Sulphoevernan, a polyanionic polysaccharide, and the narcissus lectin potently inhibit human immunodeficiency virus infection by binding to viral envelope protein

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Sulphoevernan is a sulphated α-1→3, 1→4 polyglucan (M, 20000) with a helical structure. This compound effectively inhibits both human immunodeficiency virus type 1 (HIV-1) and type 2 infection of cells in vitro at concentrations around 0.5 μg/ml. Moreover, the compound completely inhibits HIV-1-induced syncytium formation at a concentration of 1 μg/ml. Competition experiments with 35S-labelled sulphoevernan revealed that the mannose-specific lectin from Narcissus pseudonarcissus prevented binding of sulphoevernan to HIV-1, whereas the antibody OKT4A did not reduce the amount of sulphoevernan bound to MT-2 cells. These data indicate that the non-cytotoxic polymer sulphoevernan binds to the virus rather than to the host cell. In vivo studies, using Rauscher leukaemia virus in NMRI mice, revealed that, at a daily dose of 20 mg/kg, the animals were protected against virus-induced increases in spleen weight. From these in vitro and in vivo data we conclude that sulphoevernan has potential in the treatment of acquired immunodeficiency syndrome.

Introduction

Antiretroviral chemotherapy of patients with acquired immunodeficiency syndrome with dideoxynucleosides, such as azidothymidine (AZT), does help some patients (Fischl et al., 1987). However, the toxicity of AZT, a compound which presumably inhibits viral DNA polymerase in infected cells, is such that new strategies are needed (Cohen, 1987; Henderson & Gerberding, 1989). One strategy is to develop substances that interfere with viral adsorption and penetration by blocking the CD4 receptor or the viral glycoprotein (Bagasra & Lischner, 1988). It was discovered that dextran sulphate was able to block infection of cells by human immunodeficiency virus type 1 (HIV-I) (Ueno & Kuno, 1987). Subsequently, other sulphated polysaccharides, e.g. heparin sulphate, chondroitin sulphate and polysulphated polyxylan, were found to have anti-HIV activity in vitro (Biesert et al., 1988; Baba et al., 1989). These compounds inhibit virus adsorption and syncytium formation (Biesert et al., 1988; Biesert et al., 1988), although a direct influence of these drugs on the infectivity of the virus could not be demonstrated (Biesert et al., 1988).

The hitherto studied sulphated polysaccharides with in vitro anti-HIV-1 activity are linear molecules. We now show that sulphoevernan, which is an unbranched α-1→3, 1→4 sulphated polyglucan (Stefanovich, 1969a), displays a potent anti-HIV (type 1 and 2) activity in vitro. In vivo studies using the Rauscher leukaemia virus (RLV)/NMRI mouse system supported the anti-retroviral effect of sulphoevernan. The characteristic feature of this sulphopolyglucan is its helical structure and its antiviral activity, derived primarily by binding to the virus itself. Moreover, we demonstrate that a new lectin from Narcissus pseudonarcissus (NPL), which is characterized by a high specificity for (α-1,3) mannosyl residues, is equally effective against HIV infection in vitro.

Methods

Compounds. [35S]Methionine (1500 Ci/mmol) and chloro[35S]sulphonic acid (40 mCi/mmol) were obtained from Amersham. The
monoclonal antibody OKT4A was from Ortho Diagnostics and Nycomed from Nyegaard. AZT was a gift of Professor W. Prusoff (Yale University, New Haven, Conn., U.S.A.).

Preparation of everan and sulphoevernan. The polysaccharide everan was isolated from oak lichen (Evernia prunastri L. Ach.) as described previously (Stefanovich, 1960). This α-polyglucan contains approximately 80% 1→3 and 20% 1→4 linkages and has an Mₐ ranging between 18K and 34K (Stefanovich, 1969a).

Sulphonation of everan was performed with chlorosulphonic acid, as described by Stefanovich (1969b). The material obtained had an Mₐ of 20K and the degree of substitution with the sulfo group was 1. The 6-O-sulpho group is located on the outside of the helical structure of sulphoevernan (Stefanovich, 1969a). ³⁵S-labelled sulphoevernan was prepared from everan using chloro[³⁵S]sulphonic acid as a radio-labelled precursor; the specific radioactivity was 1-2 mCi/mmol of glucose.

