffective at an MOI of 0.1 or 1.0. AZT proved effective at all three MOIs. Co-cultures of uninfected and HIV-1-infected MT-4 cells were protected against destruction by dextran sulphate at a concentration of 10 and 100 μg/ml. To fully suppress viral antigen expression a concentration of 100 μg/ml was needed. When used at this concentration, a 1-h contact of dextran sulphate with the cells during the virus adsorption period sufficed to suppress HIV-1 antigen expression. In this sense, dextran sulphate behaved like the anti-HIV-1 serum. Dextran sulphate also behaved like OKT-4A in that they both inhibited HIV-1 attachment to the MT-4 cells, whereas OKT-4 failed to do so. However, dextran sulphate did not affect the binding of OKT-4A to the cells. The present results support the concept that dextran sulphate owes its anti-HIV-1 activity mainly to inhibition of virus binding to

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its target cells. The anti-HIV-1 activity of dextran sulphate is highly dependent on its sulphate content.

Anti-HIV activity; Dextran sulphate; Anti-HIV-1 serum; Sulphated polysaccharide

Introduction

Since the identification of human immunodeficiency virus (HIV) as the etiological agent of the acquired immune deficiency syndrome (AIDS) (Barré-Sinoussi et al., 1983; Popovic et al., 1984; Levy et al., 1984), various compounds have been reported to inhibit the replication of HIV in vitro, e.g. suramin (Mitsuya et al., 1984), phosphonoformic acid (Sandstrom et al., 1985), glycyrrhizin (Ito et al., 1987b), 3′-azido-2′,3′-dideoxythymidine (AzddThd, AZT) (Mitsuya et al., 1985; Nakashima et al., 1986), 2′,3′-dideoxycytidine (ddCyd, DDC) (Mitsuya and Broder, 1986), 2′,3′-dideoxycytidinene (ddeCyd, D4C) (Balzarini et al., 1986), 2′,3′-dideoxythymidinene (ddeThd, D4T) (Baba et al., 1987), and various other 2′,3′-dideoxynucleoside analogues, as recently reviewed by De Clercq (1987, 1988).

Following the suggestions by De Clercq (1986) that polyanionic substances such as dextran sulphate may be effective inhibitors of HIV adsorption to the cells, several sulphated polysaccharides, i.e. heparin (Ito et al., 1987a; Nagumo and Hoshino, 1988), dextran sulphate (Ito et al., 1987a; Ueno and Kuno, 1987; Baba et al., 1988c), pentosan polysulphate (Baba et al., 1988a), mannan sulphate (Ito et al., 1989), lentinan sulphate (Yoshida et al., 1988) and ribofuranan sulphate (Nakashima et al., 1987c) were shown to inhibit HIV infection in T4 cells at concentrations that were not toxic to the host cells.

Sulphated polysaccharides are known for their anticoagulant properties, and heparin is widely used as an anticoagulant. Furthermore, heparin has been shown to cause tumor regression in mice (Folkman et al., 1983). Heparin also inhibits DNA polymerase (i.e. reverse transcriptase) activity (DiCioccio and Sahai Srivastava, 1978), and so do sulphated polysaccharides extracted from sea algae (Nakashima et al., 1987a). Sulphated polysaccharides not only inhibited reverse transcriptase activity but also multinucleated giant cell formation (Nakashima et al., 1987c, 1988; Tochikura et al., 1988).

It has recently been demonstrated that sulphated polysaccharides, such as heparin (Baba et al., 1988b), dextran sulphate (Baba et al., 1988b; Mitsuya et al., 1988) and pentosan polysulphate (Baba et al., 1988a) are capable of blocking the binding of HIV particles to T4 lymphocytes. The present study was aimed at further deciphering the experimental conditions required for dextran sulphate to accomplish its anti-HIV activity.

Materials and Methods

Cells and virus

The HTLV-I-carrying human T cell line MT-4 was grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/ml of penicillin and 100 μg/ml of streptomycin. Viability of the cells was assessed by the trypan blue dye exclusion method, and the cells were subcultured twice a week at 3×10^5 cells/ml.

