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Naphthalene Sulfonate Polymers with CD4-Blocking and Anti-Human Immunodeficiency Virus Type 1 Activities

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PIC 024-4 and PRO 2000 are naphthalene sulfonate polymers that bind to CD4 with nanomolar affinity and block binding of gp120. Both have activity against human immunodeficiency virus type 1 in H9 cells, peripheral blood mononuclear cells, and primary monocyte/macrophages, are synergistic with zidovudine, and do not inhibit tetanus toxoid-stimulated T-cell proliferation at anti-human immunodeficiency virus type 1 concentrations.

Attachment of human immunodeficiency virus type 1 (HIV-1) to $CD4^+$ target cells is mediated through binding of the viral envelope glycoprotein gp120 to the CD4 receptor (9, 15). We have identified a class of compounds that effectively block gp120 binding to CD4 and suppress HIV-1 infection in vitro. The compounds are condensation products of 2-naphthalene sulfonic acid and formaldehyde and have the general structure shown in Fig. 1. In this study, we evaluated the biochemical and antiviral properties of a crude preparation (PIC 024-4, which contains polymers of various sizes) and of a better-defined fraction in which 60% of the polymers had molecular masses of 5 ± 1 kDa (PRO 2000).

PIC 024-4 and PRO 2000 inhibited the binding of recombinant gp120 (IIIB isolate) to recombinant CD4 in an enzyme-linked immunosorbent assay (ELISA) format in which bound gp120 was detected by an envelope-specific monoclonal antibody coupled to horseradish peroxidase (HIV gp120/CD4 Receptor EIA kit; DuPont-NEN, Boston, Mass.). Fifty percent inhibitory concentrations (IC_{50}s) were 0.3 and 0.4 μg/ml, respectively. Aurintricarboxylic acid, which has been reported to inhibit the binding of gp120 to CD4 (7, 8), had an IC_{50} of ≈1 μg/ml. By contrast, an 8-kDa preparation of dextran sulfate, which has been evaluated as an anti-HIV-1 agent in humans and found to be inactive (11), was inactive in the ELISA. Both PIC 024-4 and PRO 2000 showed much less effect on the CD2-CD58 binding interaction, giving respective IC_{50}s of 71 and 115 μg/ml in a similar ELISA format (1). This suggests that the polymers selectively block the gp120-CD4 interaction.

Fluorescence quenching experiments provided direct evidence that PIC 024-4 and PRO 2000 bind to CD4 in phosphate-buffered saline (Fig. 2). Addition of CD4 quenched the intrinsic fluorescence in a saturable manner, indicating that the naphthalene sulfonate units of the polymer bind directly to the protein. An excitation wavelength of 315 nm ensured that no inner filter effect or fluorescence signal was contributed by the added protein. Furthermore, the low concentrations of both reagents excluded significant collisional fluorescence quenching. A nonlinear curve fit to a simple biomolecular binding model (21) predicted an apparent dissociation constant of ≈20 nM and a stoichiometry of ≈1:1.

The HIV-1-inhibitory activities of PIC 024-4 were evaluated in H9 $CD4^+$ lymphoblastoid cell lines, peripheral blood mononuclear cells (PBMC), and primary monocyte/macrophages, using assays that we have described previously (14). Cell viability was assessed by trypan blue dye exclusion, and virus replication was measured by detection of HIV-1 p24 antigen in culture supernatant fluids (13). Several different HIV-1 isolates were used, including 14aPre (14), N70 (6), IIIB (20), and Ba-L (12). The IC_{50}s and other inhibitory endpoint values were determined by the dose-effect analysis described by Chou, Chou, and Talalay (3–5).

Table 1 shows IC_{50}s and IC_{90}s, as well as toxic concentrations, of PIC 024-4 and PRO 2000 against various HIV-1 isolates. PIC 024-4 and PRO 2000 inhibited infection by a wide range of HIV-1 isolates in a variety of cell types, including a $CD4^+$ cell line (H9), PBMC and monocyte/macrophages. In separate studies (19a), PIC 024-4 suppressed infection of PBMC by the clinical isolate JR-CSF and suppressed infection of macrophages by the monocytotropic isolate ADA. IC_{50}s were similar to those reported here. This activity profile appears to differ from that of dextran sulfate, which has been reported to be inactive against several macrophage-tropic isolates, including Ba-L (17). Although toxicity was not observed up to day 4 in culture, it appeared between days 4 and 11, as assessed by cell viability and proliferative capacity.

