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The Nonnucleoside Reverse Transcriptase Inhibitor UC-781 Inhibits Human Immunodeficiency Virus Type 1 Infection of Human Cervical Tissue and Dissemination by Migratory Cells

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Heterosexual transmission of human immunodeficiency virus remains the major route of transmission worldwide; thus, there is an urgent need for additional prevention strategies, particularly those that could be controlled by women. Using cellular and tissue explant models, we have evaluated the potential activity of thiocarboxanilide nonnucleoside analogue reverse transcriptase inhibitor UC-781 as a vaginal microbicide. We were able to demonstrate a potent dose-dependent effect against R5 and X4 infections of T cells. In human cervical explant cultures, UC-781 was not only able to inhibit direct infection of mucosal tissue but was able to prevent dissemination of virus by migratory cells. UC-781 formulated into a carbopol gel (0.1%) retained significant activity against both direct tissue infection and transinfection mediated by migratory cells. Furthermore, UC-781 demonstrated prolonged inhibitory effects able to prevent both localized and disseminated infections up to 6 days post compound treatment. Additional studies were carried out to determine the concentration of compound that might be required to block a primary infection within draining lymph nodes. While a greater dose of compound was required to inhibit both X4 and R5 infections of lymphoid versus cervical explants, this was equivalent to a 1:3,000 dilution of the 0.1% formulation. Furthermore, a 2-h exposure to the compound prevented infection of lymphoid tissue when challenged up to 2 days later. The prolonged protection observed following pretreatment of both genital and lymphoid tissues with UC-781 suggests that this class of inhibitors may have unique advantages over other classes of potential microbicide candidates.

As the human immunodeficiency virus (HIV)-AIDS epidemic enters its third decade, there is still a desperate need to develop preventative options to help reduce the number of new HIV type 1 (HIV-1) infections occurring each year. Globally, more than 39 million people are now infected with HIV-1 (22), with heterosexual intercourse remaining the major route of transmission. Additionally, women now account for almost half of the population infected worldwide, increasing to 57% of the infected population in Sub-Saharan Africa, where one in four women is infected by the age of 22 (23). Current suggested methods of prevention include abstinence, monogamy, a reduction in the number of sexual partners, and the use of barrier methods such as male and female condoms. There is also evidence to suggest that treatment of other sexually transmitted diseases, in particular ulcerative sexually transmitted diseases, can help reduce HIV-1 transmission rates within at-risk populations (6). Gender inequalities mean that abstinence is not a realistic choice for many women, monogamy offers no protection to women in stable partnerships where the desire for fertility outweighs concerns about HIV infection, and condom use requires partner consent (17). Thus, there is an urgent need for noncontraceptive preventative measures that could be controlled by women (17). Vaginal microbicides, topically applied formulations designed to prevent HIV-1 transmission, could represent an important new prevention option for women. Recent studies of the mechanisms of HIV dissemination through mucosal tissue to draining lymph nodes (18) indicate that such agents should be nontoxic, efficiently target various pathways of viral infection, and be active for prolonged periods.

Here we tested UC-781, a thiocarboxanilide nonnucleoside reverse transcriptase (RT) inhibitor (NNRTI) able to bind to RT with very high affinity, for potential microbicide activity (2). This class of hydrophobic, tight-binding NNRTI compounds (that includes UC-781, TMC120, DABO, and MIV-150) readily crosses membrane barriers and irreversibly inactivates RT (13). UC-781 was originally developed by Uniroyal Chemical (now Crompton) (1) as an antiretroviral agent; however, poor bioavailability prevented its development as a therapeutic treatment. For topical application, this property could prove advantageous as it may limit systemic absorption while delivering sufficient drug to prevent mucosal infection. UC-781 has been shown to protect pretreated cells from subsequent infection with HIV-1 (3, 24) in the absence of drug. In addition,

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UC-781 treatment of infected cells, whether primary peripheral blood mononuclear cells (3) or cell lines (3, 4), results in release of attenuated virus. Furthermore, previous studies have suggested that the compound may have direct virucidal activity in a concentration-dependent manner (3).

To evaluate the efficacy of UC-781, we have utilized a nonpolarized cervical explant culture model to mimic in vivo tissue that is initially exposed to virus during a heterosexual transmission event. We have previously demonstrated that intact stratified epithelium presents a barrier to infection (9); there are clear physiological reasons for such barrier effects (18). Furthermore, studies in the macaque challenge model suggest that primary infection is associated with epithelial microtrauma, providing access to underlying susceptible cells (12). Thus, we model a worst-case scenario where the virus has a maximal chance of establishing infection by being allowed to reach all potential susceptible cells within the epithelium and underlying mucosa. We demonstrate that UC-781 prevents both direct infection of human cervical explants and dissemination of virus by migratory cells. This inhibition was evident even for tissue exposed to virus several days post drug pretreatment and removal. UC-781 also demonstrated potent activity against infection of lymphoid tissue, indicating that it may be effective against HIV-1 dissemination to draining lymph nodes. The models described in the present work may be used to evaluate the potential of candidate microbicides to both prevent direct infection of cervical tissue and inhibit viral dissemination to lymph nodes by migratory cells.

