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# In Vitro Evaluation of Nonnucleoside Reverse Transcriptase Inhibitors UC-781 and TMC120-R147681 as Human Immunodeficiency Virus Microbicides†

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**The nonnucleoside reverse transcriptase inhibitors UC-781 and TMC120-R147681 (Dapivirine) effectively prevented human immunodeficiency virus (HIV) infection in cocultures of monocyte-derived dendritic cells and T cells, representing primary targets in sexual transmission. Both drugs had a favorable therapeutic index. A 24-h treatment with 1,000 nM UC-781 or 100 nM TMC120-R147681 prevented cell-free HIV infection, whereas 10-fold-higher concentrations blocked cell-associated HIV.**

Condom use is effective in preventing sexual human immunodeficiency virus (HIV) transmission but is male controlled and often not negotiable (9, 10, 12, 15, 18). To offer women more control, vaginal microbicides need to be developed.

We evaluated the nonnucleoside reverse transcriptase inhibitors UC-781 (Crompton Corp., Middleburg, Conn.) and TMC120-R147681 (Tibotec-Virco, Mechlin, Belgium), both in preclinical development as microbicides. UC-781 was reported as a reverse transcriptase tight-binding thiocarboxanilide (4, 6), while TMC120-R147681 is a diarylpyrimidine with high activity against wild-type and mutant HIV (13; B. Gruzdev, A. Horban, A. Boron-Kaczmarska, D. Gille, G. Van't Klooster, and R. Pauwels, 8th Conf. Retrovir. Opportunistic Infect., abstr. 13, 2001).

Since early microbicide trials raised concerns about testing incompletely characterized compounds in humans (17), we propose an in vitro model using monocyte-derived dendritic cells (MO-DC) and autologous $CD4^+$ T cells (20), representing early targets during sexual transmission (14, 16).

Reference data on antiviral activities and cellular toxicities of the two drugs were obtained using CEM T cells (American Type Culture Collection, Manassas, Va.), infected with the lymphotropic HIV strain $HTLV\text{-}III_B$ under previously standardized conditions (1). Both drugs prevented HIV-induced syncytium formation in the nanomolar range and showed a low cytostatic activity (Table 1), evaluated by cell counting (Coulter Counter, Harpenden, Hertfordshire, United Kingdom) of mock-infected, drug-exposed cell cultures. Inhibition of HIV type 1 (HIV-1) reverse transcriptase activity was determined in a cell-free assay according to a previously published description (3), resulting in similar 50% inhibitory concentrations for the two drugs (Table 1).

Using a T-cell line and a lymphotropic strain is physiologically less relevant; therefore, we focused on MO-DC plus $CD4^+$ T-cell cocultures, infected with monotropic HIV-1 Ba-L.

We evaluated cell-free and cell-associated virus because both are present in semen (21, 22) and cervical mucus from HIV-1-infected persons (11). MO-DC and $CD4^+$ T cells were generated from buffy coat peripheral blood mononuclear cells (PBMCs) as previously described (20).

To mimic a sustained drug release (e.g., a microbicide formulated in an intravaginal device), virus was drug treated for 1 h before infection, during the 2-h incubation of the virus with the MO-DC, and after infection (during primary culture of MO-DC and autologous $CD4^+$ T cells). Culture medium was refreshed twice weekly with drug. After 2 weeks of primary culture, cells were washed and cultured for 2 weeks with activated PBMCs to assess viral rescue (secondary cultures), as described previously (20). Secondary culture supernatants were tested in an enzyme-linked immunosorbent assay (ELISA), while cells were processed for HIV DNA measurement with a PCR-based HIV proviral DNA quantitation kit developed from the Amplicor HIV-1 Monitor test, version 1.5 (Roche Molecular Systems, Branchburg, N.J.), the modifications of which have been described elsewhere (7).

Infection of MO-DC plus $CD4^+$ T-cell cocultures with cell-associated virus was apparently blocked with 100 nM UC-781 (Table 2). However, secondary culture revealed latent infection in four of six cultures. Treatment with 1,000 nM UC-781 in the primary culture rescued virus in one of six wells. No rescue was found after treatment with a 10,000 nM concentration.

TMC120-R147681 apparently blocked infection in the primary cultures at a 10 nM concentration, but secondary cultures revealed that a 100 nM concentration was needed to completely prevent proviral integration.

When cell-free virus was used, proviral integration could not be blocked by continuous treatment (during primary culture) with up to 1,000 nM UC-781 (one of six wells positive in an

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† This work is dedicated to the memory of Paul A. J. Janssen, founder of Janssen Pharmaceutica and the Center for Molecular Design.