Narcissus lectin. Lectin NPL was purified as described (Van Damme et al., 1988). This lectin is specific for Man(α-1,3)Man residues (Van Damme et al., 1988).

Virus strains. The human T-lymphotropic virus, type IIIB (HTLV-IIIB) (Popovic et al., 1984) strain of HIV-1 and two HIV-2 strains, HIV-2ag (Kong et al., 1988) and HIV-2as (Kanki et al., 1988) were tested. HIV-1 particles were prepared from the medium of HTLV-IIIB-infected H9 cells as described by Popovic et al. (1984).

Radiolabelled virus was prepared as follows. Two week old HTLV-IIIB-infected H9 cells (Popovic et al., 1984) were incubated in medium containing 50 nCi/ml [³⁵S]methionine for 2 days. A cell-free supernatant was obtained by low-speed centrifugation (4000 g, 10 min, 4 °C). This was then dialysed against a 0.1 M-Tris-HCl buffer (pH 7.4, 0.1 M-NaCl, 0.001 M-EDTA) for 5 h at 4 °C, and virus particles were collected by centrifugation at 30000 r.p.m in a Beckman Ti-45 rotor for 2 h at 4 °C. The resulting pellet was centrifuged over a 3 m linear sucrose gradient for 24 h as described (Popovic et al., 1984); the fractions within the density range 1.18 to 1.15 g/ml were collected. The specific radioactivity varied between 1.3 × 10⁻⁶ and 2.9 × 10⁻⁶ Ci/virion.

The particles were counted by electron microscopy (Popovic et al., 1984). A second purification procedure, the Nycozenz gradient centrifugation technique, was applied to prepare the viruses (Vilmer et al., 1984). After this procedure, the specific activity of the virus preparation was found to be identical to that obtained by sucrose gradient centrifugation. This is one indication that no cellular membranes had cosedimented with the virus particles.

Cells and virus infection. The human T cell lines MT-2 (Harada et al., 1985), CEM (Nara & Fischinger, 1988), AT8 (Mitsuya & Broder, 1986) and H9 (Popovic et al., 1984), the human monocye line U937 (Ezekovitz et al., 1989) and the somatic cell hybrid culture between CEM and the B cell line 174 (Kong et al., 1988) were grown in RPMI 1640 medium supplemented with 15% (v/v) foetal calf serum. Cultures were maintained at 37 °C in a humidified atmosphere at 5% CO₂ in air.

HIV-1 infection. Cells were routinely seeded at a concentration of 1 × 10⁵ cells/ml and HIV-1 virus was added to give an m.o.i. of 3 TCID₅₀ per MT-2 cell and 12 TCID₅₀ per CEM or U937 cell. The cells were incubated in the presence or absence of the test compounds for 7 days. During that time, the uninfected MT-2 cells underwent 2±1 doubling steps, CEM cells 2±1 doublings and U937 cells 1±3 doublings. The infected cells underwent the following doubling steps: MT-2/HIV-1, 0.99, CEM/HIV-1, 0.04, and U937/HIV-1, 0.08.

Except where otherwise mentioned, clarified conditioned HIV-1 culture fluid was pretreated (30 min at 36 °C) with the different compounds and then added to the cells to provide the indicated compound and virus concentrations. The virus preparation was preincubated together with the compound in a volume of 100 µl, resulting in a 10-fold higher concentration than that finally present in the cell culture experiment.

HIV-2 infection. HIV-2ag-producing CEMx174 cells and HIV-2as-producing U937 cells were irradiated with 10000 rads and then cocultivated at a density of 4 × 10⁶ cells/ml, final assay volume, with 2 × 10⁶ ATH8 cells. After 7 days incubation, only the ATH8 cells were alive and their densities were estimated. The test compounds were added to the irradiated cells 30 min prior to the addition of the ATH8 cells. In the uninfected control cultures, the ATH8 cells underwent 2±1 doubling steps during the 7 day incubation.

Evaluation. Cell concentrations were routinely evaluated using the XTT colorimetric assay system (Scudiero et al., 1988), followed by evaluation of the ELISA reader (Bio-Rad, model 3550, equipped with the program NCIMR IIIB). To standardize growth curves, the cells were counted electronically (Cytocomp Counter; model Michaelis). The number of doubling steps was determined as described (Müller et al., 1975).