MATERIALS AND METHODS

Cell and virus culture. PM-1 cells (AIDS reagent project, National Institute for Biological Standards and Control, Potters Bar, United Kingdom) were grown in continual culture (RPMI 10% [RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin, streptomycin, and L-glutamine]) and passaged every 3 to 4 days. The HIV-1 strains used were grown in either phytohemagglutinin-stimulated peripheral blood mononuclear cells (HIV-1_{BaL}) or PM-1 cells (HIV-1_{BaL} and HIV-1_{RF}). Cell-free viral stocks were passed through 0.2-μm-pore-size filters. Infection was monitored by viral p24 antigen (HIV-1 p24 enzyme-linked immunosorbent assay [ELISA; SAIC-Frederick, Inc., Frederick, MD], carried out according to the manufacturer's protocol) or RT (16) release into culture supernatants.

Drug substances and drug formulations. UC-781 {*N*-[4-chloro-3-(3-methyl-2-butenyloxy)phenyl]-2,3-furancarbothioamide] } and its immediate unthionylated synthetic precursor, UC-22, were prepared by Crompton Corp. (Middlebury, CT). Both UC-781 and UC-22 were formulated as suspensions in carbopol-based gel formulations at pH 4.5 to 5.0. UC-22 formulation additionally contained propylene glycol (PG). Placebo formulations were identical to their drug product formulations, except for the exclusion of the active pharmaceutical ingredient.

Solid-phase immobilization of HIV-1. A monoclonal antibody to human HLA-DR was produced from the mouse hybridoma L243 (American Type Culture Collection). Briefly, the hybridoma was grown to confluence in serum-free medium (protein-free hybridoma medium; Life Technologies, United Kingdom) and then for a further 3 days following the addition of OptiMAb, in accordance with the manufacturer's guidelines (Life Technologies, United Kingdom). This monoclonal antibody was then bound to 96-well flat-bottom tissue culture plates (Nunc or Falcon) for 1 h at room temperature. Any unbound antibody was washed off with 1 volume phosphate-buffered saline (PBS) prior to the addition of an excess of viral stock (HIV-1_{BaL} or HIV-1_{RF} grown in PM-1 cells, 10^3 50% tissue culture infective doses). Plates were centrifuged for a minimum of 1 h (room temperature) at 3,200 rpm. Unbound virus was washed away with 2 volumes of PBS and immobilized virus treated with test compounds (UC-781, 1% UC-781 in carbopol gel, 0.1% UC-781 in carbopol gel, 1% UC-22 in carbopol-PG gel, 0.1% UC-22 in carbopol-PG gel, carbopol gel placebo, and carbopol-PG placebo gel, kindly supplied by Biosyn, Inc.) for 1 h at 37°C. Compounds were left in culture without washing prior to adding 4×10^4 PM-1 cells/well. Viral replication was determined following 10 days in culture by measurement of viral RT in culture supernatants. The described assay allows topical administration of candidate compounds: previous studies have demonstrated no difference in UC-781 activity against virus immobilized or in suspension (data not shown).

Culture of human genital tract tissue explants and HIV infection. Cervical tissue was obtained from women undergoing planned therapeutic hysterectomy at St George's, St Helier's, and Kingston Hospitals (London, United Kingdom) (written consent was obtained from all tissue donors according to the local Research Ethics Committee). Cervical tissue comprising both epithelium and stromal tissue was cut into 3-mm^3 explants prior to culture in 96-well, flat-bottom tissue culture plates in RPMI 10% as previously described (9). Test compounds were diluted into culture medium and used to make a 10-fold dilution series. Explants were treated with medium or compound (100 μl—2× final concentration) for 1 h prior to exposure to HIV-1_{BaL} (10^3 to 10^5 50% tissue culture infective doses) for 2 h at 37°C. After incubation with infectious virus and compound, explants were washed with 4 volumes of PBS. Explants were then cultured overnight prior to transfer to fresh plates and further culture for 12 to 14 days, with 50% medium feeds every 2 to 3 days. Migratory cells present in the overnight culture plate were cocultured with 4×10^4 PM-1 cells/well to assess blockade of virus transfer by migrating cells. At the end of the assay, HIV-1 infection was determined by the measurement of p24 in culture supernatants (ELISA; SAIC-Frederick or Beckman Coulter). Proviral DNA (long terminal repeat) was assessed in the tissue by multiplex quantitative real-time PCR for HIV-1 DNA and β-actin as previously described (9). To determine any long-term effects of pretreatment of tissue with compound prior to exposure to virus in the absence of compound, explants were pretreated with compound for 2 or 24 h. Compound was then removed with four PBS washes. Following transfer of tissue explants to a fresh plate, explants were exposed to virus (as previously described) on day 0, 2, 4, or 6 posttreatment. Virus was removed and explants cultured as described above.

Culture of human lymphoid tonsil tissue explants and HIV infection. Human tonsils, removed during routine tonsillectomy and not required for diagnostic purposes, were received within 5 h of excision. Tonsils were dissected into 2- to 3-mm^3 blocks and cultured as described previously (7, 8). Briefly, tissue blocks were placed on collagen sponge gels at the air-medium interface. Tissue blocks were pretreated with test compound for 2 h prior to exposure to HIV-1_{SF162} or HIV-$1_{LAV.04}$ (1 ng of HIV-1 p24 inoculated directly onto each tissue block) in the presence or absence of test compound and cultured for 12 days. To determine any long-term effects of pretreatment of tissue with compound prior to exposure to virus in the absence of compound, tissue blocks were pretreated with the test compound for 2 h before the compound was removed by washing and tissue transferred to fresh collagen sponge gels. Tissue blocks were then exposed to virus (HIV-1_{SF162} or HIV-$1_{LAV.04}$) as previously described on day 0, 1, or 2 following compound treatment. Tissue blocks were cultured in the absence of the test compound for 12 days. Productive infection was determined by measurement of HIV-1 p24 in culture supernatants using p24 ELISA (Beckman Coulter).