Since PIC 024-4 and PRO 2000 showed time-dependent cytotoxicities in vitro, experiments were conducted with PRO 2000 in H9 cells to determine the minimal duration and concentration required to achieve an antiviral effect and circumvent toxicity. Limited exposure to a concentration of 100, 40, 20, or 10 μg/ml administered once and followed by either drug removal after 3 days or postwash maintenance in low drug concentrations (1 μg/ml) was effective in suppressing p24 antigen production following acute infection as well as in circumventing toxicity. Complete inhibition was observed with PRO 2000 at 100 μg/ml; there was no viral breakthrough seen over 28 days in culture (Fig. 3). Partial inhibition occured at concentrations of 10 to 40 μg/ml. No toxicity was observed at concentrations of ≤100 μg/ml to day 28 after these limited exposures. In the case of established infection, however, in which cells were exposed to the virus 4 h prior to the addition of PRO 2000, there was only transient suppression of viral

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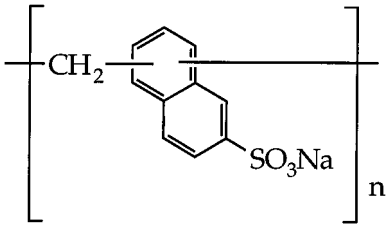

FIG. 1. General structure of PIC 024-4 and PRO 2000. Methylene bridges may connect different ring positions. For PRO 2000, $n \approx 20$.

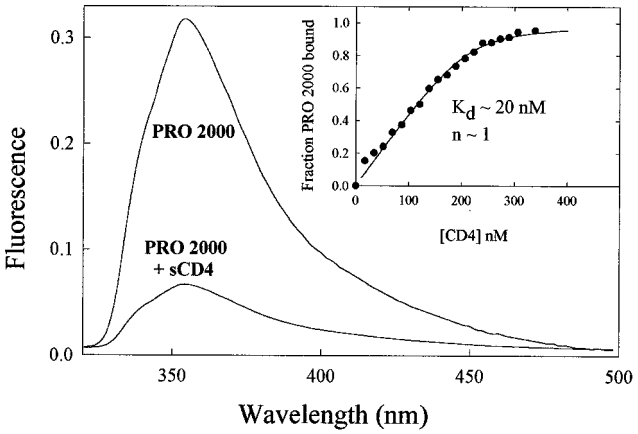

FIG. 2. Fluorescence emission spectrum of PRO 2000 in the absence and presence of recombinant soluble CD4 (sCD4) (domains 1 and 2). (Inset) Fraction of PRO 2000 bound as a function of recombinant soluble CD4 concentration. K_d, apparent dissociation constant.

replication to day 7, and viral breakthrough was observed by day 11 (data not shown).

Limited exposure to PRO 2000 was also evaluated in PBMC. No toxicity was observed with up to 50 μg/ml with 24-h exposures, and the IC_{50} and IC_{90} with HIV-1 14aPre were 12.8 and 30.3 μg/ml, respectively.

HIV-1 was passaged in the presence of increasing concentrations of PIC 024-4 (3 to 12 μg/ml) over a 212-day culture period. Higher concentrations of the compound could not be used because of toxicity. Decreased susceptibility of passaged virus did not develop under these conditions.

We also evaluated combinations of PIC 024-4 or PRO 2000 with zidovudine against HIV-1 14aPre by using techniques previously described (14). Antiviral synergy was observed between PIC 024-4 and zidovudine at the IC_{75}, IC_{90}, and IC_{95} (combination indices of 0.7, 0.4, and 0.2, respectively); synergy between PRO 2000 and zidovudine occured at the IC_{90} and IC_{95} (combination indices of 0.8 and 0.8). No toxicity was observed at the concentrations tested over the 4-day assay period.

Since the CD4 receptor plays a role in the normal immune response by interacting with the T-cell receptor–HLA class II–antigen complex during T-cell activation, PIC 024-4 and PRO 2000 were evaluated for their effects on tetanus toxoid-induced stimulation of PBMC from normal tetanus toxoid responders. Stimulation, as measured by [^{3}H]thymidine incorporation after 5 days in culture, was unaffected by concentrations of up to 50 μg/ml, which is well above the average IC_{50} for antiviral activity (data not shown).

Several organic compounds, including a series of naphthalene disulfonates, have been reported to suppress HIV-1 infection by disrupting the gp120-CD4 binding interaction (10, 18, 19). Recent data suggest that many of these compounds may not disrupt gp120-CD4 binding but rather may affect the cationic V3 loop of gp120, which appears to mediate a post-binding fusion event (2, 16). Although they are structurally related to other anionic compounds that suppress HIV-1 infection, the naphthalene sulfonic acid polymers PIC 024-4 and PRO 2000 show distinct antiviral and biochemical properties in vitro. In contrast to dextran sulfate and the low-molecular-weight naphthalene disulfonates, the polymers effectively block the gp120-CD4 binding interaction and bind tightly to recombinant CD4 under physiological conditions. Additional antiviral mechanisms, including interference with CD4-mediated postbinding events or interactions with gp120, cannot yet be excluded.