The 50% cytoprotective concentration (IC₅₀) represents that concentration at which growth of HIV-infected cells reached 50% of the growth rate of the uninfected cells during an incubation period of 7 days. The 50% cytotoxic concentration (TC₅₀) represents that concentration at which the growth rate of the infected cells was reduced by 50%; this value is usually similar to the TC₅₀ of uninfected cells. The 50% values were estimated by logit regression (Sachs, 1984). The antiviral index (AI) is calculated from the ratio TC₅₀:IC₅₀.

To test for virus release after the 7 days incubation period, cells were removed by centrifugation and the supernatant was assayed for reverse transcriptase (RT) activity (Schröder et al., 1989). The percentage of cells expressing the p24 gag proteins of HIV-1 was determined by indirect immunofluorescence microscopy, with the use of mouse monoclonal antibodies to HIV-1 p24. The positive cells were visualized by treatment with fluorescein-labelled goat anti-mouse IgG (Poeisz et al., 1980); the reactivity of the antibodies with HIV-1 infected cells was in the range of 30 to 40%.

Syncytium induction assay. This assay was performed as described (Matthews et al., 1987). A total of 1 × 10⁵ HIV-1-infected H9 cells were mixed with 1 × 10⁵ uninfected Jurkat cells in a final volume of 100 µl, either in the presence or the absence of the compounds. Syncytium formation (defined as > 4 nuclei within a common cell membrane) was semiquantitatively scored 5 and 24 h later (Lifson et al., 1986): — = no syncytia; 1++; rare small syncytia; 2++; multiple moderately sized syncytia; 3++; large syncytia in most, but not all microscope fields (magnification 400×), 4++; numerous large syncytia in all fields examined.

Virus-cell binding studies. MT-2 cells (1 × 10⁵) were suspended in 1 ml of binding assay buffer [20 mM-sodium phosphate, 1 mM-CaCl₂, 130 mM-NaCl, 2 % w/v bovine serum albumin (Müller et al., 1982)]. Then, 20 µl of labelled virus (approximately 25 × 10⁴ d.p.m./assay [final]) was added and incubated for 0 to 60 min at 37 °C in 5% CO₂. The cells were subsequently washed by centrifugation (2000 g, 10 min, 4 °C) and their radioactivity counted. Where indicated, the virus preparation (20 µl) was preincubated for 1 h at 4 °C with a 10 µl solution of sulphoevernan, evernan or NPL and then added to the cells.

[³⁵S]Sulphoevernan binding studies. Binding of sulphoevernan to HIV-1 was elucidated as follows. Purified HIV-1 was incubated in a final volume of 200 µl of binding assay buffer, in the presence or absence of CaCl₂, for 30 min at 4 °C together with 0.4 µg of [³⁵S]sulphoevernan (equivalent to 5.8 × 10⁴ d.p.m.). Then the suspension was centrifuged in an Airforge (Beckman, rotor A100/18) at 110000 g (1 h, 4 °C). The virus suspension was washed twice by centrifugation (110000 g, 1 h, 4 °C) and the radioactivity in the pelleted material was counted. Where indicated, the virus particles (20 µg/20 µl) were
preincubated in the binding buffer (in the presence or absence of 1 mM CaCl₂) with 10 µg/20 µl of NPL for 1 h at 4 °C. The background value (assay without virus protein and in the presence of Ca²⁺) was < 70 d.p.m./200 µl.

Binding of [³⁵S]sulphoevernan to uninfected MT-2 cells or HIV-1-infected MT-2 cells [infection was performed for 3 days as described above] was determined similarly. MT-2 cells (1 x 10⁵), in a final volume of 1 ml binding buffer, were preincubated with 0 or 100 µg/ml of OKT4A antibody, in the presence or absence of 1 mM-CaCl₂, for 30 min at 4 °C. Then, the cell suspension was washed twice by centrifugation (2000 g, 10 min, 4 °C) and the cells were incubated (1 h at 4 °C) in a 1 ml volume, in the presence or absence of 1 mM-Ca²⁺, with 0-2 µg of [³⁵S]sulphoevernan. Finally, the cells were washed twice with binding buffer and the cell-associated radioactivity was determined. The background value (assay without cells but in the presence of Ca²⁺) was <50 d.p.m./1 ml.