The HIV-1 strain HTLV-III_B was prepared from the supernatant of MOLT-4/HIV HTLV-III_B cells and stored in small aliquots (1.0 ml) at −80°C until used. The virus titer was determined by a plaque forming assay which has been described elsewhere (Nakashima et al., 1986, 1987a). The virus stock titer was 3×10^5 plaque-forming units (PFU)/ml.

Compounds

Dextran sulphate samples with a varying degree of sulphation were purchased from Kowa Co., Ltd. (Tokyo, Japan), and AZT was obtained from Yamasa Shoyu Co., Ltd. (Chiba, Japan). Unless stated otherwise, the dextran sulphate sample that was used in most experiments had a molecular weight of 7000 and a sulphur content of 18.1%.

Antisera

The anti-HIV-1 serum (He-1) was obtained from a patient with AIDS-related complex (ARC). The monoclonal antibodies OKT-4 and OKT-4A were purchased from Ortho Diagnostic Systems, Inc. (Raritan, NJ).

HIV-1 infection

MT-4 cells were infected with HIV-1 at different multiplicities of infection (MOI: 0.01, 0.1, 1.0) and incubated for 1 h at 37°C. After this virus adsorption period, the cells were washed and resuspended in fresh medium to a concentration of 3×10^5 cells/ml. The HIV-1-infected cell suspensions were then cultured in the presence or absence of the test compounds in a CO_2 incubator at 37°C. One half to one third of culture medium was changed every 3 days.

HIV-1 antigen expression

HIV-1-infected MT-4 cells were examined for virus-specific antigen expression by an indirect immunofluorescence (IF) method. To this end, the cells were fixed with methanol and incubated with human anti-HIV-1 serum (antibody titer, 1:4096), diluted 1:1000, for 30 min at 37°C. The cells were then washed for 15 min with phosphate-buffered saline (PBS, pH 7.4), incubated with fluorescein isothio-

cyanate (FITC)-conjugated rabbit anti-human IgG (Dakoppatts A/S, Copenhagen, Denmark) for 30 min at 37°C, and washed again with PBS. More than 500 cells were counted under a fluorescence microscope, and the percentage of IF-positive cells was determined.

HIV-1-induced cytopathogenicity

HIV-1-induced cytopathogenicity was monitored based on the decrease in the number of viable cells. Viability was assessed by the trypan blue dye exclusion method and viable cells were counted in an hemocytometer.

HIV-1 binding to the target cells

The supernatant of MOLT-4/HIV HTLV-III$_B$ cells was ultracentrifuged at 30000 $\times$ *g* for 1 h and the pellet was suspended in a 100-fold smaller volume of fresh medium. MT-4 cells (10^5 cells/ml) were seeded in 96-well microtiter plates, and 50 μl of the concentrated virus stock and 50 μl of varying concentrations of OKT-4, OKT-4A or dextran sulphate were added. After 1 h incubation at 37°C, the cells were washed 4 times with 0.5% bovine serum albumin (BSA) in PBS, and incubated with 50 μl anti-HIV-1 serum (diluted 1:100). After the cells were washed 4 times, 1 μCi of ^{125}I-labeled sheep F(ab)$_2$ antibody to human IgG (Amersham) was added, and the mixture was incubated for 1 h at 37°C. The cells were washed 4 times, dried, and cell-associated radioactivity was examined in a gamma radioactivity counter. All experiments were carried out in triplicate.