Statistical analyses. The 50% inhibitory concentration (IC_{50}) analysis was done using nonlinear regression analysis (GraphPad PRISM; GraphPad Software, Inc.).

RESULTS

UC-781 inhibits HIV infection in isolated cells. UC-781 inhibits infection by both HIV-1_{BaL} (R5) and HIV-1_{RF} (X4) in PM-1 T cells as assessed with solid-phase plate-based assays (see Materials and Methods) (Table 1). In addition, we evaluated UC-781 formulated into different gels or creams. These formulations were assessed for toxicity (data not shown), and only gels showing high selectivity were chosen for further study of anti-HIV activity. The anti-HIV-1 activities of the selected carbopol formulations were assessed as described above for native UC-781. Two formulations, one containing 1% UC-781 and another containing 0.1% UC-781, were evaluated. Both exhibited potent activity against R5 and X4 infections of T cells (Table 1), with the 0.1% product being a log less active than the 1% product when the concentration of the active compound was accounted for. The carbopol placebo gel itself was active against the X4 isolate but not against the R5 isolate.

A related compound, UC-22, produced as the unthionylated

TABLE 1. Inhibitory concentrations of native and formulated UC-781 and UC-22 against HIV-1_{BaL} and HIV-1_{RF} infection of T cells[a]

Compound	Concn units	IC_{50} (95% CI)	
		HIV-1_{BaL}	HIV-1_{RF}
Native UC-781	nM	<0.2	<0.2
1% UC-781 (carbopol gel)	μg/ml gel	0.02 (0.007–0.03)	0.04 (0.02–0.07)
	nM compound	0.04 (0.02–0.09)	0.1 (0.06–0.2)
	Gel dilution	69,000,000	28,000,000
0.1% UC-781 (carbopol gel)	μg/ml gel	3.5 (1.1–11)	0.7 (0.5–1.0)
	nM compound	1.1 (0.3–3.4)	0.2 (0.1–0.3)
	Gel dilution	300,000	1,500,000
Carbopol placebo gel	μg/ml gel	>100,000	1 (0.3–3.7)
	Gel dilution	<10	1,000,000
1% UC-22 (carbopol-PG gel)	μg/ml gel	170 (60–490)	7 (1–54)
	nM compound	534 (160–1,500)	21 (3–160)
	Gel dilution	6,000	141,000
0.1% UC-22 (carbopol-PG gel)	μg/ml gel	434 (139–1,350)	0.8 (0.4–1.6)
	nM compound	136 (40–420)	0.2 (0.1–0.5)
	Gel dilution	2,300	1,300,000
Carbopol-PG placebo gel	μg/ml gel	7,600 (470–124,400)	7 (4–14)
	Gel dilution	130	143,000

[a] HIV-1 was immobilized on a solid phase using an anti-HLA-DR antibody as described in Materials and Methods. Immobilized virus was treated with the test compound for 1 h before the addition of target T cells (PM-1). Plates were then cultured for 10 days, when viral replication was determined by supernatant RT activity. Data are expressed as the absolute amount of compound (nM), the total amount of gel (mg/ml), or the respective dilution of the formulated gel. Data represent the mean IC_{50} and 95% confidence interval (CI) of three (HIV-1_{BaL}) or two (HIV-1_{RF}) independent experiments where each condition was tested in triplicate.

immediate precursor in the manufacture of UC-781 was also tested in 1% and 0.1% formulations in a carbopol-PG gel. UC-22 formulations exhibited antiviral activity, albeit significantly lower than UC-781, with one exception, where a 0.1% formulation of UC-22 inhibited HIV-1_{RF} activity similarly to 0.1% UC-781. The carbopol-PG placebo gel for UC-22 suppressed the R5 isolate but less efficiently than carbopol–UC-22. However, the activity of the placebo gel against the X4 isolate was equivalent to that of 1% UC-22.

UC-781 inhibits HIV-1 infection of human cervical tissue and dissemination of virus by migratory cells. The potential of UC-781 to block infection of the female genital mucosa was investigated in vitro using mucosal tissue explants obtained from seronegative women undergoing therapeutic hysterectomy (9, 14). We used a modification of this system, which allows detection of viral dissemination by migratory cells (10). Ectocervical explants were cultured in a nonpolarized manner (9) and treated with UC-781 (alone or in a 0.1% formulation) for 1 h prior to exposure to HIV-1_{BaL}. Tissue explants were exposed to virus for 2 h in the presence of the compound, after which the unbound drug and virus were washed away. Viral infection was evaluated by p24 release and by quantitative real-time PCR for proviral DNA within the tissue following 12 to 14 days in culture. UC-781 potently inhibited HIV-1_{BaL} (R5) infection of the tissue in a dose-dependent manner (Fig. 1A, ii), as evaluated by the reduction of provirus in cultured explants. The IC_{50} was estimated to be 0.006 nM by proviral DNA detection and 3.2 nM by p24 release (Table 2). Supernatant p24 levels were at the limits of detection; therefore, proviral formation, essential for de novo viral replication, was taken as the definitive marker of infection. Differences with p24 antigen release may reflect low levels of input p24 residing within tissues. Although complete inhibition of integrated provirus was observed at a concentration of 100 μM, all other concentrations tested resulted in a mild breakthrough of provirus. Nevertheless, inhibition of greater than 99% was observed at a concentration of 1 nM.