RLV infection of mice. RLV, obtained from the spleens of virus-infected BALB/c mice, was injected intraperitoneally into NMRI mice. Groups of five animals were each treated subcutaneously with different doses of sulphoevernan as indicated; the treatment started 1 or 5 days post-infection. At day 20 the animals were killed and the weight of the spleens was determined.

Protein concentration. This was determined as described (Lowry et al., 1951), using bovine serum albumin as a standard.

Statistics. For the statistical evaluation, Student's t-test was applied (Sachs, 1984).

Results

Anti-HIV activity of sulphoevernan and NPL

The compounds evernan, sulphoevernan, a sulphated polyglucan (Fig. 1), and NPL were tested for anti-HIV activity by the cytoprotection assay; cell density was determined by the XTT tetrazolium/formazan assay. As shown in Table 1, sulphoevernan displayed a strong cytoprotective activity both on HIV-1- and HIV-2-infected cells. The dose–response curves show that the compound was not at all toxic to the cells up to the maximum concentration tested of 100 µg/ml. The IC₅₀ in the assays with HIV-1-infected cells was determined to be 0-32 µg/ml (equivalent to 16 nM) and for HIV-2-infected cells 0-38 µg/ml (equivalent to 19 nM). In both virus-infected systems, sulphoevernan at concentrations above 1 µg/ml gave a 100% cytoprotective effect.

In addition to U937 monocytes, we tested the human T cell lines MT-2 and CEM in order to determine whether the anti-HIV-1 cytoprotective effect was dependent on the host cell line. As summarized in Table 1, the antiviral index for sulphoevernan varied from >192 to >312. Although on a weight/concentration basis it was much less effective, on a molar basis sulphoevernan [IC₅₀ 0-52 µg/ml (equivalent to 26 nM)] was as effective as AZT [IC₅₀ 0-008 µg/ml (equivalent to 30 nM)] in the MT-2/HIV-1 system. The non-sulphonated evernan was without any effect, whereas the mannose-specific NPL displayed a considerable anti-HIV-1 cytoprotective effect, with an AI between >14 and >46.

The anti-HIV-2 effect of sulphoevernan was equally strong in both the CEM × 174/HIV-2₅₅ and the U937/HIV-2₅₅ systems, where the AI was determined to be >137 and >263, respectively. Moreover, this compound is characterized by an IC₅₀ value of 0-73 µg/ml.

Table 1. Cytoprotective effects of AZT, evernan, sulphoevernan and NPL on growth of different HIV-1- and HIV-2-infected cells

<table>
<thead>
<tr>
<th>HIV-1</th>
<th>MT-2/HIV-1</th>
<th>CEM/HIV-1</th>
<th>U937/HIV-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>IC₅₀ (µg/ml)</td>
<td>TC₅₀ (µg/ml)</td>
<td>AI</td>
</tr>
<tr>
<td>AZT</td>
<td>0-008</td>
<td>11-7</td>
<td>1462</td>
</tr>
<tr>
<td>Evernan</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Sulphoevernan</td>
<td>0-52</td>
<td>&gt;100</td>
<td>&gt;192</td>
</tr>
<tr>
<td>NPL</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HIV-2</th>
<th>CEM×174/HIV-2₅₅</th>
<th>U937/HIV-2₅₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound</td>
<td>IC₅₀ (µg/ml)</td>
<td>TC₅₀ (µg/ml)</td>
</tr>
<tr>
<td>AZT</td>
<td>0-023</td>
<td>8-7</td>
</tr>
<tr>
<td>Evernan</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Sulphoevernan</td>
<td>0-73</td>
<td>&gt;100</td>
</tr>
<tr>
<td>NPL</td>
<td>6-58</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Fig. 1. Structure of the sulphated polyglucan, sulphoevernan.
Table 2. Neutralization of HIV-1-induced Jurkat cell fusion by sulphoevernan and NPL*

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µg/ml)</th>
<th>5 h</th>
<th>24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>–</td>
<td>4+</td>
<td>4+</td>
</tr>
<tr>
<td>Sulphoevernan</td>
<td>0.1</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>1+</td>
<td>2+</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Evernan</td>
<td>50.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>NPL</td>
<td>0.3</td>
<td>3+</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1+</td>
<td>1+</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Syncytium were scored 5 and 24 h after addition of uninfected Jurkat cells to HIV-1-producing H9 cells. The compounds were added at the beginning of the co-incubation period and remained in the assays until the evaluation.