Results

Inhibition of viral antigen expression in MT-4 cells infected with HIV-1 at different multiplicities of infection

In the first set of experiments the inhibitory effect of dextran sulphate on HIV-1 replication was compared to that of AZT and anti-HIV-1 serum, which are assumed to inhibit reverse (RNA-to-DNA) transcription and virus-cell binding, respectively. When MT-4 cells were infected with HIV-1 at an MOI of 0.01, 0.1 or 1.0, the percentage of the viral antigen-positive cells one day after the infection was 2.5, 25 and 100%, respectively. All three cell cultures, whether infected at an MOI of 0.01, 0.1 or 1.0, became 100% positive for HIV-1 antigen expression within 3 days after infection (Fig. 1A). When added at a concentration of 2 μM after the 1-h virus adsorption period, AZT completely suppressed HIV-1 antigen expression, irrespective of the MOI (0.01, 0.1 or 1) (Fig. 1B). The marked contrast with AZT, anti-HIV-1 serum did not cause any change of viral antigen expression in cells which had been infected with an MOI of 1.0 or 0.1 (Fig. 1B). A delay in viral antigen expression was achieved by anti-HIV-1 serum in cells which had been infected with HIV-1 at an MOI of 0.01 (Fig. 1B). In cells which had been infected

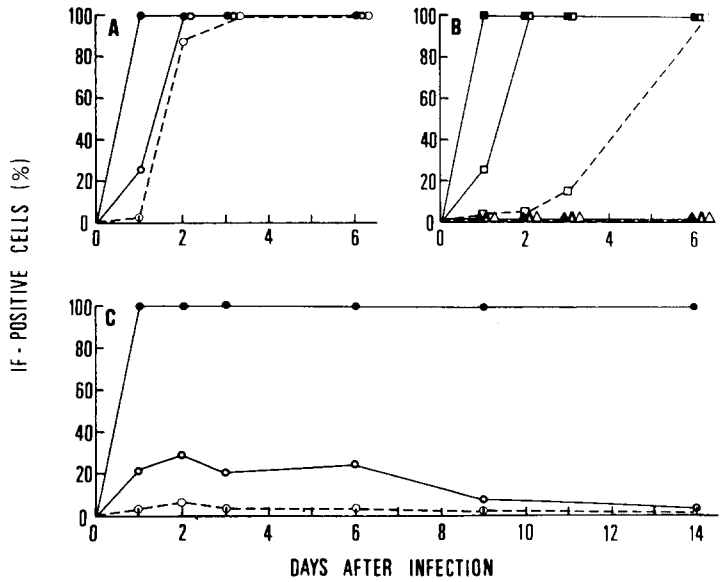

Fig. 1. Inhibition of HIV-1 antigen expression in HIV-1-infected MT-4 cells by AZT, anti-HIV-1 serum (He-1) or dextran sulphate. *Panel A*: cells were infected with HIV-1 at different multiplicities of infection [MOI = 1.0 (●—●), 0.1 (○—○), or 0.01 (○---○)]. *Panel B*: as in panel A, but following the 1-h virus adsorption period either 2 μM AZT [MOI = 1.0 (▲—▲), 0.1 (△—△) or 0.01 (△---△)] or 1% anti-HIV-1 serum [MOI = 1.0 (■—■), 0.1 (□—□) or 0.01 (□---□)] were added. *Panel C*: as in panel A, but following the 1-h virus adsorption period dextran sulphate at 100 μg/ml [MOI = 1.0 (●—●), 0.1 (○—○), or 0.01 (○---○)] was added.

with HIV-1 at an MOI of 1.0, dextran sulphate did not inhibit viral antigen expression, even at a concentration of 100 μg/ml (Fig. 1C). However, dextran sulphate reduced the number of antigen-positive cells, infected at an MOI of 0.1, to 20–30% and the number of antigen-positive cells, infected at an MOI of 0.01, to 2–3% (as evaluated 6 days after infection) (Fig. 1C). The percentage of viral antigen-positive cells in the cell cultures that had been infected at an MOI of 0.1 or 0.01 and then treated with dextran sulphate at 100 μg/ml became undetectable upon incubation of the cells for more than 6 days (Fig. 1C).