We also determined whether UC-781 could prevent HIV-1 transinfection by $CD4^+$ dendritic cells (DCs) that spontaneously migrate out of cervical explants (10). These cells were harvested from the same cultures following overnight culture of cervical explants (exposed to drug and virus as described above) and then cocultured with the permissive T-cell line PM-1. UC-781 inhibited transmission of virus to permissive T cells with an IC_{50} of 0.2 nM (Table 2), as determined by coculture. Again, this effect was dose dependent (Fig. 1A, iii).

A similar dose-dependent inhibition was also seen with formulated UC-781 (0.1%) (Fig. 1B). At a concentration of 0.5 mg/ml (equivalent to 1.5 μM UC-781), this gel almost completely (>99%) inhibited infection of cervical explants. A subsequent 10-fold dilution (0.05 mg/ml gel, 0.15 μM UC-781) reduced the effect to around 95%, with significant activity being lost by a 0.005-mg/ml gel (0.015 μM UC-781). Overall, the 0.1% formulated product had an IC_{50} (as evaluated by proviral DNA detection) of 0.3 nM active compound (0.09 μg/ml gel; Table 2). Furthermore, the formulated product, although able to inhibit dissemination of the virus with an IC_{50} of 106 nM (35 μg/ml gel) active compound, was significantly less active than the unformulated compound. In contrast, the placebo gel (vehicle control) had an IC_{50} of 14.8 mg/ml gel against proviral accumulation within tissue and an IC_{50} of 3 mg/ml against dissemination of the virus by migratory cells (Table 2).