(equivalent to 37 nM), which is again superior to the IC_{50} value for AZT [0.023 µg/ml (equivalent to 86 nM)] under the in vitro conditions (CEM × 174/HIV-2(ß)) used. In addition, sulphoevernan displayed no cytotoxic effects up to 100 µg/ml, whereas AZT caused a 50% cytotoxic effect at concentrations around 9 µg/ml (Table 1). For comparison, we have included in the tables the inhibitory activity of AZT against HIV-1 and HIV-2.

To demonstrate that the cytoprotective effect displayed by sulphoevernan was due to a direct anti-HIV effect, the reduction in the expression of HIV-1 protein p24 as well as the reduction in virus particles (measured by the determination of RT level) was determined in the CEM/HIV-1 system (data not shown). At a concentration of 0.5 µg/ml of sulphoevernan, a 49% inhibition of HIV-1 p24 expression and 43% reduction of the RT level in the culture fluid was detectable. At 1 µg/ml, both the inhibition and the reduction were complete.

Inhibition of syncytium formation by sulphoevernan and NPL

Addition of sulphoevernan or NPL during the co-incubation period of Jurkat cells with HIV-1-producing H9 cells strongly inhibited syncytium formation (Table 2). At a concentration of 1 µg/ml of sulphoevernan or 3 µg/ml of NPL no syncytium formation could be measured. Again, evernan gave no protection.

Inhibition of HIV-1 binding to MT-2 cells by sulphoevernan

Addition of both sulphoevernan (3 µg/ml) and NPL (20 µg/ml) almost completely abolished the binding of HIV-1 particles to MT-2 cells (Fig. 2), whereas evernan had no effect on this system. Furthermore, antibody OKT4A, which prevents the binding of HIV to CD4 receptors on cells (Bagasra & Lischner, 1988), also inhibited virus binding; 1·5 × 10^3 virus particles were estimated to be bound to each cell.

Binding of sulphoevernan to HIV-1 particles

To determine whether sulphoevernan protects MT-2 cells against HIV-1 infection by binding to receptors on the virus particles, competition experiments with NPL and antibody OKT4A were performed. The rationale for selecting these two tools was that NPL, with its high specificity for Man(α-1,3)Man residues, would mask the carbohydrate structures of the virus gpl20 (Geyer et al., 1988; Mizuochi et al., 1988) and OKT4A would mask the CD4 virus-binding sites on cells (Bagasra & Lischner, 1988).

The experiments revealed that [35S]sulphoevernan bound only to a small extent to both virus particles and MT-2 cells in the absence of Ca^{2+} (Table 3). Addition of 1 mM-CaCl_2 strongly enhanced the affinity of sulphoevernan for HIV-1; 120 d.p.m. of [35S]sulphoevernan was determined to bind to virus particles in the absence of Ca^{2+} and 1730 d.p.m. in its presence. In a control experiment it was established that addition of a 20-fold surplus of unlabelled sulphoevernan (8 µg/200 µl) to the labelled compound (0·4 µg/200 µl) reduced the amount of radioactivity bound to the virus by 95% (not shown). Preincubation of the virus particles with NPL prevented binding of sulphoevernan to the virus (Table 3); only 8% of the radioactivity was found to be associated with the
Table 3. Binding affinity of [35S]sulphoevernan to HIV-1 or MT-2 cells in the presence of NPL or antibody OKT4A

<table>
<thead>
<tr>
<th>Preincubation components</th>
<th>Incubation with [35S]sulphoevernan</th>
<th>Radioactivity†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D.p.m./20 μg HIV (×10^3)</td>
<td>D.p.m./10^5 cells (×10^3)</td>
</tr>
<tr>
<td>HIV-1 cells</td>
<td>MT-2 cells</td>
<td>NPL</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>-</td>
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<tr>
<td>+</td>
<td>-</td>
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<td>*</td>
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</tbody>
</table>

* HIV-1-infected MT-2 cells.
† Results given are the means of five experiments (± SD).