The activity of the naphthalene sulfonate polymers in vivo may be affected by pharmacokinetics, binding to serum components, or the ability to reach sites of viral replication. In addition, toxicity may be a problem with these compounds with prolonged use. Although toxicity was not observed at therapeutic drug concentrations up to day 4 in culture, it was apparent between days 4 and 11, thereby narrowing the therapeutic effect/toxic effect ratios after prolonged exposure. This effect could be overcome by limiting the time of cell exposure to either drug. If drugs were removed after 24 to 72 h, antiviral effects persisted and toxicity was minimized. Whether less-frequent dosing will be necessary in vivo remains to be determined.

Multiple-dose toxicity studies of PRO 2000 in rodents and

TABLE 1. IC_{50}s, IC_{90}s, and toxicities of PIC 024-4 and PRO 2000 against HIV-1

Drug and no. of expts	Cells	Virus	IC_{50} (μg/ml)	IC_{90} (μg/ml)	Toxicity (μg/ml) after:	
					Prolonged exposure[a]	Limited exposure[b]
PIC 024-4						
3	H9	IIIB	0.4–0.7	0.7–1.4	>15	ND[c]
1	H9	14aPre	1.3	4.9	>15	ND
5	PBMC	14aPre	8.4–14.3	17.9–29.2	>12	ND
1	PBMC	N70	3.9	12.8	>12	ND
1	Monocyte/macrophage	Ba-L	1.2	4.5	>16.7	ND
PRO 2000						
2	H9	IIIB	0.7–1.0	ND	>5	>100
1	PBMC	14aPre	12.8[d]	30.3[d]	>5	>50

[a] Toxicity was observed between 4 and 11 days in culture.
[b] Drug was removed from culture after 24 to 72 h of exposure.
[c] ND, not determined.
[d] PRO 2000 activity against HIV-1 was evaluated by limited drug exposure of 24 h.

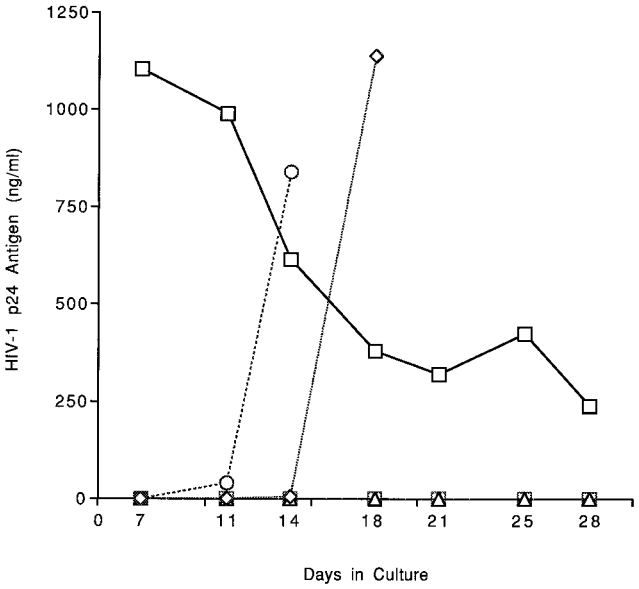

FIG. 3. Inhibition of HIV-1 replication by limited exposure (3 days) to PRO 2000 at 10 (◇), 20 (○), 40 (△), or 100 (⊞) μg/ml following simultaneous infection of H9 cells. □, infected control. For the 40-μg/ml preparation, one of two culture wells showed viral breakthrough by day 18, and the second culture well showed suppression of virus through day 28 in culture.

cynomolgus monkeys have shown that the most sensitive endpoints for high-dose effects are prolongation of activated partial thromboplastin time and elevation of alanine aminotransferase levels (20a). In vitro, the concentration of PRO 2000 in citrated human plasma required to double the activated partial thromboplastin time is 10-fold greater than the corresponding concentration of dextran sulfate (8 kDa), suggesting that the higher doses of PRO 2000 can be safely administered. In mice, total T-cell levels, T-cell subset ratios, and T-cell mitogenic responses to concanavalin A were largely unaffected by PRO 2000 administration (20a). Details of these studies will be reported elsewhere. Clinical testing of PRO 2000 in humans is now under way.

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