Inhibition of viral cytopathogenicity and viral antigen expression by dextran sulphate in co-cultures of HIV-1-infected and uninfected MT-4 cells

MT-4 cells were infected with HIV-1 at an MOI of 1.0 and cultured for 20 h at 37°C. Under these conditions, almost all cells became viral antigen-positive while not losing their viability. The HIV-1-infected MT-4 cells were then mixed with uninfected MT-4 cells at a ratio of 1:4 and cultured in the presence of dextran sulphate at 100 μg/ml or 10 μg/ml, or in the absence of dextran sulphate (control). In the control, the cell number rapidly decreased, and after 6 days of co-culturing, almost all cells had died (Fig. 2A). In co-cultures which were incubated in the

presence of dextran sulphate at 100 μg/ml the cell number increased at the same rate for uninfected MT-4 cells. Co-cultures of HIV-1-infected and uninfected MT-4 cells that were incubated with dextran sulphate at 10 μg/ml also continued to increase in cell number, albeit at a reduced pace, as compared to those incubated with 100 μg/ml of dextran sulphate. The expression of viral antigen was inversely correlated to the number of viable cells. The number of HIV-1 antigen-positive cells reached 100% within 1-day incubation of the co-cultures in drug-free medium (Fig. 2B). It took 3 days for co-culture to reach 100% viral antigen expression in the presence of 10 μg/ml of dextran sulphate. In co-cultures which had been incubated with 100 μg/ml of dextran sulphate the number of viral antigen-positive cells gradually decreased and from the 9th day of co-culturing viral antigen-positive cells were no longer detectable (Fig. 2B).

Inhibition of viral antigen expression in HIV-1-infected MT-4 cells by AZT, anti-HIV-1 serum or dextran sulphate exposed to the cells before and/or during virus adsorption

To ascertain whether dextran sulphate behaved like AZT or anti-HIV-1 serum in their dependence on the time of addition to the cells, the compounds were exposed to the cells either before virus adsorption, during virus adsorption, or both before and during virus adsorption. All experiments were carried out at an MOI of 1.0. Under these conditions, the number of HIV-1 antigen-positive cells attains 100% within 1 day after infection (Fig. 1A). When the MT-4 cells were treated for 18 h with 2 μM AZT, then washed and infected with HIV-1, the number of viral

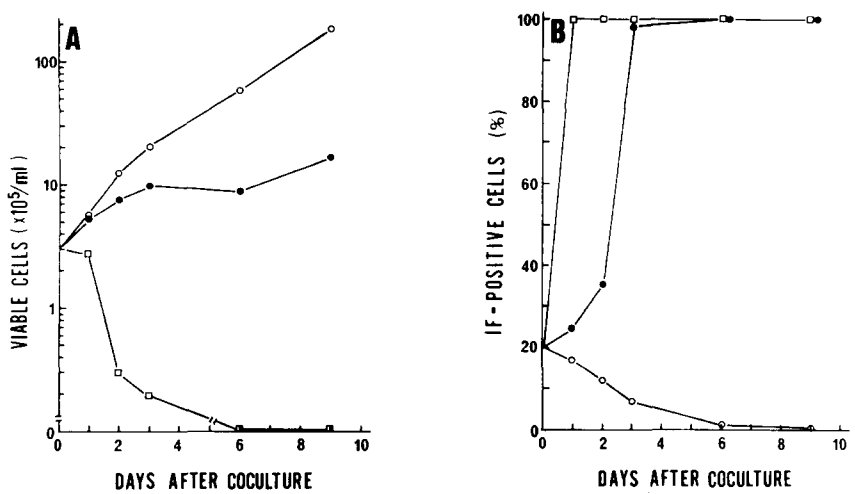

Fig. 2. Inhibition of HIV-1-induced cytopathogenicity (*panel A*) and viral antigen expression (*panel B*) by dextran sulphate in co-cultures of HIV-1-infected MT-4 cells with uninfected MT-4 cells. The cell co-cultures were incubated in the presence of dextran sulphate at 100 μg/ml (○—○) or 10 μg/ml (●—●) or in the absence of dextran sulphate (□—□).