TABLE 1. Antiviral activities, cytotoxicities, and HIV-1 reverse transcriptase inhibitory capacities of UC-781 and TMC120-R147681 in CEM T cells[a]

| Drug | Treatment | HIV-1 ($III_B$) | $EC_{50}$ (nM) | $CC_{50}$ (nM) | $IC_{50}$ (nM) |
|---|---|---|---|---|---|
| UC-781 | Continuous | Cell free | 6 | >20,000 | 23 |
| TMC120-R147681 | Continuous | Cell free | 1 | 1,367 | 24 |

[a] $EC_{50}$ (50% effective concentration), drug concentration required to inhibit syncytium formation of HIV-1 $III_B$-infected CEM T cells by 50%. $CC_{50}$, 50% cytostatic concentration, drug concentration required to inhibit proliferation of mock-infected CEM T cells by 50%. $IC_{50}$, 50% inhibitory concentration, drug concentration required to inhibit HIV-1 reverse transcriptase activity by 50%. All results are the means of two experiments.

ELISA of secondary culture; data not shown). In contrast, continuous treatment with 10 nM TMC120-R147681 sufficed to completely block HIV infection (Table 3).

We next investigated whether viral infection and integration (measured by ELISA and PCR, respectively) were prevented by a short drug treatment (24 h) of virus and cells, mimicking a microbicide formulated in a gel. After 24 h, cells were washed and incubated without drug (primary and secondary cultures). Compared to continuous treatment, similar concentrations of UC-781 were needed to completely block cell-free or cell-associated virus, whereas TMC120-R147681 blocked infection at concentrations 10 times higher than those used for the continuous treatment (Table 3).

If treatment was further limited to pretreatment of the virus (1 h) and treatment of the MO-DC during infection (2 h), but not during the MO-DC plus $CD4^+$ T-cell cocultures, up to 10,000 nM (either) UC-781 or TMC120-R147681 failed to completely block infection (data not shown). Nevertheless, previous studies by Borkow et al. showed that UC-781 acts as a "virucidal agent" (4). Possible explanations for this discrepancy include the use of a different viral strain (the lymphotropic HIV-1 $III_B$ instead of HIV-1 Ba-L) and different target cells (cord blood mononuclear cells instead of MO-DC). Borkow et al. themselves reported that UC-781 was unable to establish a "chemical barrier" to protect epithelial cells from HIV infection (5). Moreover, other labs also were unable to reproduce the memory and virucidal effects of UC-781 (19).

TABLE 2. Inhibition of infection of MO-DC plus $CD4^+$ T-cell cocultures with cell-associated HIV Ba-L

| Drug | Concn (nM) | HIV antigen (no. of positive wells)[a] | | HIV proviral DNA[d] (2nd culture) |
|---|---|---|---|---|
| | | Primary culture[b] | Secondary culture[c] | |
| UC-781 | 10,000 | 0 | 0 | Neg |
| | 1,000 | 0 | 1 | 4.66 |
| | 100 | 0 | 4 | ND |
| | 10 | 5 | 5 | ND |
| TMC120-R147681 | 10,000 | 0 | 0 | Neg |
| | 1,000 | 0 | 0 | Neg |
| | 100 | 0 | 0 | Neg |
| | 10 | 0 | 3 | 4.74 |
| No drug | 0 | 6 | 6 | 4.85 |

[a] Culture supernatant was tested for HIV antigen by ELISA. Every condition was evaluated in sixfold replicates. Results representative of two experiments are shown. The numbers of antigen- positive microcultures (out of six) at the end of the primary and secondary cultures are represented.

[b] Cell-associated HIV Ba-L was preincubated with drug, washed, and added to cocultures of MO-DC and autologous $CD4^+$ T cells. Cells were cultured for 2 weeks, in the continuous presence of drug (primary culture).

[c] After the primary culture, cells were washed and phytohemagglutinin–interleukin-2-activated PBMCs were added and maintained in interleukin-2-containing medium during a secondary culture of 2 weeks (no drug present).

[d] After the secondary culture, cells were pooled and analyzed by PCR for the presence of proviral DNA; results are expressed as log(number of DNA copies/$10^6$ cells). ND, not done; Neg, negative.