The related compound UC-22, formulated into a car-

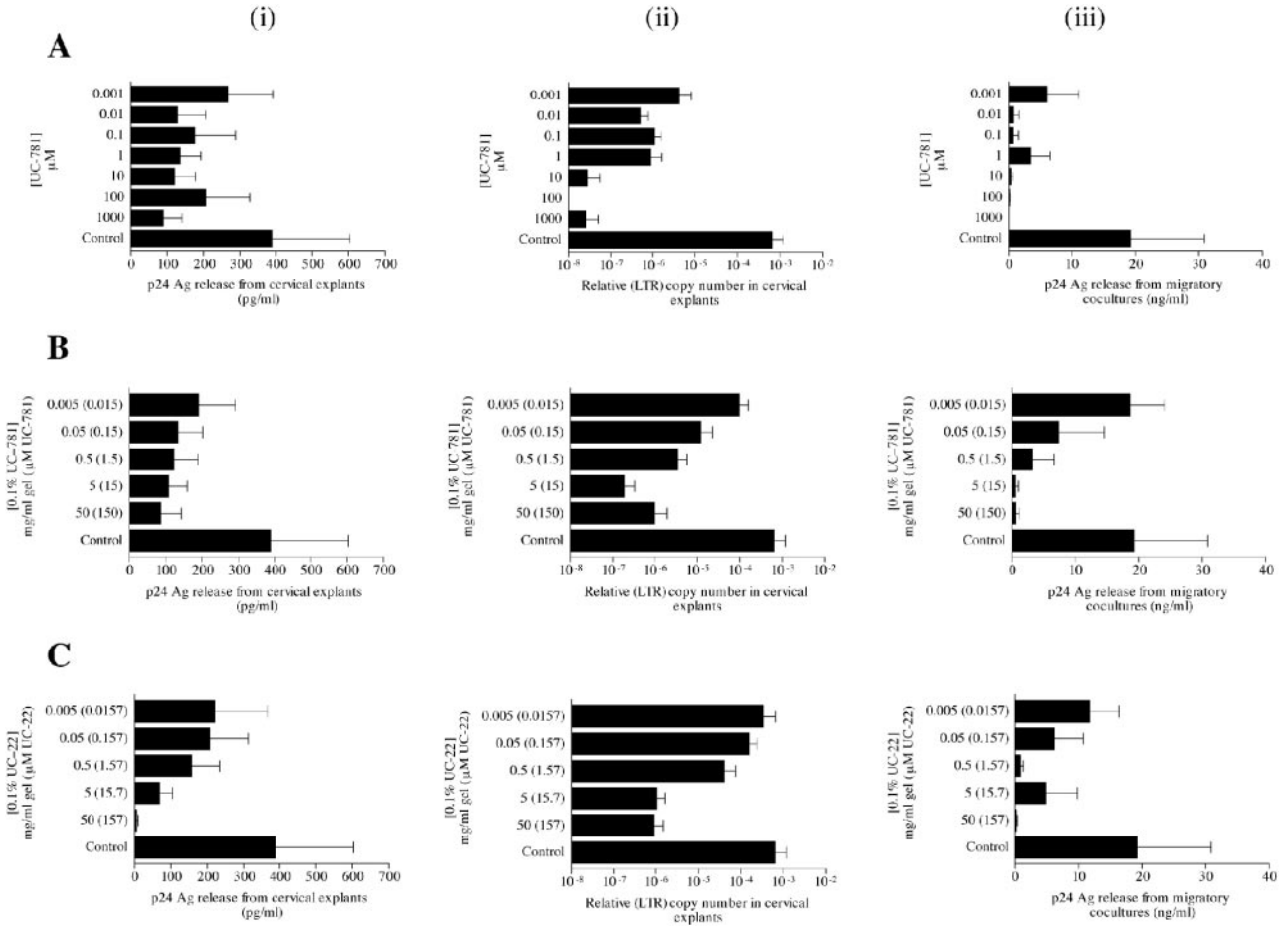

FIG. 1. UC-781 inhibits HIV-1_{BaL} infection of cervical explants and transfer of virus from migrating cells. Ectocervical tissue explants were exposed to HIV-1_{BaL} for 2 h in the presence of (A) UC-781 (native compound), (B) 0.1% UC-781 gel (formulated in carbopol gel), and (C) 0.1% UC-22 gel (formulated in carbopol-PG gel). Following overnight culture, explants were separated from any cells that had migrated from the tissue and cultured separately for 10 days. Infection was determined by (i) p24 Ag in explant culture supernatants, (ii) relative copy number of HIV-1 proviral DNA in proteinase K-digested tissue (note log scale), and (iii) p24 antigen (Ag) in migratory cell coculture supernatants. Data represent the mean ± the standard error of the mean of six or fewer individual donors where each condition was tested in triplicate.

bopol-PG gel, was also active against HIV-1 in cervical tissue but to a lesser extent (Fig. 1C). Here, formulated UC-22 was able to inhibit (>99%) viral integration at 5 mg/ml (15.7 μM UC-22) and had an IC_{50} of 22 nM active compound (7 μg/ml gel; Table 2). This formulated compound was also less active against viral dissemination than native UC-781 (IC_{50} of 12 μg/ml gel) (Fig. 2, iii). Interestingly, the placebo gel (vehicle control) showed similar activity to formulated UC-22 against proviral accumulation within tissue (IC_{50} of 1.4 μg/ml gel) but far less activity against dissemination of the virus by migratory cells (IC_{50} of 1.7 mg/ml gel) (Table 2).

Prolonged inhibitory effect of UC-781. To determine the duration of the protection provided by UC-781, cervical explants were treated with native UC-781 for 2 or 24 h, after which the compound was washed away and explants cultured in the absence of UC-781. Explants were subsequently exposed to HIV-1_{BaL} following 0, 2, 4, or 6 days in culture. Two-hour pretreatment with UC-781 resulted in significant inhibition of HIV-1 infection when challenged with virus on days 0, 2, 4, and 6 post drug removal (Fig. 2A). Interestingly, a more pronounced effect was noted on days 2 and 4 post drug removal compared to virus exposure immediately following UC-781 treatment (day 0). A similar trend was seen with 24-h pretreatment with UC-781 (Fig. 2B); however, lower doses of the drug appeared more active against provirus formation in tissue exposed to virus immediately post drug treatment. In addition to suppression of infection in mucosal tissue, UC-781 was also able to completely inhibit virus dissemination by migratory cells up to 6 days post drug removal (Fig. 2, iii) at concentrations down to 100 μM following 2- or 24-h compound treatment.

UC-781 inhibits HIV-1 infection of lymphoid tissue. Further experiments were carried out to determine the concentration of UC-781 that might be required to prevent HIV-1 replication in lymphoid tissue should viral dissemination occur. Here, human lymphoid tissue explants were exposed to HIV-$1_{LAV.04}$ (X4) or HIV-1_{SF162} (R5) in the presence of compound following a 2-h pretreatment with UC-781. When the compound was present throughout the experiment, both X4 and R5 infections of lymphoid tissue were inhibited at a concentration of 0.1 μM

TABLE 2. Inhibitory concentrations of native and formulated UC-781 and UC-22 against HIV-1_{BaL} infection of cervical tissue[a]

Compound	Concn units	IC_{50} (95% CI)		
		Cervix culture p24 release	Cervix culture proviral DNA (LTR)	Migratory cell coculture p24 release
UC-781 (native)	nM	3.2 (0.4–24)	0.006 (0.004–0.01)	0.2 (0.04–0.7)
0.1% UC-781 gel (carbopol)	μg/ml gel	0.3 (0.04–1.6)	0.09 (0.03–0.2)	35 (6–209)
	nM compound	0.8 (0.1–5)	0.3 (0.1–0.7)	106 (18–627)
	Gel dilution	4,000,000	12,000,000	28,000
0.1% UC-22 gel (carbopol-PG)	μg/ml gel	64 (11–358)	7 (1.4–36)	12 (3–54)
	nM compound	200 (35–1,120)	22 (4–114)	39 (9–168)
	Gel dilution	16,000	141,000	81,000
Carbopol placebo gel	μg/ml gel	872 (150–5,000)	14,800 ($4e^{-5}$–$6e^{12}$)	3,000 (80–115,000)
	Gel dilution	1,150	70	330
Carbopol-PG placebo gel	μg/ml gel	626 (133–3,000)	1.4 (0.04–44)	1,723 (43–70,000)
	Gel dilution	1,600	714,000	600

[a] Ectocervical tissue explants were exposed to HIV-1_{BaL} for 2 h in the presence of compound. Following overnight culture, explants were separated from any cells that had migrated from the tissue and cultured separately. Infection of explants was determined by ELISA measurement of p24 antigen in culture supernatants or the relative copy number of HIV-1 proviral DNA in proteinase K-digested tissue by real-time quantitative PCR. Migratory cells were cocultured with permissive T cells (PM-1) and infection determined by detection of p24 antigen in culture supernatants. Data are expressed as the absolute amount of compound (nM), the total amount of gel (mg/ml), and the respective dilution of formulated gel. Data represent the mean IC_{50} and 95% confidence interval (CI) of six or fewer individual donors where each condition was tested in triplicate. LTR, long terminal repeat.