antigen-positive cells was less than 1% and 16% at 3 days and 6 days after infection, respectively (Fig. 3B). When the cells were treated with 2 μM AZT for only 1 h during the virus adsorption period, expression of viral antigen was reduced to a similar extent as following pretreatment with AZT. In cultures which were treated with AZT both before and during virus adsorption, the number of viral antigen-positive cells at 6 days after infection did not exceed 2%. However, all these cells became HIV-1 antigen-positive at 9 days after infection (Fig. 3B). Pretreatment of the cells with 1% anti-HIV-1 serum did not affect viral antigen expression (Fig. 3A). Treatment of the cells with the anti-HIV-1 serum for 1 h during the virus adsorption period resulted in a virtually complete inhibition of viral antigen expres-

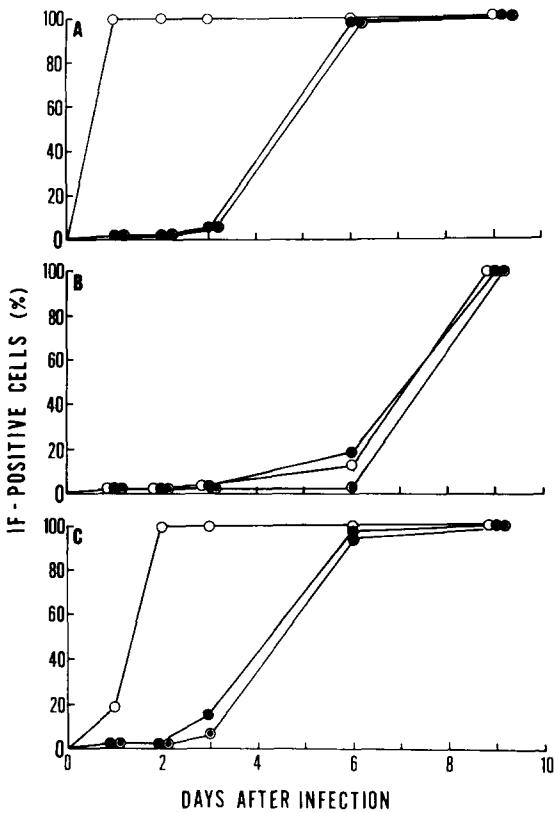

Fig. 3. Inhibition of viral antigen expression in HIV-1-infected MT-4 cells following treatment of the cells before and/or during virus adsorption with AZT, anti-HIV-1 serum or dextran sulphate. *Panel A*: cells were treated with 1% anti-HIV-1 serum for 18 h before HIV-1 infection (○—○), or for 1 h during HIV-1 adsorption (●—●), or for 18 h before virus adsorption and for 1 h during virus adsorption (◉—◉). *Panel B*: cells were treated with 2 μM AZT for 18 h before HIV-1 infection (○—○), or for 1 h during HIV-1 adsorption (●—●), or for 18 h before virus adsorption and for 1 h during virus adsorption (◉—◉). *Panel C*: cells were treated with dextran sulphate at 100 μg/ml for 18 h before HIV-1 infection (○—○), or for 1 h during HIV-1 adsorption (●—●), or for 18 h before virus adsorption and for 1 h during virus adsorption (◉—◉). MOI was 1.0 in all the experiments.

sion till the 3rd day after infection. However, all cells became antigen-positive at 6 days after infection (Fig. 3A). When the cells were treated with dextran sulphate at 100 μg/ml for 1 h during the virus adsorption period, viral antigen expression was significantly delayed (Fig. 3C), as compared to the untreated cells, in which viral antigen expression reaches 100% within 1 day after infection (Fig. 1A). When dextran sulphate was added at 18 h before virus infection, and removed again immediately before virus infection, it retarded viral antigen expression by 1 day (100% antigen expression was now attained at 2 days after infection) (Fig. 3C). Treatment of the cells with dextran sulphate before and during virus adsorption afforded not much more protection than treatment during the virus adsorption period only (Fig. 3C). From the kinetics of the curves obtained for treatment before and/or during virus adsorption (Fig. 3), it is evident that dextran sulphate behaved very much like anti-HIV-1 antibody.