Sulphoevernan, in vitro HIV inhibitor

- Sulphoevernan, in vitro HIV inhibitor

Antiretroviral activity of sulphoevernan in vivo

To test for antiretroviral activity in vivo, the RLV/NMRI mouse system was used. As summarized in Table 4, sulphoevernan administered subcutaneously, beginning 1 day after RLV inoculation and then given daily for 8 days at a dose of 20 mg/kg, completely prevented increases in spleen weight. The lower dose of 5 mg/kg suppressed the weight increase by 95%. If administration of the compound was started at day 5, the antiretroviral activity was complete at a daily dose of 20 mg/kg and already significant (P < 0.01) at a daily dose of 5 mg/kg.

Discussion

Like dextran sulphate (Mitsuya et al., 1988; Baba et al., 1988) and polysulphated polyxylan (Biesert et al., 1988), sulphoevernan displays potent anti-HIV activity in vitro. The antiviral effect, measured on the basis of 50% cytoprotection, was observed at a concentration as low as 0.3 μg/ml for both HIV-1 and HIV-2 strains. Hence sulphoevernan displays a higher antiviral potency than dextran sulphate (ED₅₀ 9.1 μg/ml) (Baba et al., 1988). In contrast to dextran sulphate, sulphoevernan completely inhibits syncytium formation at a concentration of 1 μg/ml. Moreover, sulphoevernan is 20 times more potent than the polysulphated polyxylan (minimum inhibitory concentration 20 μg/ml) in this assay system (Biesert et al., 1988). At present we attribute this effect to the helical structure of sulphoevernan which might facilitate its interaction with viral gp120 (see below). This polysaccharide was not toxic to cells, a property which is shared with other sulphated polysaccharides (Mitsuya et al., 1988; Baba et al., 1988; Biesert et al., 1988).

We have addressed the question of which structure sulphoevernan binds to, thereby preventing HIV-infection. HIV-1 gp120 and the cellular CD4 are glycoproteins that are considered to interact with one other and to allow the binding of the virus to CD4+ cells (Klatzmann...
et al., 1984; Dalgleish et al., 1984). Both the protein backbone and oligosaccharide side-chains of gp120 contribute to its ability to bind to CD4 (Putney et al., 1986; Fennie & Lasky, 1989). The carbohydrate structures of gp120 can be subdivided into high-mannose and complex types; the latter glycans terminate with sialic acid residues (Geyer et al., 1988; Mizuochi et al., 1988). In a previous study, it was shown that Gerardia savaglia lectin, which specifically recognizes (α1,2)-mannosyl residues, prevents binding of HIV-1 to H9 cells via complex formation with gp120 (Müller et al., 1988a, b). We now show that NPL, with its high specificity for (α1,3)-mannosyl residues (Van Damme et al., 1988), also prevents HIV-1 and HIV-2 infection of cells in vitro. Both the (α1,2)- and the (α1,3)-mannose residues reside in oligomannosidic side-chains of the high-mannose type (Geyer et al., 1988). Using NPL and OKT4A antibodies, we also demonstrated that binding of sulphoevernan to HIV is prevented by NPL whereas the antibodies displayed no effect on the interaction between sulphoevernan and cells.

This finding indicates that sulphoevernan inhibits HIV infection by binding to the virus, provided that Ca²⁺ ions are present. In the absence of Ca²⁺ ions the binding of sulphoevernan was negligible. Hence, we suppose that Ca²⁺ allows binding of sulphoevernan to the sialic acid residues of complex type carbohydrates of gp120, a binding which is both feasible and strong (Wang, 1986). At present we cannot decide to which molecule on the cell surface the virus binds via Ca²⁺ ions.

The results presented here, namely that sulphoevernan exhibits potent anti-HIV activity, might be of practical significance in view of earlier findings which revealed a series of important biopharmaceutical properties of sulphoevernan, e.g. complex formation with a human β-lipoprotein and with histamine, increase of prothrombin time and enhancement of hydrolysis of triolein (Stefanovich, 1969a). Moreover, sulphoevernan also inhibits the esterification of cholesterol (Stefanovich, 1969b) and decreases experimental aortic arteriosclerosis in rabbits (Stefanovich et al., 1970). A more important aspect, in the context of the anti-HIV effect of sulphoevernan in vitro, was our finding that, in the in vitro retrovirus model system, RLV-infected mice, sulphoevernan protected the animals against virus-induced disseminated lymphoma. At a daily dose of 20 mg/kg, sulphoevernan completely suppressed elevation of spleen weight.

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