(Fig. 3A). Also, HIV inhibition was observed when explants were pretreated with 10 μM UC-781 and then exposed to virus in the absence of the compound. Breakthrough infection occurred when UC-781 was used at a concentration lower than 1 μM (Fig. 3B). Additionally, lymphoid explants that had been treated with UC-781 for 2 h, cultured in the absence of the compound, and exposed to virus on days 0, 1, and 2 posttreatment with UC-781 were also protected against infection by

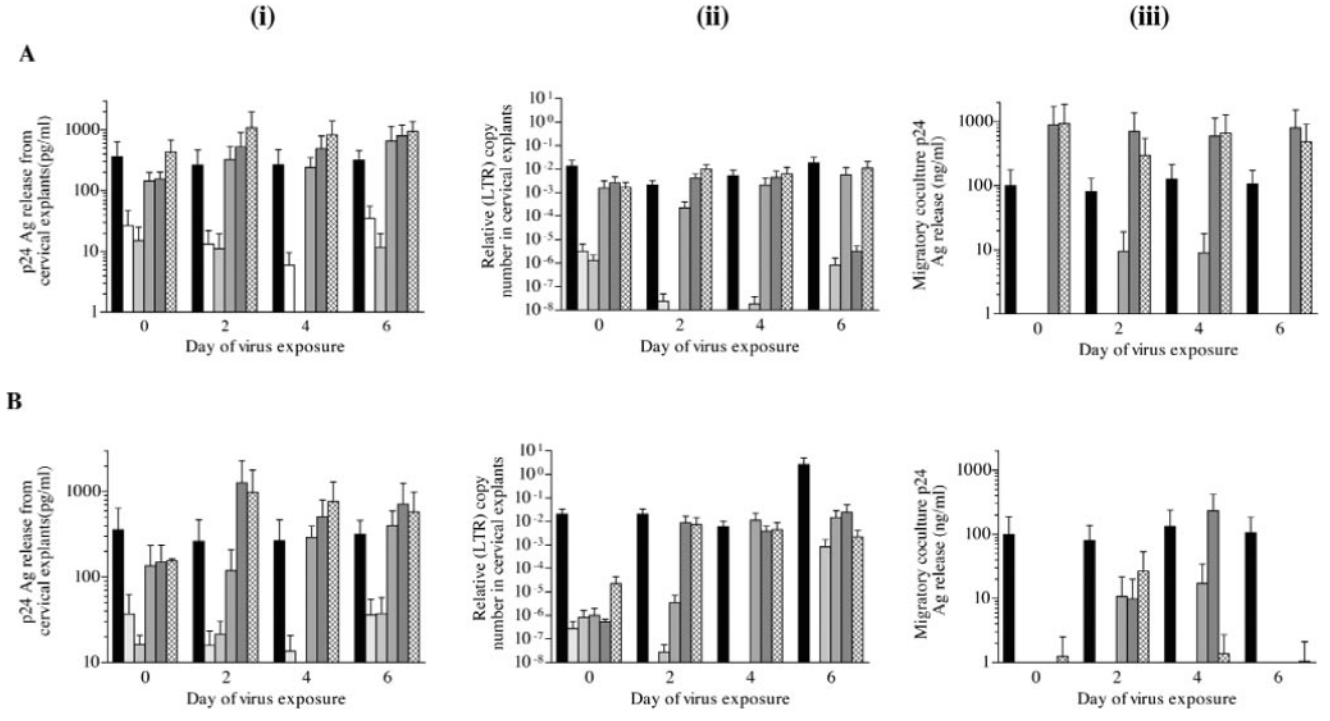

FIG. 2. UC781 demonstrates a memory effect in cervical explants. Ectocervical explants were exposed to UC781 for (A) 2 or (B) 24 h. Bars (from the left): no-compound control, 1,000 μM, 100 μM, 10 μM, 1 μM, and 0.1 μM UC781. Following compound removal, explants were exposed to HIV-1_{BaL} (2 h) either immediately (0) or following 2, 4, or 6 days in culture. Virus was removed and explants cultured as described in Materials and Methods for a further 10 days, when infection was determined by (i) p24 antigen (Ag) content of explant culture supernatants, (ii) HIV-1 proviral DNA content of proteinase K-digested explants (note log scale), and (iii) p24 antigen content of migratory cell coculture supernatants of explants. Data represent the mean ± the standard error of the mean for three individual donors where each condition was tested in triplicate.

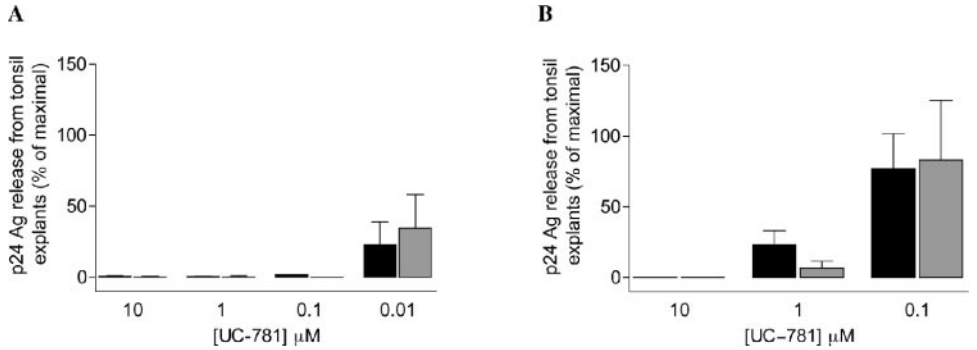

FIG. 3. UC-781 inhibits HIV-1 infection of lymphoid tissue. Tonsil explants were exposed to HIV-$1_{LAV.04}$ (black bars) or HIV-1_{SF162} (gray bars) (A) in the presence of UC-781 or (B) in the absence of the compound following a 2-h pretreatment of tissue with UC-781. Explants were cultured for 12 days when HIV-1 infection was determined by p24 antigen content of culture supernatants. Data represent the mean ± the standard error of the mean of three to nine (two in the case of 0.1 μM in panel A) measurements. Maximal replication of untreated controls was used as 100%. Ag, antigen.