Inhibition of HIV-1 binding to MT-4 cells by anti-CD4 monoclonal antibody (OKT-4, OKT-4A) and dextran sulphate

A novel method was developed to monitor the binding of HIV-1 to the cells (see Materials and Methods), and this method was used to assess whether dextran sul-

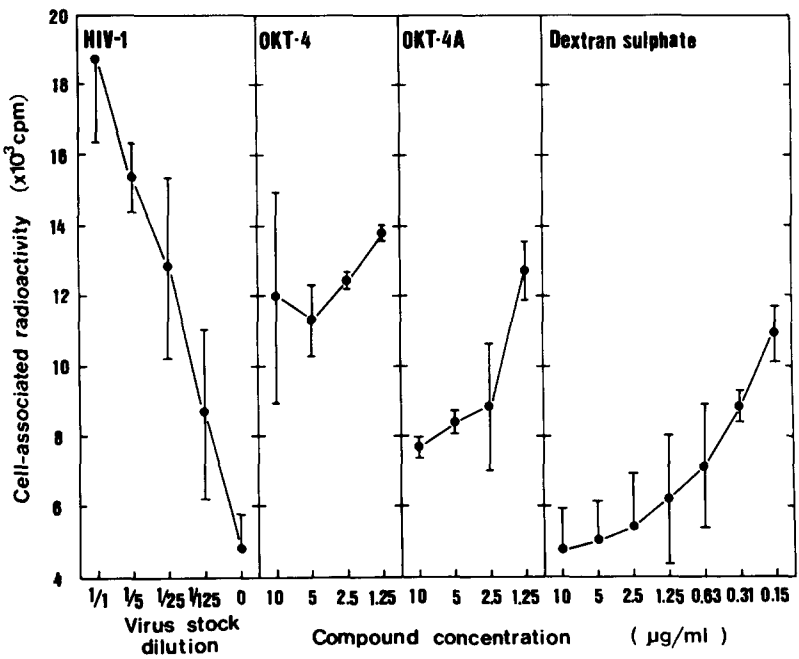

Fig. 4. Inhibition of HIV-1 binding to MT-4 cells by monoclonal antibody to the cell receptor for HIV-1 (OKT-4, OKT-4A) and dextran sulphate. Left panel, virus dose-response curve, as established with various dilutions of virus stock. Dilution 1/1 was used in the experiments with OKT-4, OKT-4A and dextran sulphate. Results represent the mean value ± SD of two experiments, each performed in triplicate.

phate was able to block binding of HIV-1 particles to MT-4 cells. In parallel with dextran sulphate, monoclonal antibodies (OKT-4 and OKT-4A) to the cell receptor (CD4) for HIV-1 were also examined for their ability to block virus adsorption. MT-4 cells were exposed to concentrated HIV-1 (× 100) in the presence of either OKT-4, OKT-4A or dextran sulphate at different concentrations; binding of the virus particles to the cells was examined by a radioimmunoassay and is expressed as cell-associated radioactivity (Fig. 4). When the concentrated virus stock was diluted, cell-associated radioactivity decreased in a dose-dependent fashion. OKT-4 effected a partial reduction in the amount of radioactivity that became associated with the cells. OKT-4A clearly had a more pronounced effect on virus binding than OKT-4; but the clearest inhibitory effect was shown by dextran sulphate, which suppressed virus binding by more than 95% at a concentration of 10, 5 and 2.5 μg/ml (Fig. 4).

In additional experiments, the possibility was examined whether dextran sulphate might interfere with the binding of OKT-4A monoclonal antibody to MT-4 cells. Dextran sulphate did not inhibit the binding of OKT-4A to the surface receptor of MT-4 cells when 10 μg/ml of the compound was added (data not shown).