TABLE 3. Minimal drug concentrations for prevention of replicative HIV infection in MO-DC plus $CD4^+$ T-cell cocultures

| Drug | Treatment[a] | HIV | Concn (nM)[b] |
|---|---|---|---|
| UC-781 | 24 h | Cell free | 1,000 |
| | | Cell associated | 10,000 |
| | Continuous | Cell free | >1,000[c] |
| | | Cell associated | 10,000 |
| TMC120-R147681 | 24 h | Cell free | 100 |
| | | Cell associated | 1,000 |
| | Continuous | Cell free | 10 |
| | | Cell associated | 100 |

[a] For 24-h treatment, MO-DC plus $CD4^+$ T-cell cocultures were incubated with cell-free or cell- associated HIV and drug for 24 h. Cocultures were then washed and cultured for 2 weeks of primary culture (no drug present). For continuous treatment, MO-DC were incubated with drug- treated cell-free or cell-associated HIV, cocultured with autologous $CD4^+$ T cells, and continuously drug treated during the primary culture. After the primary culture of 24 h and continuous treatment, cells were washed and used for the secondary cultures (no drug present).

[b] Drug concentrations that prevent replicative HIV infection, as measured by ELISA of culture supernatants and DNA PCR in cells after the secondary culture. Every condition was evaluated in sixfold replicates. Results representative of two experiments are shown.

[c] The 10,000 nM concentration was not used in these parts of the experiments.

Besides antiviral activity, we evaluated whether both drugs inhibited cell proliferation in mixed leukocyte cultures with MO-DC as stimulators and allogeneic $CD4^+$ T cells as responders. This model was chosen to mimic the induction of an immune response, as happens during HIV infection. In the case that an anti-HIV drug fails, it should at least not inhibit the generation of a potentially beneficial immune response. If drug was present during the whole 5-day culture period, the 50% immune suppressive concentration ($ISC_{50}$) was over 45,000 nM for UC-781 and about 1,500 nM for TMC120-R147681. If the drugs were present during the first 24 h only, the $ISC_{50}$ of UC-781 was similar, but it increased to almost 25,000 nM for TMC120-R147681 (Table 4). Thus, the immune suppressive activity of UC-781 was low, irrespective of the duration of exposure, whereas TMC120-R147681 was clearly less suppressive in the 24-h treatment. We don't know which mechanism causes this effect.

To evaluate the relation of antiviral and immune suppressive activities, the 50% effective concentrations ($EC_{50}$) were calculated and the therapeutic indices (TI; $ISC_{50}/EC_{50}$) were determined (Table 4). Both drugs, but especially TMC120-R147681, showed favorable TIs which were higher during a continuous treatment than during a 24-h treatment. This is mainly due to the lower $EC_{50}$ (higher antiviral activity) of drugs during a continuous treatment, while the $ISC_{50}$ were less affected.

With regard to in vivo vaginal toxicity, Balzarini et al. reported on the toxicity of UC-781 in a rabbit model, in which a gel containing 148 mM UC-781 (5%) did not cause local inflammation or damage of the vaginal mucosa and epithelia (2).

TABLE 4. Overview of the antiviral and immune suppressive activities of UC-781 and TMC120- R147681 in cocultures of MO-DC plus $CD4^+$ T cells

| Drug | Treatment | HIV | $EC_{50}$ (nM)[a] | $ISC_{50}$ (nM)[b] | TI[d] |
|---|---|---|---|---|---|
| UC-781 | 24 h | Cell free | 550 | 45,873 | 83 |
| | | Cell associated | 1,588 | | 29 |
| | Continuous | Cell free | 5 | 45,850 | 9,170 |
| | | Cell associated | 55 | | 834 |
| TMC120-R147681 | 24 h | Cell free | 42 | 24,886 | 592 |
| | | Cell associated | 63 | | 395 |
| | Continuous | Cell free | <0.1 | 1,515 | >15,150 |
| | | Cell associated | <1[c] | | >1,515 |

[a] $EC_{50}$, drug concentration inhibiting 50% of HIV Ba-L replication. Results representative of two experiments are shown.
[b] $ISC_{50}$ (50% immune suppressive concentration), drug concentration inhibiting 50% of T- lymphocyte proliferation. Results representative of two experiments are shown.
[c] The 0.1 nM concentration was not used in these parts of the experiments.
[d] TI, therapeutic index ($ISC_{50}/EC_{50}$).

Similarly, a 10-day daily application of a gel containing up to 10 mM TMC120-R147681 (0.3%) showed no irritation, evaluated either macroscopically at necropsy or microscopically after histopathologic examination (J. Van Roey, personal communication).

Di Fabio et al. recently showed that vaginal infection of SCID mice with cell-associated HIV-1 is blocked with a nontoxic, 0.00225 mM TMC120-R147681-containing gel (8). We provide in vitro evidence that a short treatment (24 h) with UC-781 or TMC120-R147681 can block cell-free or cell-associated HIV infection of MO-DC plus $CD4^+$ T-cell cocultures, representing the early targets during transmission. From this viewpoint, our results encourage the further development of these drugs as microbicides.

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