both isolates tested (Fig. 4). A concentration of 10 μM was sufficient to completely block infection by HIV-$1_{LAV.04}$ (Fig. 4A) up to 2 days post drug removal, and in similar experiments 1 μM protected against infection by HIV-1_{SF162} (Fig. 4B).

DISCUSSION

Effective microbicide formulations need to protect against all potential routes of HIV transmission across mucosal surfaces. We have previously shown that blockade of cell surface receptors (CD4, CCR5, and CXCR4) within the mucosa may be sufficient to prevent localized infection of T cells, macrophages, and DCs. Furthermore, viral uptake and dissemination by migratory DCs occurs through CD4 and mannose binding C-type lectin receptors and may be more resistant to microbicides than the establishment of localized infection (10). Thus, effective microbicides may need to target both localized mucosal infection and virus dissemination to draining lymph nodes (18).

We have shown that UC-781 alone and formulated in a vaginal gel potently inhibits viral replication in indicator T cells when present during viral exposure (Table 1). The activity of the placebo gel against X4 virus is likely to be due to the polyanionic carbopol contained within the base formulation. Lack of activity against R5 virus by the placebo formulation probably reflects differences in envelope charge previously shown to reduce susceptibility to polyanion inhibition (19). UC-22, an intermediate of UC-781, was considerably less active than UC-781 against R5 HIV-1_{BaL}. However, there was little difference in activity between the placebo and 1% UC-22 against X4 virus, again reflecting the effects of the carbopol base.

In contrast to previous reports (3), we were unable to demonstrate direct inactivation of viral particles by UC-781; indeed, high-titer virus exposed for 1 h to 200 times the IC_{50} of UC-781 (required to inhibit infection of cells) was still infectious when diluted beyond inhibitory concentrations of UC-781 (data not shown). Furthermore, we were unable to show inhibition using size exclusion columns to separate virus from unbound UC-781 as previously described (13) (data not shown). Other groups have also failed to demonstrate direct virucidal activity for UC-781 (20, 24). Thus, in this study we have focused on the ability of UC-781 to block infection of susceptible cells.

Unformulated UC-781 potently inhibited HIV-1 infection of

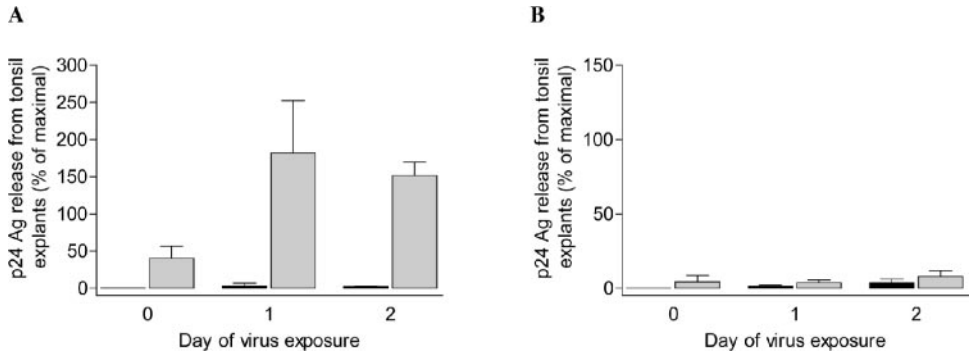

FIG. 4. UC-781 demonstrates a memory effect in lymphoid tissue. Tonsil explants were exposed to UC-781 for 2 h (black bars, 10 μM UC-781; gray bars, 1 μM UC-781). Following compound removal, explants were exposed to (A) HIV-$1_{LAV.04}$ or (B) HIV-1_{SF162} either immediately (day zero) or following 1 or 2 days in culture. Explants were cultured for 12 days when infection was determined by p24 antigen (Ag) content of culture supernatants. Data represent the mean ± the standard error of the mean for three individual donors. Maximal replication of untreated controls was used as 100%.

cervical explant tissue when the tissue was exposed to the compound for only 2 h; indeed, 10 μM was sufficient to block infection 99.99%. We also investigated the effects of UC-781 on virus dissemination by DCs that spontaneously migrate out of cervical explants. We observed that 2-h exposure to compound was sufficient to inhibit this pathway, with 10 μM providing complete protection. Formulated UC-781 was slightly less active than the unformulated compound. The observed differences most likely reflect rates of compound release from the gel formulation. It is possible that different formulations provide faster release rates; nevertheless, the 0.1% gel formulation provided 99.9% protection at a 1/200 dilution. Thus, it is unlikely to be affected by any dilution effects associated with intercourse. Furthermore, controlled release of the compound from the gel might provide a reservoir effect, sustaining drug levels for a prolonged period. A 0.1% UC-22 concentration showed similar activity, although the IC_{50} necessary to block infection of cervical tissue was approximately 100-fold greater then that observed for UC-781. However, the placebo gel demonstrated similar activity against proviral accumulation within cervical tissue, suggesting that the carbopol base itself had potent inhibitory effects. In contrast, the placebo gel had much lower activity against dissemination via migratory cells, suggesting that the activity of the 0.1% gel formulation against this pathway was predominantly dependent upon the activity of UC-22 itself.