Inhibition of HIV-1 infection by dextran sulphate samples with varying sulphate content

To assess the influence of degree of sulphation, dextran sulphate samples of similar polymer length (16 to 20 glucose units) but varying sulphate content (4.8, 9.1 and 18.1% sulphur) were investigated for their protective activity against virus-induced cytopathogenicity. As shown in Fig. 5, the sample with the highest sulphate content (S: 18.1%) displayed the most marked anti-HIV-1 activity (full

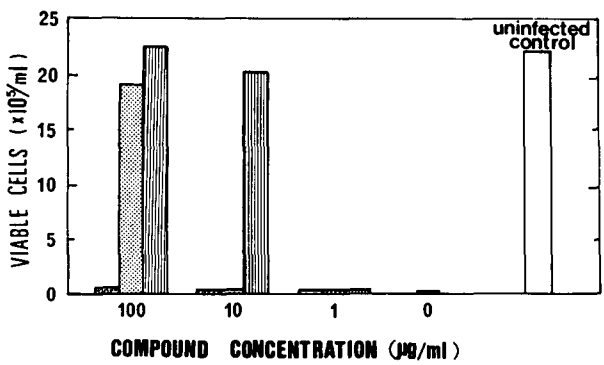

Fig. 5. Effect of dextran sulphate samples with varying sulphate content on HIV-1-induced cytopathogenicity. HIV-1-infected MT-4 cells (MOI: 0.002) were adjusted to 3 × 10^5 cells/ml and further incubated in the presence of dextran sulphate samples with different sulphate content: 18.1% (▥); 9.1% (▨); or 4.8% (▤). The number of viable cells was determined by trypan blue dye exclusion at 6 days after infection. The number of viable cells in the uninfected control (□) (without dextran sulphate) was 22.5 × 10^5/ml; whereas the corresponding cell number was < 10^4/ml for the infected untreated MT-4 cells (■).

protection at a concentration of 10 μg/ml). The sample with S: 9.1% exhibited anti-HIV-1 activity only at a concentration of 100 μg/ml, and the sample with S: 4.8% had no effect whatsoever. When the sulphate content remained constant and the length of the polymer chain was varied, anti-HIV-1 activity increased as the molecular weight increased from 1000 to 10000 and remained virtually constant when the molecular weight further increased to 500000 (Baba et al., 1988c).

Discussion

As stated in the Introduction, various sulphated polysaccharides, e.g. dextran sulphate, heparin, pentosan polysulphate, mannan sulphate and carrageenans have proven to be potent and selective inhibitors of HIV-1. Although dextran sulphate is inhibitory to the reverse transcriptase of HIV-1 (Nakashima et al., 1987c) it is unlikely that inhibition of reverse transcriptase activity plays any role in the mechanism of action of the sulphated polysaccharides: *first*, because it is questionable that these high-molecular-weight compounds readily penetrate the cells and reach the reverse transcriptase inside the cells; and *second*, inhibition of reverse transcriptase is achieved at a concentration which is much higher than that required for inhibition of virus adsorption and virus replication. In fact, the doses at which dextran sulphate inhibit HIV-1 replication closely correlate with those that are required to inhibit HIV-1 attachment to the cells (Schols et al., 1989).

Thus, dextran sulphate, and other sulphated polysaccharides as well, may owe their mechanism of action to an interaction with the first step in the virus replicative cycle, that is virus attachment to the cells. That dextran sulphate, heparin and pentosan polysulphate are able to block virus binding to the cells has been directly demonstrated by using radiolabeled HIV particles (Baba et al., 1988a,b; Mitsuya et al., 1988) as well as immunofluorescence flow cytometry (Schols et al., 1989). As a result of their inhibitory effect on virus-cell binding, sulphated polysaccharides may also be expected to block virus-induced cell fusion (syncytium formation) (Nakashima et al., 1987b,c, 1988; Tochikura et al., 1988).