Exposure to UC-781 for only 2 h was sufficient to prevent localized infection within cervical explants and inhibit viral dissemination by migratory cells harvested 18 h later. A two-phase transfer model has been proposed for DCs in mucosal infection and dissemination (21). The first involves HIV-1 capture, endosomal internalization, and rapid transinfection of localized T cells, within hours (DC endosomal transfer); the second is dependent upon primary HIV-1 infection of DCs, increasing over 24 to 48 h, and subsequent secondary transinfection of T cells (DC replication-dependent transfer). We have been unable to demonstrate direct inactivation of free virions by UC-781 at the concentrations tested. Furthermore, we have observed that UC-781 does not prevent binding of virus to DC-SIGN-positive cells (unpublished data), and this is likely to be true for other mannose binding C-type lectin receptors. Therefore, it is highly unlikely that UC-781 would prevent HIV-1 capture and endosomal internalization in vivo. However, the ability of UC-781 to prevent HIV-1 infection within cervical explants indicates that while these compounds may not prevent uptake of virus, sufficient compound remains within the tissue to prevent both localized DC endosomal or DC replication-dependent transinfection of localized T cells.

DCs are thought to disseminate localized HIV-1 infection to draining lymph nodes within days (15), and consequently this pathway is most likely mediated by DC replication-dependent transfer rather than endosomal transfer, the latter having a half-life of 4 h (21). The inability to rescue infectious virus from migratory cells harvested 18 h post viral exposure suggests that UC-781 would prevent viral dissemination via DC replication-dependent transfer. Nevertheless, we cannot exclude the possibility that more rapid migration of DC (within hours) might still facilitate viral dissemination via DC endosomal transfer. It is interesting that while previous studies by our group demonstrated that CCR5 antagonists effectively blocked localized infection of cervical explants, in contrast to UC-781, they were unable to inhibit dissemination by migratory cells (10). More recent work has shown that topical PSC-RANTES can prevent SHIV-162P infection of rhesus macaques (11). These data suggest either that blockade of localized infection is sufficient to prevent transmission or that sufficient compound is required to protect both localized mucosa and draining lymph nodes.

UC-781 demonstrated prolonged activity with inhibition of both localized infection and dissemination by migratory cells still observed 6 days after the compound was removed. A 24-h pretreatment was more effective than 2 h, suggesting increased tissue adsorption of UC-781 with time. Nevertheless, it should be noted that only 2 h of pretreatment with 10 μM UC-781 was sufficient to inhibit 99.9% of provirus formation and completely block dissemination of virus by migratory cells. As a class, tight-binding inhibitors of HIV-1 RT are very hydrophobic and readily cross cell membranes. Due to the hydrophobic nature of UC-781, it is highly likely that sufficient drug remains associated with the tissue to prevent infection for up to 6 days post compound removal.

We carried out subsequent experiments to determine the concentration of UC-781 that would be required to prevent HIV-1 in lymphoid tissue. Lymphoid tissue required higher concentrations of UC-781 to prevent infection with the X4 and R5 viruses than was seen in cervical explants. Furthermore, there was greater efficacy against R5 over X4 virus. This likely reflects the higher number of potential target cells, their activation status, and the relative proportions of CXCR4 versus CCR5 target cells present in lymphoid tissue (8).

It is unclear how much compound would need to be applied vaginally to achieve these levels in draining lymphoid tissue; however, the 0.1% formulation is 30,000 times higher than the concentration required to block infection of lymphoid tissue (0.1 μM; Fig. 3A). Thus, it is highly probable that suppressive levels of the drug could be achieved by passive diffusion. These findings may be important in guiding rational product development through subsequent animal and human studies. Protection or lack of protection in the macaque challenge model should be correlated to mucosal and lymphoid tissue levels of UC-781. Should protection be dependent upon tissue levels of the compound shown in this study to protect infection of lymphoid tissue, then success in human clinical trials would likely be dependent upon efficient delivery of such a dose via vaginal administration.

The prolonged protection observed in both cervical and lymphoid tissues pretreated with compound may give this class of inhibitors (tight-binding NNRTIs) a significant advantage over earlier polyanion-based microbicide compounds, currently in human phase III trials, that are only active when present during virus-cell interaction (18). If the duration of protection (>6 days for cervical tissue) observed in this study can be translated to the in vivo situation, it may be possible to develop microbicide formulations that will be applied on a less frequent basis (daily to weekly) and independently of coitus. This would provide significant advantages for user compliance. Another important issue in regard to the use of UC-781 and similar compounds as microbicides is their potential to act against viruses containing mutations associated with resistance to this family of drugs. While we have not specifically addressed the activity

of UC-781 against resistant strains, previous studies have demonstrated that the *n*-fold resistances of virus containing the Y181C, L100I, and K103N mutations were 13-, 67-, and 17-fold, respectively, compared to wild-type virus (5). However, these resistant strains were completely inhibited at low micromolar concentrations equivalent to a 1/100 to 1/1,000 dilution of a 0.1% gel. These data suggest that, if formulated at a sufficiently high concentration, UC-781 could be used to prevent infection with resistant virus.

In summary, these data suggest that UC-781 is an attractive candidate for development as a topical vaginal microbicide against HIV-1.

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