In the present study, the anti-HIV activity of dextran sulphate was explored under varying experimental conditions and compared with that of AZT, which is supposed to act as a reverse transcriptase inhibitor following intracellular phosphorylation to its 5′-triphosphate (Furman et al., 1986), and anti-HIV-1 serum, which may be expected to interact with the attachment of the virions to the cells. In the absence of any inhibitor, viral antigen expression by HIV-1-infected MT-4 cells was highly dependent on the MOI: i.e., 100% viral antigen expression was achieved at 1, 2 and 3 days after infection with an MOI of 1.0, 0.1 and 0.01, respectively. When the neutralizing antibody was added after HIV-1 exposure, no significant difference in the number of antigen-positive cells was observed in cell cultures infected at an MOI of 1.0 or 0.1. Only at an MOI of 0.01 was the expression of viral antigens delayed. On the other hand, viral antigen expression was completely suppressed by AZT, even at an MOI of 1.0. The pattern of anti-HIV activity shown by dextran sulphate (Fig. 1) was similar to that of the anti-HIV-1

serum, which suggests that dextran sulphate acted in a similar fashion as the neutralizing antibody, i.e. interfered with virus binding to the cells.

When AZT, anti-HIV-1 serum and dextran sulphate were added at different times, i.e. before or during virus adsorption (Fig. 3), again the pattern of anti-HIV activity shown by dextran sulphate was similar to that of the neutralizing antibody but different from that of AZT. From these results again it can be postulated that dextran sulphate interferes with the virus binding to the cells.

HIV binding to CD4+ T cells is inhibited by monoclonal antibodies to the cellular receptor (CD4) for HIV, and by human polyclonal and murine monoclonal antibodies to the viral glycoprotein gp 120 (McDougal et al., 1986). Dextran sulphate inhibited binding of HIV-1 to MT-4 cells (Fig. 4), and it did so with even greater efficiency than did the monoclonal antibody to CD4 (OKT-4A). However, dextran sulphate did not interfere with the binding of OKT-4A to MT-4 cells, which is in agreement with our previous observations (Baba et al., 1988b).

Our results thus corroborate the hypothesis that the anti-HIV activity of dextran sulphate is mainly due to inhibition of virus binding to the cells. In this respect dextran sulphate acts in a similar fashion as anti-HIV-1 serum and OKT-4A monoclonal antibody. What cannot be excluded, however, is that dextran sulphate, in addition to its inhibitory effect on virus attachment, may also act at an intracellular stage of the virus replicative cycle. Although little is known about the uptake of sulphated polysaccharides by the cells, the data of González et al. (1987) suggest that they may actually penetrate into the cells. According to these authors, carrageenans would inhibit a step in the herpes simplex virus replicative cycle that is subsequent to viral internalization but prior to the onset of late viral protein synthesis.

Where dextran sulphate proved superior to neutralizing antibody was in its capacity to suppress viral antigen expression in cells which had been infected with HIV at an MOI of 0.1 or 0.01 (Fig. 1C). This phenomenon became particularly evident after a prolonged incubation time and suggests that dextran sulphate may be able to block virus spread from infected to uninfected cells and thus keep uninfected cells protected against HIV-1 infection. This property of dextran sulphate may have interesting therapeutic implications, i.e. in HIV-infected individuals.

The antiviral activity of dextran sulphate is highly dependent on its sulphate content (Fig. 5) and molecular weight (Baba et al., 1988c). Dextran sulphate is used in Japan for the treatment of hyperlipidemia and side effects are well documented (Uchida et al., 1975; Ohji et al., 1978). Oral administration of high-molecular-weight dextran sulphate may cause ulcerative colitis. On the other hand, intravenous administration of high-molecular-weight dextran sulphate may lead to allergic reactions and renal toxicity. For this reason, a dextran sulphate preparation of 3000 dalton is used for intravenous administration in Japan. However, of dextran sulphate this molecular weight has only weak anti-HIV-1 activity (Baba et al., 1988c; Nakashima et al., unpublished data). In accordance with the report of phase I trial (Abrams et al., 1989), oral dextran sulphate is well tolerated. Further synthetic approaches should be directed toward the development of sulphated polysaccharides with the appropriate molecular weight and sulphate content so as to yield maximal antiviral activity with minimal toxicity.

Acknowledgements

This work was supported by Grants-in-Aid for Cancer Research from the Ministry of Education, Science, and Culture of Japan, and Grant-in-Aid from the Mochida Memorial Foundation for Medical and Pharmaceutical Research. We thank Christiane Callebaut for her excellent editorial assistance.

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